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Part I
Epigenetic Alterations in Hypoxia

Chapter 1
Epigenetic Mechanisms as an Interface Between the Environment and Genome

Zdenko Herceg

Abstract Recent advances in epigenetics have had tremendous impact on our thinking and understanding of biological phenomena and the impact of environmental stressors on complex diseases, notably cancer. Environmental and lifestyle factors are thought to be implicated in the development of a wide range of human cancers by eliciting epigenetic changes, however, the underlying mechanisms remain poorly understood. Epigenetic mechanisms can be viewed as an interface between the genome and environmental influence, therefore aberrant epigenetic events associated with environmental stressors and factors in the cell microenvironment are likely to play an important role in the onset and progression of different human malignancies. At the cellular level, aberrant epigenetic events influence critical cellular events (such as gene expression, carcinogen detoxification, DNA repair, and cell cycle), which are further modulated by risk factor exposures and thus may define the severity/subtype of cancer. This review summarizes recent progress in our understanding of the epigenetic mechanisms through which environmental stressors and endogenous factors may promote tumor development and progression.

Keywords Epigenome • Environment • DNA methylation • Histone modifications • Noncoding RNAs • Cancer

1.1 Introduction

Epigenetics represents a rapidly expanding field of cancer research, as epigenetic changes have emerged as key mechanisms in cancer development. The term "epigenetic" refers to all heritable changes in gene expression and chromatin organization that are independent of the DNA sequence itself and that can be propagated over cell divisions [4]. The key events associated with cancer development and progression can be caused not only by genetic changes but also by epigenetic deregulation. The ubiquity and intrinsic reversibility of epigenetic changes, as well as their

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early appearance in virtually all types of human cancer, makes them attractive subjects for biomarker discovery and strategies for cancer treatment and prevention.

All critical changes in cancer cells, such as silencing of tumor suppressor genes, activation of oncogenes, and defects in DNA repair, can be induced by deregulated epigenetic mechanisms. Therefore, understanding epigenetic mechanisms that promote cancer onset, progression, and metastasis is fundamental to improving our ability to successfully prevent and treat cancer.

1.2 Epigenetic Mechanisms

There are three distinct classes of epigenetic information that can be inherited over cell generations: DNA methylation, histone modifications, and RNA-mediated gene silencing (Fig. 1).

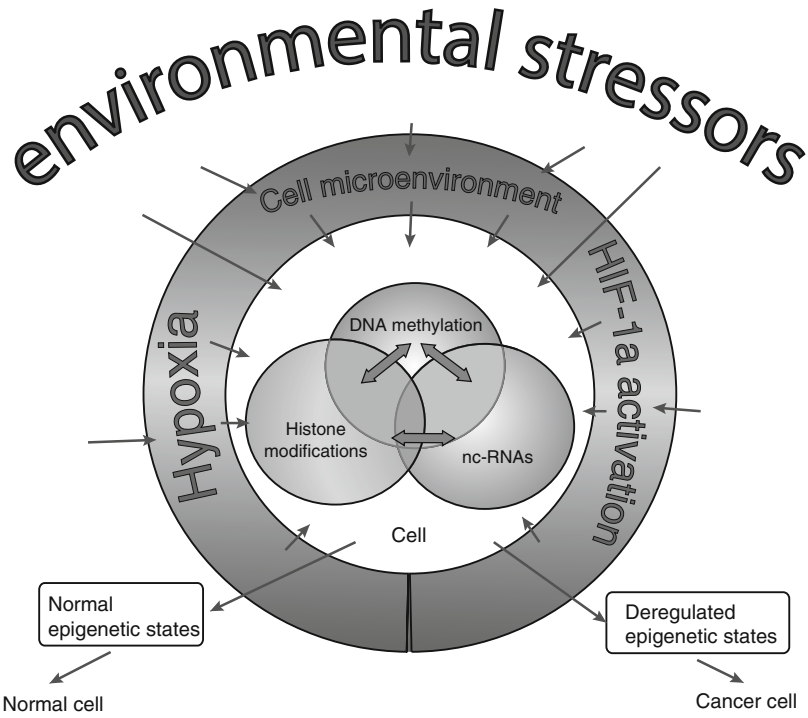

Fig. 1 Signals that trigger epigenetic events and mechanisms of initiation and maintenance of epigenetic states. Theoretically, epigenetic initiators and maintainers respond to epigenators (external and endogenous signals) resulting in initiation and maintenance of a change in epigenetic state. This cascade of events may dictate cellular outcomes by regulating cellular processes such as gene transcription, proliferation, and DNA repair. Deregulation of epigenetic mechanisms may promote the development of abnormal phenotypes and diseases including cancer

DNA methylation. Methylation of DNA refers to the covalent addition of a methyl group (-CH3) on the cytosine pyrimidine ring in DNA by a number of DNA methyltransferases. It occurs almost exclusively at cytosines that are located 5′ to a guanine in a CpG dinucleotide [3], although there is growing evidence for the presence of methylated cytosine which is not in a CpG configuration [3, 10, 15, 39, 42, 54]. DNA methylation is a physiological process that participates in the maintenance of gene activity states (imprinting, differentiation) and cell identity as well as in genome defence mechanisms, acting against potentially deleterious mobile genetic elements. However, unscheduled hypermethylation of small stretches of DNA, known as CpG islands, that are often located within the promoter regions of human genes and frequently free of DNA methylation in normal cells, tend to be associated with aberrant transcriptional inactivation in cancer cells. However, the precise molecular mechanism by which DNA methylation brings about gene silencing is not fully understood.

Histone modifications. Histone modifications include a variety of posttranslational modifications of the histones (specialized proteins associated with genomic DNA forming the chromatin, a DNA–protein complex). Histone modifications include acetylation, phosphorylation, ubuiquitination, and methylation of histone proteins at specific amino-acid residues. Previous studies have suggested that different histone marks may act in a combinatorial fashion to regulate cellular processes and consistently dictate the outcome, a concept known as the "histone code" [25].

Noncoding RNAs. Noncoding RNAs, found in the form of small RNAs (microRNAs) or long noncoding RNAs (lncRNAs), represent the most recent epigenetic mechanism playing an important role in the regulation of the gene transcription [7]. Deregulation of noncoding RNA expression has been associated with human diseases, including cancer [34].

Recent studies have provided evidence that different epigenetic mechanisms work together to establish and maintain gene activity states over many cell generations and that deregulation of one may cross-influence other epigenetic mechanism [66] (Fig. 1).

1.3 Epigenetic Changes in Cancer

A wealth of evidence indicates that all three classes of epigenetic modifications are profoundly altered in human malignancies. Deregulation of DNA methylation is found in two distinct forms: global hypomethylation and promoter-specific hypermethylation. Global hypomethylation refers to a total loss of 5-methyl cytosine that is found in virtually all human cancers [29]. This consistent, although relatively moderate, demethylation is caused by the loss of methyl-cytosine in the regions of the genome containing transposons and repetitive sequences. Although it has been proposed that global hypomethylation may act through the induction of chromosomal instability and activation of cellular proto-oncogenes, the precise mechanism

by which the global loss of DNA methylation contributes to the oncogenic transformation and tumor development remains unknown [14].

DNA hypermethylation occurs at CpG islands in the promoter regions of large numbers of genes and is usually associated with gene inactivation [14, 30]. A large number of human genes, including tumor suppressor genes and other cancer-associated genes, have been found hypermethylated and epigenetically silenced in most, if not all, human malignancies. However, with the advent of new epigenomic tools that allow high-resolution and cost-effective profiling of DNA methylation the list of genes targeted by aberrant hypermethylation is likely to grow steadily. Although it is well established that the predominant consequence of methylation is transcriptional silencing, it is less clear whether this is mediated through a direct or indirect mechanism [66]. Direct inhibition of transcription may be through blocking transcription factors from binding to promoters containing methylated CpG sites, while indirect repression may involve proteins that bind to methylated DNA via a methyl-CpG-binding domain.

1.4 Epigenators, Initiators, and Maintainers

The vast majority of epigenetics and epigenomics studies have focused on the analysis of epigenetic states in different cell types (including normal and tumor tissues) and pathophysiologic conditions. While these studies have provided important information on the maintenance and heritability of epigenetic patterns, they have had a limited impact on our understanding of the mechanisms involved in the initiation of epigenetic changes. Also, little is known on the agents and conditions that trigger epigenetic events. Three types of signals (known as epigenators, initiators, and maintainers) are thought to participate in triggering and establishing epigenetic states that are transmitted over cell generations [2]. Epigenators are defined as the signals originating from the cell's external environment that trigger an intracellular pathway leading to epigenetic change. In contrast to plants, the role of epigenators in human tissues and how their deregulation promotes human diseases is largely unknown [2, 57]. For example, cell–cell and cell–extracellular matrix changes induced by environmental exposures and endogenous cues could be considered as epigenators. However, further studies are needed to determine the identity of epigenators and elucidate the mechanisms involved in the initiation of epigenetic states. Application of new epigenomics tools combined with appropriate experimental models may prove valuable in providing important information on epigenators and their mode of action.

It has been proposed that an epigenetic initiator responds to an epigenator signal and defines the location of the epigenetic change on the chromosome [2]. DNA binding proteins and noncoding RNAs (ncRNAs) can be considered epigenetic initiators. In this respect, lncRNAs have been shown to play an important role in determining how chromatin modifications are distributed along chromosomes. For example, HOTAIR (a long noncoding RNA) was shown to participate in silencing a

chromosomal region by binding to the RNA binding domain of Polycomb repressive complex, thereby dictating epigenetic silencing through Polycomb-mediated deposition of repressive histone marks [17]. Because ncRNAs have been implicated in the mechanisms underlying silencing of large chromatin regions (heterochromatin) this class of epigenetic modifications may be a bridge between initiator signals and epigenetic maintainers. lncRNAs are currently under intensive research and the near future is likely to provide information on the role of ncRNAs as epigenetic initiators.

In contrast to epigenators and epigenetic initiators, a great deal is known about the molecules involved in maintaining epigenetic states. This is particularly true for DNA methylation and histone modifications. Epigenetic maintainers are defined as molecules and complexes that respond to initiators and ensure the maintenance of epigenetic states [2]. DNA methylation and histone modifications are carriers of epigenetic signals through cell division, and can thus be considered epigenetic maintainers. Deregulation of epigenetic maintainers has been found in complex human diseases, notably cancer; however, the identity of the epigenators and initiator signals that presumably precede and promote maintaining aberrant epigenetic states remains to be established. Screening for potential epigenators, initiators, and maintainers in parallel in the same model system combined with focused functional studies may prove informative in identifying possible interactions and interdependencies between epigenators, initiators, and maintainers in physiological and pathological conditions.

1.5 Epigenome as an Interface Between the Environment and Genetic Code

Epigenetic modifications are considered an interface between genotype and phenotype [4, 13, 20, 24]; however, the epigenome could also be viewed as an interface between the environment and the genome (Fig. 1). This epigenetic interface may "buffer" the impact of environmental exposures on the genome. It also modulates the response of the genome to environmental cues. The implications of this concept are twofold: first, defects in the epigenetic interface components may deregulate key cellular processes (such as gene transcription or DNA repair and replication) following environmental exposure, which may result in cell death or oncogenic transformation. Second, environmental factors may leave "exposure sequelae" on the epigenetic interface that could be exploited in biomarker discovery.

A number of agents in the environment have been suggested to alter epigenetic states [20]. Evidence is accumulating that environmental agents may affect cellular functions through their impact on DNA methylation, histone modifications and noncoding RNAs. Several recent studies suggest that the hypermethylation and unscheduled silencing of several key cellular genes in lung cancer are associated with exposure to tobacco smoke [1, 48, 65]. Therefore, tobacco smoke may, in addition

to inducing gene mutations, contribute to oncogenic transformation by inactivating key cancer-associated genes through epigenetic disruption. Environmental toxins, such as arsenic and nickel, may also deregulate epigenetic states (including chromatin modifications and DNA methylation) [9, 33, 59, 60, 73, 74], and promote cancer development through epigenetic mechanisms.

Biological agents, such as viruses, like Human papillomavirus (HPV), Epstein-Barr virus (EBV), and Human hepatitis virus (HBV), and bacteria may also alter the expression of host genes via an epigenetic strategy [11, 20, 37, 41, 67]. Epigenetic mechanisms including DNA methylation, chromatin modifications, and RNA-mediated gene silencing are believed to be important in protecting against viral genomes [3, 20, 24]. However, viruses also use epigenetic mechanisms to regulate expression of their own genes [75]. Importantly, previous studies have shown that HBV infection and integration of viral genomes may lead to epigenetic changes at the level of both viral and host genomes [20]. Therefore, epigenetic changes associated with viral infection and integration of viral genomes may trigger aberrant events that lead to oncogenic transformation and cancer development. In other words, different viruses may abrogate cellular defence systems and induce silencing of host genes through epigenetic deregulation; however, the role of epigenetic events associated with viral infection and their role in cancer remains largely unknown. In particular, it is unclear whether viral infections promote carcinogenesis directly through deregulation of key genes and pathways or indirectly, through inflammatory processes. For example, chronic production of cytokines during inflammation may alter epigenetic states in affected cells. Alternatively, low oxygenation (hypoxia) that commonly occurs in inflamed tissue may induce Hypoxia-Induced Factors (HIFs) that may have an impact on histone modifications [5, 51] (Fig. 1).

In addition to viruses, bacterial infection (such as that induced by *H. pylori*) has been associated with aberrant epigenetic states (DNA methylation) in human gastric cancer. *H. pylori* infection appears to induce DNA methylation changes in promoters of many key genes in gastric mucosa, thus promoting the development and progression of gastric cancer [41]. Although, the mechanism by which *H. pylori* infection induces changes in DNA methylation remains poorly understood, chronic inflammation and cell proliferation associated with bacterial infection, rather than the presence of bacterial agents, may trigger aberrant hypermethylation [23, 61, 68]. For example, the suppression of gene expression in the host genome, a phenomenon frequently observed in tissues affected by inflammation, may promote aberrant DNA methylation [12, 22, 45, 58].

1.6 Dietary Factors and One-Carbon Metabolism

Diet influences DNA methylation levels in cells in several ways, but mainly via the one-carbon metabolism pathway. The DNA methylation reaction involves the use of methyl groups, therefore the establishing and maintaining of DNA methylation

relies on dietary methyl donors [63]. In a DNA methylation reaction, the final methyl donor produced by one-carbon metabolism, S-adenosylmethionine (SAM), is used. The primary methyl donors and key mediators of one-carbon metabolic pathways are dietary folates, although choline and other cofactors such as vitamins B6 and B12 represent important methyl donors [72]. For the production of tetrahydrofolate (THF), a precursor for homocysteine conversion to methionine, cells use methyl-THF which serves as a methyl group donor. In cells, methionine is converted to SAM by methionine adenosyltransferase, whereas SAM serves as the principal methyl donor [35]. Therefore, conversion of SAM to S-adenosylhomocysteine (SAH) is critical for the methylation process. Because SAH is a potent competitive inhibitor of methylation reactions, disruption of the SAH/SAM ratio, through an increase in SAH or a decrease in SAM, leads to inhibition of methylation reactions. The SAM:SAH ratio is regulated via inhibition of SAM by 5,10-methylene-THF reductase (MTHFR), and of GNMT by folate compounds [16]. Therefore, intake of dietary folates is important for reactions in one-carbon metabolism, and low dietary intake of folate and choline may decrease concentrations of SAM, potentially triggering DNA hypomethylation.

Consistent with the essential role of dietary folate in DNA methylation, deficiencies in folate, methionine, and vitamin B6 have been associated with an increased risk of cancer at different sites. Recent studies suggested that serum levels of one-carbon metabolites are associated with cancer risk [28] and DNA methylation states in blood cells [69]. These results support the notion that dietary intake of folates, and subsequently plasma levels of one-carbon metabolites and B vitamins, could influence the methylation level of key cellular genes; however, the precise mechanism underlying the modulation of DNA methylation levels by one-carbon metabolites and B vitamins remains to be established.

1.7 DNA Demethylation

DNA methylation has long been considered as a highly stable epigenetic modification. However, recent studies suggest that cycles of DNA methylation and demethylation may take place during the life of a cell, arguing that DNA methylation mark may be more dynamic than previously thought. For example, Kangaspeska et al. reported a cyclical pattern of DNA methylation and demethylation at a set of promoters during the initial cycle of transcription, and again after the second cycle of productive transcription [32]. Another study also demonstrated that cyclical DNA methylation occurs at several CpG sites and that this process may be strand specific, with only the transcribed strand being demethylated after the first cycle of transcription [43]. Consistent with these observations, DNMT enzymes were found at the active promoter and their recruitment coincides with both phases of DNA methylation and demethylation. These intriguing results argue that DNMTs may be involved in both the addition and removal of methyl groups. Furthermore, DNA methyltransferases DNMT3A and DNMT3B were found capable of deaminating

methylated cytosines, thereby generating mismatches that are cleaved by a glycosylase and repaired by machinery involved in the base-excision repair [43].

These studies, together with those showing a rapid wave of DNA demethylation of the paternal genome after fertilization [36], argue that the mechanism of DNA demethylation is operational in mammalian cells. One of the proposed mechanisms involves passive DNA demethylation through rounds of DNA replication, brought about by inhibition of DNMT1. This process can be chemically induced by 5-Aza-2′-deoxycytidine, an inhibitor of DNMT, which blocks DNMT1 activity, compromising the maintenance of the DNA methylation mark through cell division [49, 71]. However, a passive mechanism for DNA demethylation may not be the only mechanism and active DNA demethylation might exist. There are several possible active mechanisms of DNA demethylation that may operate in mammalian cells. These include the direct removal of the methyl group via hydrolytic attack, oxidation, or a DNA demethylase enzyme [55]. Removal of methyl groups from cytosines is considered unlikely, arguing that alternative pathways involving DNA glycosylases and deaminases may operate in mammalian cells [38, 56, 64], although many reported DNA demethylase activities have been challenged [19, 26, 47] and it is not clear which protein(s) may function as an active DNA demethylase in mammals [6].

Recent studies suggested that the Tet family of proteins may be involved in active DNA demethylation. The capacity of Tet proteins to hydrolyse methyl cytosines (producing 5-hydroxymethylcytosine, 5hmC) and to act on fully methylated or hemi-methylated DNA has been reported [62]. Moreover, Tet1 blocks DNMTs and DNA demethylation is followed by chromatin remodelling including loss of H1 and H2Az [18, 62]. Other potential players such as GADD45 and ELP3 have been investigated in the context of DNA demethylation [40, 46, 47, 52, 70]. Together, these results provide compelling evidence that DNA methylation mark is more dynamic than previously thought. These observations may also suggest that establishing and maintaining DNA methylation may be highly susceptible to modulation by environmental and extracellular influences.

1.8 Environmental Exposure and Transgenerational Epigenetic Inheritance

Exposure to environmental and dietary factors during embryonic life as well as during childhood and adolescence is associated with a change in risk of developing specific human cancers in adulthood. Epigenetic deregulation during this critical period of growth and development might explain such observations. This notion is supported by recent studies showing an association between early life energy restriction and DNA methylation states in adult colorectal cancer [21]. Therefore, exposure to a transient environmental factor during early life may result in persistent epigenetic changes that later influence cancer development. Furthermore, it is possible that epigenetic changes induced by environmental exposures might be

transmitted to future generations. It is widely believed that epigenetic states are cleared on passage through the germ line in mammals and that only genetic features are passed on to subsequent generations. However, accumulating evidence argues that in both animals and plants epigenetic modifications are not completely erased between generations. The phenomenon of incomplete erasure of epigenetic marks between generations resulting in a detectable phenotype is known as transgenerational epigenetic inheritance [27].

Perhaps surprisingly, recent studies indicate that epigenetic states can be inherited transgenerationally after not only maternal but also paternal transmission [8, 53]. Epigenomic screening revealed that paternal diet may induce changes in DNA methylation and consequently expression of specific genes and pathways in offspring of inbred mice and that carriers of epigenetic information that reside in sperm may respond to environmental exposures [8]. In addition, epigenetic intergenerational transmission of metabolic changes from father to offspring in rats have also been described [44]. Therefore, epigenetic changes induced by environmental exposures may be inherited through the germline and this could be a plausible transgenerational carrier of environmental "memory", although future studies are required to substantiate these observations and to define their underlying mechanism.

This intriguing concept, derived from experimental studies, is further supported by epidemiological observations suggesting that environmental and dietary exposures in men may influence health and susceptibility to diseases in following generations [31, 50]. However, to what extent the parental environmental exposures contribute to cancer risk through transgenerational epigenetic inheritance remains to be establishes and warrants further study.

1.9 Conclusions and Perspectives

The field of cancer epigenetics has been expanding rapidly over the past decade and numerous conceptual advances have dramatically accelerated research in this and related fields. Both the scientific and medical communities now recognize the key role of epigenetic mechanisms in the development and control of normal cellular processes as well as abnormal events associated with disease development, notably human cancer. Epigenetic modifications can be viewed as an interface between the environment and the genome, the deregulation of which may disrupt key cellular processes, leading to disease. This interface may also be a memory system that "records" and transmits information about past exposures to the subsequent generations of cells, and could thus be exploited in biomarker discovery. Recent studies also indicated that epigenetic states may be more dynamic than previously thought, and that establishing and maintaining them may be influenced by environmental and dietary factors and endogenous cues. However, further studies are needed to elucidate the molecular mechanisms by which the environmental and extracellular signals impair initiation, establishment, and maintenance of normal patterns of epigenetic modifications as well as aberrant epigenetic states associated with cancer

development. The almost spectacular advances in epigenomics and the emergence of powerful technologies that allow the analysis of epigenetic events in high-throughput and genome-wide settings should facilitate this task.

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Chapter 2
Developmental Origins of Hypoxic Pulmonary Hypertension and Systemic Vascular Dysfunction: Evidence from Humans

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Abstract Epidemiological studies have shown an association between pathologic events occurring during fetal/perinatal life and the development of cardiovascular and metabolic disease in adulthood. These observations have led to the so-called developmental origin of adult disease hypothesis. More recently, evidence has been provided that the pulmonary circulation is also an important target for the developmental programming of adult disease in both experimental animal models and in humans. Here we will review this evidence and provide insight into mechanisms that may play a pathogenic role.

Keywords Barker hypothesis • Epigenetics • Perinatal insult

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2.1 Introduction

2.1.1 The Barker Hypothesis

The initial observations made by Barker and colleagues [1], that individuals born with a low birth weight present increased cardiovascular mortality in adulthood, gave rise to the "Barker hypothesis." This hypothesis postulated that environmental factors, in particular nutritional, could act during the early phases of life and determine the risk to suffer from metabolic and/or cardiovascular disease later in life. Since then, many epidemiological studies have confirmed the association between impaired fetal growth (deduced from birth weight or body composition) and an increased incidence of cardiovascular diseases, type 2 diabetes mellitus, or their precursors: dyslipidemia, impaired glucose tolerance, or vascular endothelial dysfunction. The terms "fetal programming" and "developmental origin of adult diseases" were coined to describe these associations. Interestingly, this association is not only present in children with extremely low birth weight, since in children with normal birth weight the cardiovascular risk is also inversely related to birth weight. In some conditions, adverse developmental influences could also affect disease risk without birth size affected [12].

Developmental plasticity provides organisms with the ability to change structure and function in response to environmental cues. These changes usually take place during critical time windows, and then become permanent, and thereby permit a range of phenotypes to develop from a single genotype. The predictive developmental adaptive responses are thought to optimize the phenotype for the probable environment of the mature organism. Where there is a match between the predicted and actual mature environment, these predictive adaptive responses are appropriate and assist survival. Conversely, inappropriate predictions increase the risk of disease. Modeling suggests that such lagged responses aid the survival of the species [11].

To explain his observations, Barker postulated that when the fetal environment is low in nutrients, the fetus adapts its metabolism to increase its chances of survival after the birth in presumably similarly poor conditions. However, if the actual environment will be richer in food than predicted, then the adaptations programmed during the pregnancy might be deleterious and predispose to disease in adulthood [15].

In humans, such a situation occurred towards the end of World War II. A Dutch epidemiological study showed that an insufficient caloric intake in pregnant mothers during the period of famine of the winter 1944–1945 increased the risk of the offspring to develop cardiovascular or metabolic diseases in adulthood, and this even in the presence of a normal birth weight [30]. Noteworthily, the girls born from these pregnancies in period of famine gave themselves birth to children of lower than normal weight, suggesting the possibility of a transgenerational transmission of the consequences of a perinatal insult [28].

2.2 Underlying Mechanisms

A diet restricted in caloric or protein intake is the most widely used experimental animal model to study underlying mechanisms by which environmental cues may influence the developmental program. These studies have indicated two potential candidate mechanisms.

2.2.1 Altered Tissue Differentiation

When the fetus does not have sufficient substrates for its development, differentiation and growth of certain tissues may be altered. For example, in the rat, a low caloric diet during pregnancy induces a reduction in the number of β cells in the pancreas. This may explain, at least in part, the increased risk of diabetes in the adult offspring.

Similarly, a reduction in the number of nephrons has been suggested to be responsible for the increased risk of hypertension, whereas a reduction of the quantity of cardiomyocytes may explain the increased risk of cardiovascular disease in the adult offspring of restrictive diet pregnancies [26].

2.2.2 Epigenetic Alterations

The term "epigenetic" indicates changes of gene expression that are not related to modifications of the DNA sequence.

Gene expression is controlled by the epigenome, which comprises chromatine structure and DNA methylation. Methylation at the 5′ position of cytosine occurs in 60–90 % of CpG dinucleotides within the vertebrate genome and is associated with stable variation in gene expression. Methylation of CpG-rich clusters, termed CpG islands, is associated with transcriptional repression, whereas hypomethylation is associated with transcriptional activation [6, 29]. DNA methylation inversely correlates with histone acetylation [22]. Acting mainly on promoters, these covalent changes in DNA and histone structure affect the extent to which the transcription machinery is able to access specific regions of the DNA over extended periods of time. These modifications are maintained during cell division, may persist throughout the life span of the individual [13, 21, 23] and transmitted to the next generation, although the mechanism for epigenetic inheritance is not yet well understood.

Recent studies have provided evidence for the potential role of epigenetic mechanisms underpinning the fetal origin of adult diseases. In rodents, uteroplacental insufficiency and hypoxia increase acetylated histone H3 and alter DNA methylation in vitro, and may cause DNA hypomethylation and increased histone acetylation in the postnatal rat liver [25].

In humans, individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944–45 had, 6 decades later, less DNA methylation of the imprinted *IGF2* gene compared with their unexposed, same-sex siblings. This association was specific for periconceptional exposure, reinforcing the concept that very early mammalian development is a crucial period for establishing epigenetic marks that may persist throughout life [17].

Until recently, the DNA methylation pattern was thought to be irreversible in adult post-mitotic cells. However, recent data suggest that histone deacetylase inhibitors are capable of inducing replication-independent demethylation of ectopically methylated genes by increasing histone acetylation [42]. Accordingly, supplementation of pregnant mice with methyl-donor and cofactors (folic acid, vitamin B12) increases CpG methylation in the offspring, and this pattern is retained into adulthood [40].

Epigenetic alterations may have important functional consequences later in life. For example, offspring of mothers that show increased pup licking/grooming and arched-back nursing (high LG-ABN mothers) exhibit reduced fearfulness, decreased hypothalamic CRF expression, and more modest hypothalamic–pituitary–adrenal responses to stress during the first week of postnatal life. As adults, offspring of high LG-ABN mothers show increased hippocampal glucocorticoid receptor expression and enhanced glucocorticoid feedback sensitivity compared to offspring of low LG-ABN mothers [7]. This protective maternal behavior during the first week of life is associated with global DNA demethylation, and increased histone acetylation in the hippocampus of the offspring [38].

In line with this concept, central infusion of the histone deacetylase inhibitor richostatin A normalized histone acetylation, DNA methylation, hippocampal glucocorticoid receptor expression, and hypothalamic–pituitary–adrenal responses to stress in the adult offspring of high LG-ABN dams [43].

Interestingly, although the effects of maternal care or Trichostatin A administration involve a large number of genes, these effects were quite specific and limited to a small number of genes suggesting that these interventions did not result in a general collapse of gene expression programming.

Although the basis for this specificity remains unknown, these observations may have important implications for the potential use of such interventions for the treatment of diseases associated with epigenetic alterations (Fig. 2.1).

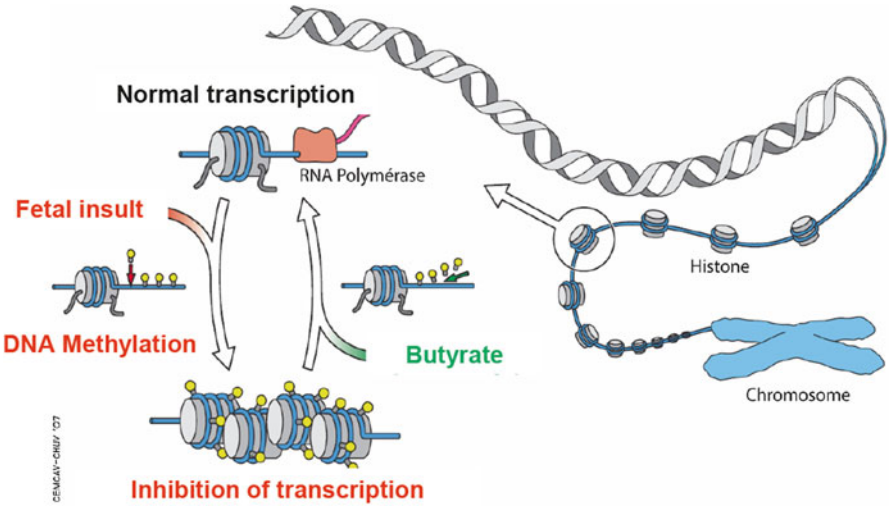

Fig. 2.1 Fetal/perinatal insults may induce epigenetic alterations (DNA methylation, histone deacetylation) that can alter transcription. Despite being stable and maintained during cell division throughout the life span, epigenetic alteration may be reversed by pharmacological agents, such as histone deacetylase inhibitors (Sodium Butyrate, Trichostatin A, Valproic Acid)

2.3 Pulmonary Arterial Hypertension and Fetal Programming

Very recently, we provided evidence, in both experimental animal models and in humans, that the pulmonary circulation is also an important target for the developmental programming of adult disease.

2.3.1 Environmental Insult During the Perinatal Period

During the perinatal period, the pulmonary circulation undergoes important structural and functional changes to allow the sudden transition from gas exchange by the placenta to gas exchange by the lungs. During this period, the pulmonary circulation is particularly vulnerable to noxious stimuli.

In line with this concept, in rats, exposure to hypoxia during the first days of life induces a transient increase of pulmonary artery pressure, and predisposes to exaggerated pulmonary vasoconstrictor responses to hypoxia and monocrotaline in adulthood [14].

During studies at the high-altitude research laboratory Capanna Regina Margherita in the Alps (4559 m), we have demonstrated a similar phenomenon in young healthy adults who had suffered from transient lack of oxygen during the first few days after birth [31]. Indeed, in these subjects, the altitude-induced

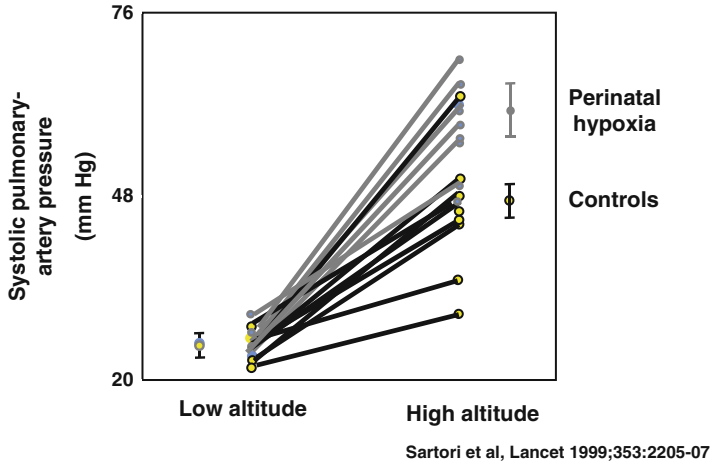

Fig. 2.2 Pulmonary-artery pressure measured at low (560 m) and at high altitude (4550 m) in young adults having suffered from transient perinatal hypoxia and control subjects

increase in pulmonary artery pressure was more than 50 % larger than in control subjects (Fig. 2.2).

The mechanism underlying this exaggerated vasoconstrictor response is not known yet, but there is evidence that this pathologic response is related to a functional rather than a structural defect. Data in rats show that transient hypoxia during the first few days leads to decreased eNOS expression in the lungs [36]. Thus, impaired NO synthesis may represent a potential mechanism. In line with this hypothesis, NO inhalation caused a substantially larger decrease in pulmonary artery pressure in the subjects with a perinatal insult than in control subjects.

These findings provided the first evidence in humans that a transient insult to the pulmonary circulation during the perinatal period leaves a persistent imprint (possibly defective NO synthesis) which, when activated later in the life, predisposes to a pathological response. This observation also suggests that survivors of perinatal pulmonary hypertension may be at risk of developing this disorder later in life.

Based on these findings, we wondered whether an insult occurring earlier during gestation may have similar long-term effects on the pulmonary circulation and, if so, may predispose to chronic hypoxic pulmonary hypertension.

To answer these questions, in collaboration with Bolivian researchers at the Instituto Boliviano de Biologia de Altura, we studied cardiopulmonary adaptation in high-altitude dwellers living in La Paz, Bolivia (3600–4000 m).

2.3.2 *Environmental Insults During Late Fetal Period*

Preeclampsia is the most frequent complication of pregnancy, and its prevalence is particularly high in high-altitude populations. Preeclampsia refers to the new onset of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman. It occurs in approximately 5–15 % of pregnancies worldwide and is associated with endothelial dysfunction in the mother which is related to the release of circulating vasculotoxic factors and the induction of augmented oxidative stress by the diseased placenta [16, 20, 24, 39]. We speculated that these circulating factors may pass the placental barrier and leave a persistent imprint in the pulmonary circulation of the offspring that may predispose to a pathological response later in life. Accordingly, we found that offspring of preeclampsia had exaggerated pulmonary hypertension. These important observations provide the first evidence in humans that a pathological event during late fetal development predisposes the offspring to pulmonary vascular dysfunction.

The preeclampsia-induced predisposition for exaggerated hypoxic pulmonary hypertension may have clinical consequences. Exaggerated hypoxic pulmonary hypertension is an important underlying mechanism of high-altitude pulmonary edema [32, 33]. Offspring of preeclampsia may be at risk for this problem. In line with this speculation, several offspring of preeclampsia had suffered from re-entry high-altitude pulmonary edema. Moreover, offspring of preeclampsia living at high altitude, or living at low altitude and suffering from disease states associated with chronic hypoxemia, may be at greater risk for developing sustained pulmonary hypertension and right heart failure.

The underlying mechanisms are not known.

Augmented oxidative stress may represent a candidate mechanism. Fetal insults are associated with a persistent increase of oxidative stress in the offspring in humans and experimental animals [9, 10]. Oxidative stress causes endothelial dysfunction and facilitates hypoxic pulmonary vasoconstriction in experimental animal models [8, 19]. Exaggerated oxidative stress during the fetal period may induce endothelial dysfunction in the offspring by causing epigenetically-induced alterations of the expression of genes involved in the regulation of endothelial function [44].

Consistent with this hypothesis, adult offspring of restrictive diet pregnancies, a mouse model of exaggerated oxidative stress during gestation [9] display pulmonary endothelial dysfunction in vitro and exaggerated hypoxia-induced pulmonary hypertension and right ventricular hypertrophy in vivo. This pulmonary vascular dysfunction was related, at least in part, to augmented oxidative stress, because Tempol normalized acetylcholine-induced vasodilation in vitro in offspring of restrictive diet pregnancy, and its administration during restrictive diet pregnancy prevented the pulmonary vascular dysfunction and exaggerated hypoxia-induced right ventricular hypertrophy in the offspring.

Furthermore, preliminary data from our group show that in offspring of preeclampsia exaggerated hypoxic pulmonary hypertension was related, at least in

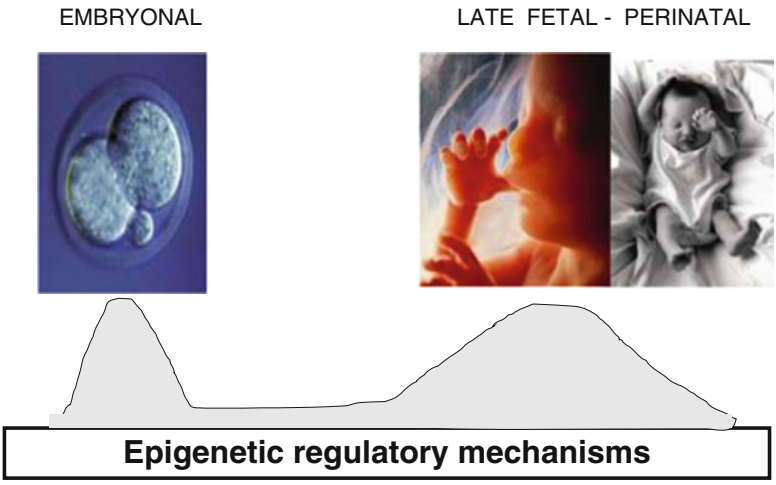

Fig. 2.3 Activity of epigenetic regulatory mechanisms during development

part, to increased oxidative stress, because TBARS plasma levels were increased and treatment with the antioxidant Vitamin C almost normalized pulmonary-artery pressure.

To test for the role of epigenetic mechanisms, we assessed pulmonary DNA methylation and examined the effects of the deacetylation inhibitor sodium butyrate on DNA methylation [3, 5, 18] and pulmonary vascular responsiveness in the offspring of restrictive diet pregnacies.

In very preliminary experiments, we observed that restrictive diet during gestation in mice was associated with altered global DNA methylation in the lung. Furthermore, administration of the histone deacetylatase inhibitor Butyrate during pregnancy prevented the alteration of DNA methylation in lung tissue of the offspring. Prevention of these pulmonary DNA methylation alterations in the offspring was associated with prevention of pulmonary endothelial dysfunction in vitro and exaggerated hypoxic pulmonary hypertension in vivo. This very interesting observation suggests that epigenetic alterations may be involved in the restrictive diet-induced impairment of pulmonary endothelial function in mice.

Epigenetic regulatory mechanisms play an important role not only during the fetal/perinatal period but also around conception during gametogenesis (Fig. 2.3).

Primordial germ cells undergo epigenetic erasure as they migrate along the genital ridge, and epigenetic marks are reestablished during gametogenesis. For example, after fertilization, there is active demethylation of the paternal pronucleus, and then a second wave of passive demethylation of the zygote genome. Imprinted genes are protected from this erasure. We therefore wondered whether environmental insults occurring during this period may have similar long-term effects in the offspring.

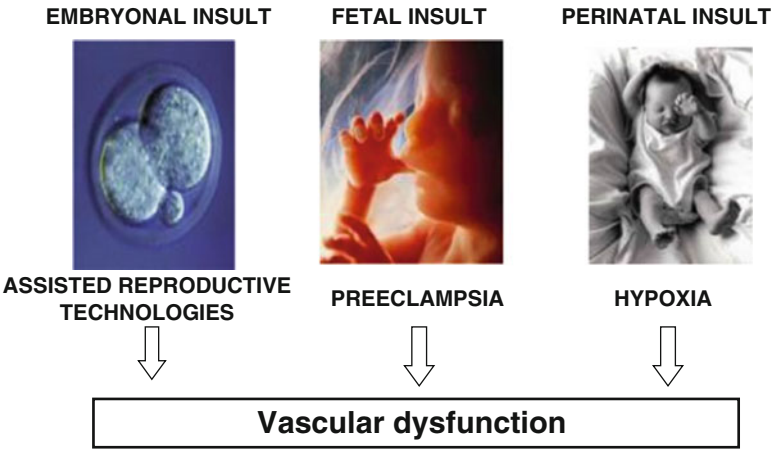

Fig. 2.4 Fetal/perinatal insults that have been shown to predispose to systemic and pulmonary vascular dysfunction in human offspring

2.3.3 Environmental Insults During Gametogenesis

Assisted reproductive technologies (ART) involve the manipulation of early embryos at a time when they may be particularly vulnerable to external disturbances. ART is a modulator of the epigenome in several animal species and in humans [2, 45]. In humans, this method is associated with a higher than expected frequency of rare imprinting disorders (i.e. Beckwith-Wiedeman and Angelman syndrome) [4]. The safety of ART for long-term health is therefore of utmost importance, but there is little information [27, 37]. This may be related at least in part to the young age of the progeny, since clinically manifest chronic disease may not yet have had time to develop.

Based on our previous observations in offspring of preeclampsia [34] and in young adults who had suffered from transient perinatal hypoxia [31] (see above), we speculated that ART might predispose to vascular dysfunction (Fig. 2.4).

Consistent with this speculation, recent studies in normal mice suggest that ART is associated with alterations of the activity of enzymes involved in the regulation of metabolic and cardiovascular homeostasis as well as arterial hypertension in the adult offspring [41]. No information was available in humans.

Data from our group now demonstrate that children born after ART present systemic and pulmonary vascular dysfunction on high-altitude exposure (a condition known to facilitate detection of vascular dysfunction in subjects with endothelial dysfunction) (See chap. 4).

2.4 Conclusion

Cardiovascular and metabolic diseases were thought to result from the interaction between the behavior of an individual and his genetic inheritance. Recent data indicate, however, that fetal programming also plays an important pathogenic role.

Among the underlying mechanisms by which fetal programming may lead to cardiovascular dysfunction, augmented oxidative stress and/or epigenetic alterations appear to play a major role. Fetal programming may occur at various stages of development and there is evidence that fetal programming-induced alterations are transmissible from cell to cell throughout life and then also to the next generation.

More importantly from a medical therapeutic standpoint, epigenetic alterations may be reversed by pharmacological interventions opening a window for the potential prevention and treatment of cardiovascular and metabolic diseases associated with fetal programming.

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Chapter 3
Acquired Mitochondrial Abnormalities, Including Epigenetic Inhibition of Superoxide Dismutase 2, in Pulmonary Hypertension and Cancer: Therapeutic Implications

Stephen L. Archer

Abstract There is no cure for non-small-cell lung cancer (NSCLC) or pulmonary arterial hypertension (PAH). Therapies lack efficacy and/or are toxic, reflecting a failure to target disease abnormalities that are distinct from processes vital to normal cells. NSCLC and PAH share reversible mitochondrial-metabolic abnormalities which may offer selective therapeutic targets. The following mutually reinforcing, mitochondrial abnormalities favor proliferation, impair apoptosis, and are relatively restricted to PAH and cancer cells: (1) Epigenetic silencing of superoxide dismutase-2 (SOD2) by methylation of CpG islands creates a pseudohypoxic redox environment that causes normoxic activation of hypoxia inducible factor (HIF-1α). (2) HIF-1α increases expression of pyruvate dehydrogenase kinase (PDK), which impairs oxidative metabolism and promotes a glycolytic metabolic state. (3) Mitochondrial fragmentation, partially due to mitofusin-2 downregulation, promotes proliferation. This review focuses on the recent discovery that decreased expression of SOD2, a putative tumor-suppressor gene and the major source of H_2O_2, results from hypermethylation of CpG islands. In cancer and PAH hypermethylation of a site in the enhancer region of intron 2 inhibits SOD2 transcription. In normal PASMC, SOD2 siRNA decreases H_2O_2 and activates HIF-1α. In PAH, reduced SOD2 expression decreases H_2O_2, reduces the cytosol and thereby activates HIF-1α. This causes a glycolytic shift in metabolism and increases the proliferation/apoptosis ratio by downregulating Kv1.5 channels, increasing cytosolic calcium, and inhibiting caspases. The DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine, which restores SOD2 expression, corrects the proliferation/apoptosis imbalance in PAH and cancer cells. The specificity of PAH for lung vessels may relate to the selective upregulation of DNA methyltransferases that mediate CpG methylation in PASMC (DNA MT-1A and -3B). SOD2 augmentation inactivates HIF-1α in PAH PASMC and therapy with the SOD mimetic, MnTBAP, regresses experimental PAH. In conclusion, cancer and PAH share acquired mitochondrial abnormalities that increase proliferation and inhibit apoptosis, suggesting new therapeutic targets.

Keywords Non-small-cell lung cancer • Gene methylation • Pyruvate dehydrogenase kinase • Hypoxia inducible factor • Warburg hypothesis

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3.1 Introduction

Pulmonary hypertension is defined simply as a mean pulmonary artery pressure (mPAP) above 25 mmHg at rest. There are five categories of PH in the current World Health Organization classification [90]. Category 1 PH, also called pulmonary arterial hypertension (PAH), is a syndrome in which obstructed, constricted, inflamed small pulmonary arteries (PA) increase pulmonary vascular resistance (PVR), leading to right ventricular hypertrophy and ultimately failure. Although category 1 PH (which includes idiopathic and familial PH and PH associated with connective tissue disease or congenital heart disease) is relatively rare, it is the only category for which there are approved medical therapies. Despite important advances in understanding the genetics of PAH, such as the discovery of mutations in bone morphogenetic protein receptors (BMPR-2) in familial PAH [49, 100] and the recognition of somatic chromosomal abnormalities in sporadic PAH [2], the cause of most cases of PAH remains unclear. Moreover, determining the genetic abnormality in PAH does not (at present) determine therapy. Despite effective intravenous therapies, such as epoprostenol, and oral agents, notably phosphodiesterase-5 inhibitors and endothelin antagonists, mortality remains high (15 % at 1-year) (reviewed in [7]), suggesting we have yet to identify an optimal therapeutic target. Phase 1 testing of drugs that attack newly identified targets, derived from an explosion of research on the Basic Science of PAH, has lagged behind the development of treatments for cancer, perhaps because PAH is an orphan disease [7]. One such unexploited target is the excessive proliferation and impaired apoptosis of pulmonary artery smooth muscle cells (PASMC) that contributes to vascular obstruction in PAH patients and Fawn-hooded rats (FHR) with PAH. This imbalance is milder than in cancer but nonetheless suggests similarities between PAH and neoplasia [104].

Lung cancer is the most common cause of cancer death in men and women, with non-small-cell lung cancer (NSCLC) accounting for over 80 % of cases. NSCLC usually presents in an advanced form (metastatic in >40 % of cases at presentation). Excessive cell proliferation and impaired apoptosis are disease hallmarks. Standard therapy entails a platinum-based regimen, but molecularly targeted therapies increasingly have a role in subsets of the patient population. Although mortality has decreased compared since the 1990s, Stage 4 NSCLC still has only a 19 % 1-year survival rate in unselected cohorts [65]. Newer therapies that target cancer-specific abnormalities, such as Erlotinib (Tarceva®), achieve good antitumor activity with acceptable tolerability. Erlotinib is a small-molecule, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor that blocks the upregulated, proliferation-promoting EGFR pathway [102]. Erlotinib acts by induction of the cyclin-dependent kinase inhibitor p27(KIP1), which suppresses the G1/S transition [52]. There is an emerging interest in testing other tyrosine kinase inhibitors used in oncology, such as Imatinib (Gleevec®) and Sorafenib (Nexavar®), in patients with Category 1 PH [29, 32].

Thus, despite 3 decades of clinical trials, the prognosis remains grim in both NSCLC and PAH. The lack of "silver bullet" for cancer or PAH reflects the fact that most therapies damage normal cells, resulting in a narrow therapeutic window. Recent discoveries in the biology of NSCLC and PAH suggest a possible therapeutic convergence around metabolic therapies as a nontoxic means of reducing cell proliferation. Our laboratory is targeting acquired mitochondrial abnormalities that impair oxidative metabolism and enhance glycolysis to treat PAH and NSCLC. We hypothesize that excessive cell proliferation and impaired apoptosis in PAH and NSCLC reflect a pseudohypoxic, mitochondrial-metabolic redox state, occurring despite adequate oxygen availability. We have identified four such abnormalities and are in the process of testing them as therapeutic targets.

3.2 Basic Science of PAH

This overview (based on a recent review [7]) provides a context in which the new mitochondrial abnormalities can be viewed. PAH is a panvasculopathy. Abnormalities in each layer of the artery contribute to this syndrome of obstructed, constricted small pulmonary arteries (PA) and right ventricular hypertrophy (RVH). Although PAH primarily effects small PAs (as judged by histology) increased stiffness of large, proximal arteries contributes to adverse outcomes in PH and alters flow in small PAs [50]. In the blood there is elevated plasma serotonin [36]. In the endothelium there is a decreased ratio of vasodilators/constrictors [19, 96, 97]. Early in PAH, endothelial apoptosis may occur, leading to selection of apoptosis-resistant endothelial precursor cells that eventually form plexiform lesions [84]. In experimental PAH, several groups find PASMC apoptosis is suppressed and proliferation is enhanced [20, 25, 47, 57, 58, 60, 62, 80], consistent with findings in human PAH [66]. Many factors drive PASMC proliferation, including mutation [66] or downregulation [59] of BMPR-2, de novo expression of the anti-apoptotic protein survivin [57, 84], increased expression/activity of the serotonin transporter (SERT) [24, 33] and increased expression/activity of platelet-derived growth factor (PDGF) receptor [86, 112]. Another proliferative, anti-apoptotic, PASMC abnormality is the selective decrease in expression of Kv1.5, a voltage-gated, O2-sensitive potassium channel. Kv1.5 downregulation occurs in human PAH [114], rat PAH models (whether induced by chronic hypoxia [62, 78], monocrotaline [57] or spontaneous PAH in fawn hooded rats, FHR [12]). Kv1.5 is also downregulated in transgenic mice with PAH due to SERT overexpression [33] or BMPR-2 mutation [111]. In PAH, loss of Kv1.5, the same channel that is inhibited by hypoxia to initiate hypoxic pulmonary vasoconstriction [6, 8], depolarizes the membrane and elevates cytosolic K^+ and Ca^{2+}. The resulting calcium overload (later reinforced by activation of trp channels [48]), leads to Ca^{2+}-calcineurin-dependent activation of the transcription factor, NFAT, which favors proliferation [13]. HIF-1α, another transcription factor

is activated in PAH [12]. In the adventitia, metalloprotease activation causes architectural disruption, permitting cell migration and generating mitogenic peptides (tenascin) [20]. Finally, infiltration of the lung with inflammatory cells, endothelial-precursor cells, and mesenchymal and bone marrow-derived stem cells occurs in PAH [21].

With the discovery of BMPR-mutations in familial PAH [22, 100] the cause of PAH appeared to have been elucidated. These loss-of-function mutations favor PASMC proliferation. Consistent with this, a transgenic mouse with SMC-specific over-expression of a human dominant-negative BMPR-2 transgene develops pulmonary hypertension [107]. Pulmonary hypertension in BMPR-2 dominant-negative mice is not initially associated with vascular remodeling; rather it manifests as a Kv1.5 deficiency (membrane depolarization activates L-type calcium channels causing vasoconstriction). PH in these mice can be reversed with an L-type calcium channel blocker [111], consistent with the observation that ~10 % of PAH patients have robust long-term response to nifedipine [82]. Eventually, impaired apoptosis and enhanced PASMC proliferation transforms PAH to a more fixed disease [58]. Indeed only 30–40 % of PAH patients have significant vasodilator responses (>20 % fall in PVR and PAP) to inhaled nitric oxide [81, 91]. However, BMPR-2 mutation does not appear to be a common cause of sporadic PAH. BMPR-2 mutations occur in 10–20 % of sporadic PAH patients and even in familial PAH, penetrance is low (~25 %) [68]. While modifier genes, such as SERT and TGFβ may explain variable penetrance, aberrant BMPR-2 function alone is neither a necessary nor sufficient precondition for most cases of PAH [70]. This literature highlights the multiplicity of putative "causes" for PAH and, in so doing, highlights the lack of a fundamental, initiating cause for sporadic PAH. While this partially reflects the fact that PAH is a syndrome, rather than a homogenous disease, it raises the question, "Does a unifying cause for PAH exist?" We believe the answer is, "perhaps." At least there is an explanation that links many of these abnormalities together. We hypothesize that upstream mitochondrial abnormalities (epigenetic downregulation of SOD2, activation of HIF-1α and PDK and an increased mitochondrial fission/fusion ratio) lead to a pseudohypoxic metabolic milieu that creates a downstream proliferative, anti-apoptotic phenotype [12]. The discovery of an epigenetic basis for PAH could also explain the heritable aspect of this syndrome in some cases.

3.3 Similarities Between PAH and Cancer

Similarities between cancer and PAH include: increased proliferation/depressed apoptosis, in cancer [11] and PAH [60, 74, 75, 80], pathological activation of HIF-1α, in cancer [53, 56, 87, 98, 108] and PAH [5, 12], PDK activation in cancer [11] and PAH (PDK) [11, 12] and mitochondrial fragmentation in cancer (unpublished data) and PAH [12]. We have also come to recognize metabolic similarities between cancer and PAH. Otto Warburg, Nobel laureate (1931) proposed that a shift in glucose metabolism from oxidative phosphorylation to glycolysis (despite

adequate oxygen supply) was central to the cause/maintenance of cancers. Although this hypothesis proved controversial, recent work from oncology and PAH investigators, including our group, suggests that PAH and cancer share this "Warburg phenotype." Teleologically, the reliance on glycolysis for energy has several "advantages" to the cancer or PAH cell (and disadvantages for the host). By taking the mitochondria out of play, glycolysis removes a powerful brake on proliferation while eliminating the "risk" of mitochondrial-induced apoptosis. The cost of this strategy is the energetically inefficient nature of glycolysis, which generates 1/18th as much ATP as oxidative metabolism. To compensate, the glucose transporter (glut1) is markedly upregulated in both conditions. This explains why both cancer and PAH manifest increased fluorodeoxyglucose (FDG) uptake on PET scans, evident in PAH both in the lung [110] and in the hypertrophied right ventricle [71, 73].

3.4 Disordered Oxygen Sensing in PAH and Cancer

We propose that subversion of a physiologic, mitochondrial O_2-sensing pathway is central to the progression of PAH and NSCLC. Relevant mitochondrial-metabolic abnormalities in cancer and PAH (highlighted in Fig. 3.1) affect enzymes and transcription factors that are participants in the lung's mechanism of oxygen sensing (reviewed in [106]). In the pulmonary vasculature, mitochondrial-derived H_2O_2, by virtue of its less toxic nature and moderate diffusion radius, serves as a signaling molecule communicating the "PO_2" (sensed in the mitochondria) to the plasma membrane (Kv1.5 and other channels [6]) and transcription factors [106]. "Normoxia" can thus be considered a reflection of mitochondrial ROS production and does not always correlate with PO_2 (a Warburgian disconnect that is evident in NSCLC and PAH).

The seminal observation that suggested shared mitochondrial pathology in cancer and PAH was the finding of that FHR had depressed hypoxic pulmonary vasoconstriction and mild polycythemia as well as a fragmented, hyperpolarized mitochondrial network that makes subphysiologic levels of H_2O_2. This redox abnormality appears to be the basis for the normoxic activation of HIF-1α in FHR and is reversed by exogenous H_2O_2 [12]. Thus it appeared that FHR had an O_2-sensing problem.

Downstream from these mitochondrial abnormalities, and presumably connected through the loss of mitochondrial H_2O_2 generation and HIF-1α activation, we observe depression of the voltage-gated K+ channel (Kv1.5). Activation of 2 transcription factors accounts for much of the downregulation of Kv1.5 in PAH (HIF-1α and the calcium-sensitive, NFAT [12, 13]). Interestingly, restoring Kv1.5 expression reduces pulmonary hypertension [75]. Recent studies identify single nucleotide polymorphisms in the Kv1.5 promoter and translated regions of KCNA5 also explain some of the depressed expression and/or function of Kv1.5 channels in PASMC from idiopathic PAH patients [79, 114]. Preussat et al. linked

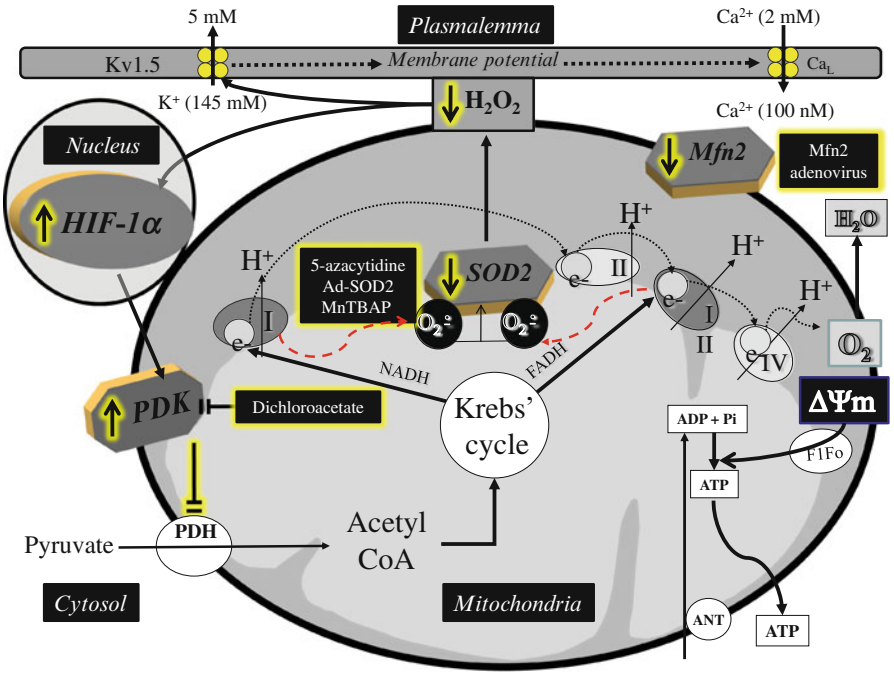

Fig. 3.1 Schematic representation of the consequences of SOD2 downregulation. Normally, SOD2 rapidly converts superoxide (produced at complexes I and III) to H_2O_2. Diffusible H_2O_2serves as a redox messenger signaling "normoxia." H_2O_2 maintains Kv1.5 in an open state and prevents HIF-1α activation. In PAH, SOD2 methylation decreases gene transcription, lowering SOD2 protein, and decreasing H_2O_2 production. This reduces the redox environment, causing HIF-1α activation. HIF-1α induces transcription of pyruvate dehydrogenase kinase (PDK), which inhibits pyruvate dehydrogenase (PDH). Inhibition of PDH blocks production of Acetyl-CoA, slowing the Krebs' cycle and decreasing production of the electron donors. The resulting decrease in passage of electrons (e-) down the transport chain further reduces ROS production. This pathologic positive feedback loop can be interrupted by restoring SOD2 activity (using 5-aza-2′-deoxycytidine to demethylate SOD2, SOD2 gene transfer, or MnTBAP) or by inhibiting PDK (using dichloroacetate) [12]. Abbreviations: ΔΨm = mitochondrial membrane potential, Mfn2 = mitofusin 2, Ad-SOD = adenoviral vector containing the superoxide dismutase 2 gene

Kv1.5 with cancer: Kv1.5 expression (but not other Kv channels) correlated with tumor grade in human glioblastoma (i.e. higher tumor grade = lower Kv1.5 expression [76]. Loss of Kv1.5 (whether due to loss of H_2O_2 which activates these channels [8] or decreased expression) depolarizes membrane potential and ultimately increases intracellular K+ and Ca2+, which inhibits apoptosis and promotes proliferation, respectively. Put another way, PAH PASMC and cancer cells behave as if they perceive themselves to be hypoxic, despite exposure to normal PO_2. The interaction between the sensors (mitochondria), mediators (transcription factors, ROS), and effectors (ion channels, caspase) is shown in schematic form in Fig. 3.2.

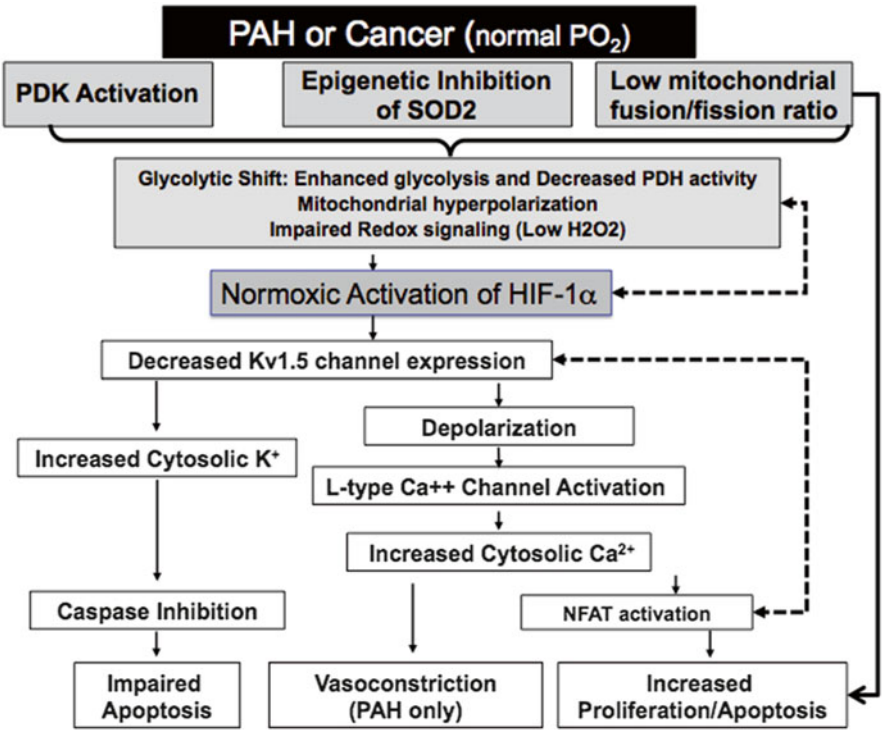

Fig. 3.2 Mitochondrial abnormalities in cancer and PAH increase proliferation and decrease apoptosis. This schematic illustrates the connections between the key mitochondrial abnormalities that are discussed in this review

3.5 Acquired Mitochondrial Abnormalities in PAH and Cancer

While excessive vasoconstriction [42, 43, 67], inflammation [23] and thrombosis [72] contribute to the pathogenesis of PAH, this new "oncologic view" of PAH, originally proposed by Tuder and Voelkel [104], may constitute a paradigm-shift from the twentieth century view of PAH as a vasospastic disease. Fawn hooded rats (FHR), and humans with idiopathic PAH (iPAH), share an unexplained PASMC mitochondrial-metabolic phenotype that underlies their proliferation/apoptosis imbalance and which may explain this neoplastic phenotype. PAH PASMC are characterized by a fragmented, hyperpolarized mitochondrial reticulum with decreased superoxide dismutase-2 (SOD2) expression/activity [5]. SOD2 deficiency reduces the generation of H_2O_2 below physiologic levels which activates of HIF-1α, which in turn induces PDK transcription (Figs. 3.1 and 3.2) [12]. SOD2 downregulation and HIF-1α activation have been described in PAH patients by other scientists, although their interpretation of the significance and mechanism differ [14, 101]. Bowers et al. have also noted decreased SOD2 in the PAs in human PAH [14]; however, their interpretation was that PAH is a condition of increased

oxidative stress (in part because other oxidant markers were elevated). Our data indicate that in FHR, H_2O_2 is subphysiologic due to both a primary effect, methylation-induced reduction of the SOD2 gene expression, and a secondary effect, PDK-mediated inhibition of oxidative metabolism [12].

The downstream consequences of this mitochondrial-metabolic abnormality include decreased production of the redox signaling molecule, H_2O_2, and reduced Kv1.5 channel expression (the latter resulting in increased cytosolic K^+ and Ca^{2+}). Ultimately this abnormal SOD2-Hydrogen peroxide-HIF-1α-PDK pathway increases proliferation and suppresses apoptosis [3]. Four related, mitochondrial-metabolic abnormalities promote PAH and each has potential as a therapeutic target. This review focuses on the epigenetic silencing of SOD2 (Figs. 3.1 and 3.3).

3.5.1 Epigenetic Silencing of SOD2

Mitochondrial SOD2 can be downregulated by two epigenetic mechanisms, methylation of CpG islands and activation of histone deacetylase. Methylation at cytosine's C5 atom inhibits gene expression by preventing the binding of transcription factors [69]. CpG methylation is established and maintained by 3 DNA methyltransferases [31]. SOD2 is a major source of endogenous H_2O_2, produced in mitochondria when SOD2 detoxifies the low basal amounts of superoxide that are generated by unpaired electron flux during normal activity of the ETC. Although toxic at high levels, at physiologic levels, H_2O_2 is a vasodilatory, antiproliferative, redox-signaling molecule [16, 61, 63, 109]. The dynamic coupling of H_2O_2 production to PO_2 and its ability to regulate redox-sensitive targets, such as HIF-1 and Kv1.5, are key to the mitochondria's role as vascular O_2 sensors [106]. Several observations lead us to investigate SOD2 as a candidate PAH gene. First, in rats the gene for this nuclear encoded mitochondrial protein resides on chromosome 1. Consomic rats (FH-BN1), which are identical to FHR except for introgression of a normal chromosome 1, lack the FHR's mitochondrial abnormalities, have normal SOD2 levels and do not develop PAH [12]. Second, serial DNA microarray analysis of resistance PA indicated that SOD2 mRNA was downregulated threefold in FHR, prior to onset of pulmonary hypertension (unpublished data). DNA sequencing revealed that the SOD2 gene and promoter were normal in FHR. Relevant to PAH and cancer, methylation can be reversed, is heritable and can be tissue-specific [77]. This, coupled with the heritable nature of FHR PAH, raised the possibility of epigenetic mechanisms for SOD2 downregulation.

Genomic bisulfite sequencing (of mRNA from lungs of FHR ± 5-azacytidine treatment in vivo), identified differential hypermethylation of SOD2 at 2 sites (one in intron 2, the other in the promoter). This was confirmed in culture PASMC from FHR. In a series of experiments we demonstrated that the FHR's SOD2 deficiency results from covalent cytosine methylation in 2 dinucleotide CpG islands (Fig. 3.3). CpG methylation is established and maintained by a family of DNA methyltransferases [31]. The SOD2 methylation in FHR results from increased DNA methyl-

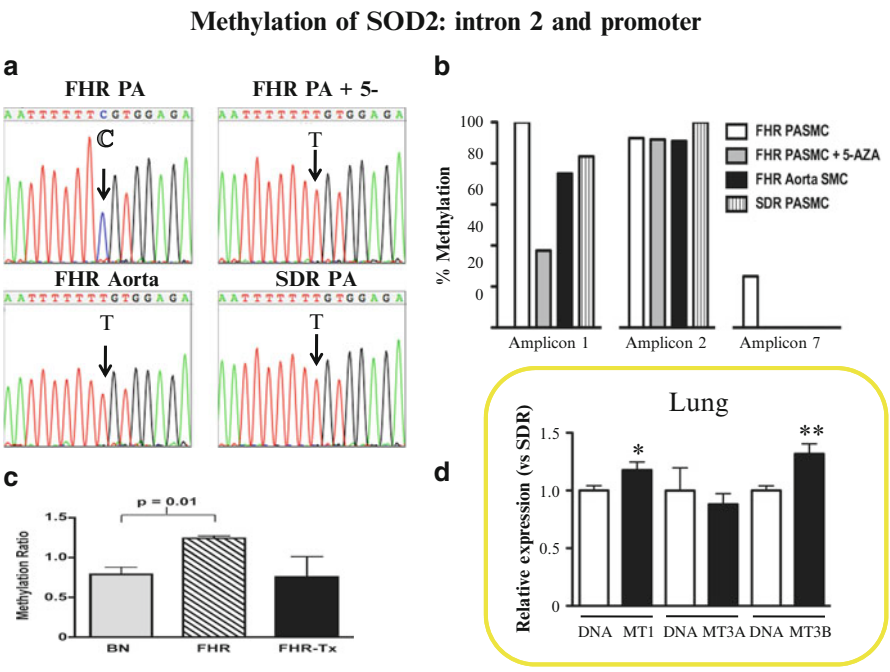

Fig. 3.3 Methylation of 2 CpG Islands in SOD2 in FHR PASMC are reversible by 5-aza-2′-deoxycytidine. (**a**) Representative sequencing traces of a differentially methylated region within intron 2. Only methylated cytidines are protected against bisulfite-mediated deamination of cytidine into uridine (which is recognized as thymidine when the PCR product is amplified). As indicated by the *arrow*, the cytidine in FHR PASMCs was methylated (and therefore remains a cytidine, *upper left panel*). This is reversed by 5-azacytidine (*upper right panel*). The site is not methylated in FHR aortic SMC (*lower left panel*) or in SDR PASMCs (*lower right panel*). (**b**) Tissue heterogeneity in SOD2 methylation in cultured PASMCs. 5-aza-2′-deoxycytidine reduced methylation in amplicon 1 but had no effect on amplicon 2. Within amplicon 7, one site was identified that was only methylated in FHR PASMCs, and this site becomes demethylated after 5-azacytidine treatment. (**c**) There is increased methylation of intron 2 in FHR lungs and this is reduced by in vivo therapy with 5-azacytidine (FHR-Tx). (**d**) Both DNA methyltransferase (DNA-MT-1 and -3B mRNA were increased in lungs of control FHR versus Sprague Dawley rats ($n=12$ each), *, ** $p<0.05$ and 0.01, respectively. In low passage [3] PASMCs ($n=8$ in each group), FHR had a significantly higher DNA methyltransferase 3B expression and a trend toward increased DNA MT1. Reproduced from Ref. [5] with permission

transferase-1 and -3B (DNA-MT1 and -3B) (Fig. 3.3). DNA-MT1 serves as a maintenance methyltransferase in proliferating tissues transferring methylation patterns from parental to daughter DNA during DNA replication. DNA-MT3A and -3B are “de novo” methyltransferases and create new methylation during adult life. DNA-MT3B expression was increased in FHR PASMC (Fig. 3.3). Interestingly this methylation occurred in FHR PA but not aortic SMC (Fig. 3.3), perhaps explaining the well-known restriction of pathology in PAH to the lung vasculature [5]. We did not inhibit DNA-MT activity in FHR, however in breast and lung cancers, depletion of DNA-MT3B can reactivate methylation-silenced genes and decrease proliferation [9].

The consequences of reduced SOD2 expression in PAH and NSCLC is decreased H2O2 production, which activates HIF-1α and increases PDK expression thereby inhibiting PDH and slowing the electron transport chain (which reduces ROS production). This positive feedback loop reinforces the pseudohypoxic environment and creates an input block at the proximal electron transport chain. SOD2 silencing (which in PAH only reduces activity ~50 %) is reversible with inhibitors of DNA methyltransferase (5-aza-2′-deoxycytidine). 5-aza-2′-deoxycytidine covalently binds and irreversibly inhibits DNA methyltransferases [99], so that, after a requisite cell division, it can restore transcription of previously methylated genes.

The effects of changes in SOD2 expression on ROS are complex, in part because expression of the enzyme varies with oxidant stress. One might anticipate increased superoxide anion in the face of low SOD2 levels. However, in PAH the activation of PDK inhibits PDH and an "input block" which lowers electron flux and the associated generation of superoxide (Fig. 3.1). To clarify the effects of SOD2 downregulation we not only measured superoxide, using L-012 chemiluminescence and the CMH spin trap (Fig. 3.4), but also measured mitochondrial versus cytosolic redox state, using compartment-specific redox probes (roGFPcyt and roGFPmito) (Fig. 3.5).

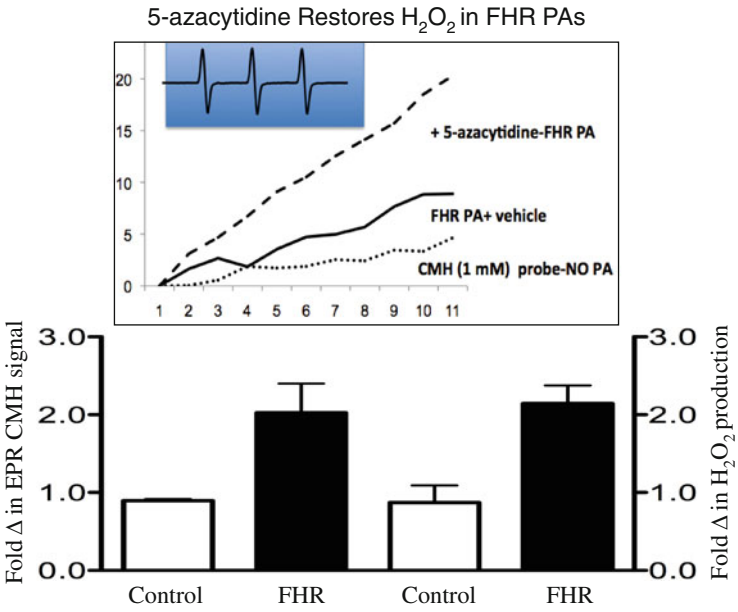

Fig. 3.4 Lower ROS levels and reduced cellular environment in FHR. (**a**) ROS mean data and representative trace (inset) showing the CMH triplet signal. Peak height (EPR amplitude units) is proportional to ROS levels and is normalized to wet weight. PAs from FHR treated with 5-aza-2′-deoxycytidine (*black bars*) have increased production of ROS (*left*) and H_2O_2 (*right*) versus non-treated FHR. Control rats show no increase in ROS or H_2O_2 in response to 5-aza-2′-deoxycytidine. Reproduced from Ref. [5] with permission

As a proof of concept we used SOD2 siRNA to decrease SOD2 expression in normal PASMC. As predicted this decreased endogenous H_2O_2 production and activated HIF-1α during normoxia (Fig. 3.6). The siSOD2 siRNA replicated other abnormalities seen in human and FHR PAH (e.g. decreased Kv1.5 expression and increased cytosolic calcium). In PAH PASMC, where there is already low SOD2 expression, the net redox state of the cytosol was reduced compared to normal PASMC and augmenting SOD2 activity caused oxidation (Fig. 3.5). This low ROS state resulted in activation of HIF-1α (Fig. 3.7). Treatment with 5-aza-2′-deoxycytidine not only caused a dose-dependent increase in SOD2 in FHR PASMC (Fig. 3.8), but also inhibited cell proliferation and increased apoptosis (Fig. 3.8). While this supports our hypothesis, it is possible (indeed probable) that 5-aza-2′-deoxycytidine had targets in addition to the SOD2 gene.

An important translational component of this study was the testing of SOD replacement strategies in cultured FHR PASMC and in FHR in vivo. We found that augmentation of SOD2 (or SOD activity), achieved by three complementary strategies, improved mitochondrial function, inhibited PASMC proliferation, and increased apoptosis in vitro [5]. In FHR PASMC, SOD2 gene therapy, application

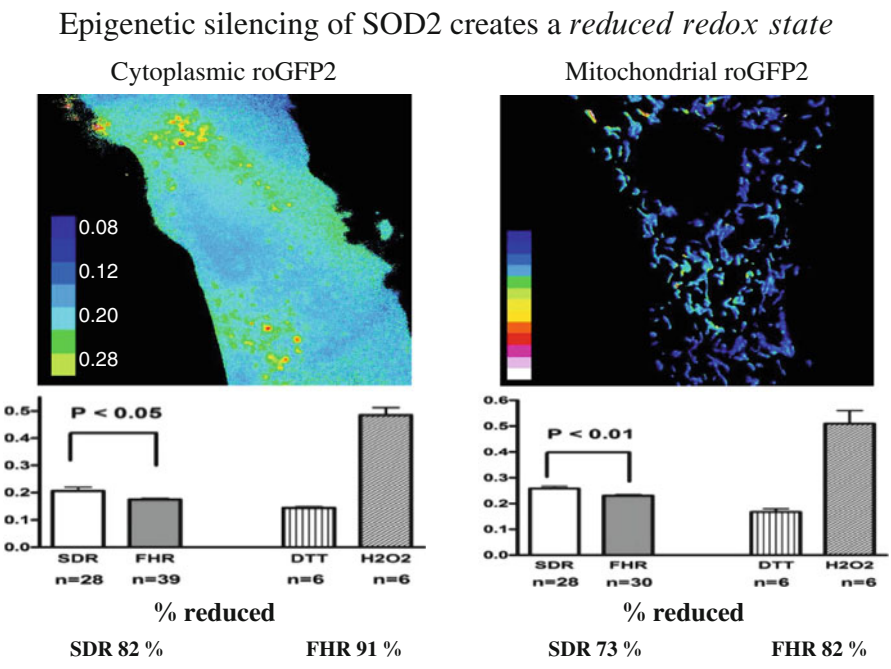

Fig. 3.5 FHR PASMC are reduced. PASMCs were transfected with redox-sensitive GFP constructs targeted to the cytoplasm (*upper panel*) and mitochondria (*lower panel*). FHR PASMC had a reduced cytoplasm and mitochondria, relative to Sprague Dawley PASMC. Dithiothreitol (DTT) and H_2O_2 were used to completely reduce and oxidize the cells, respectively. Reproduced from Ref. [5] with permission

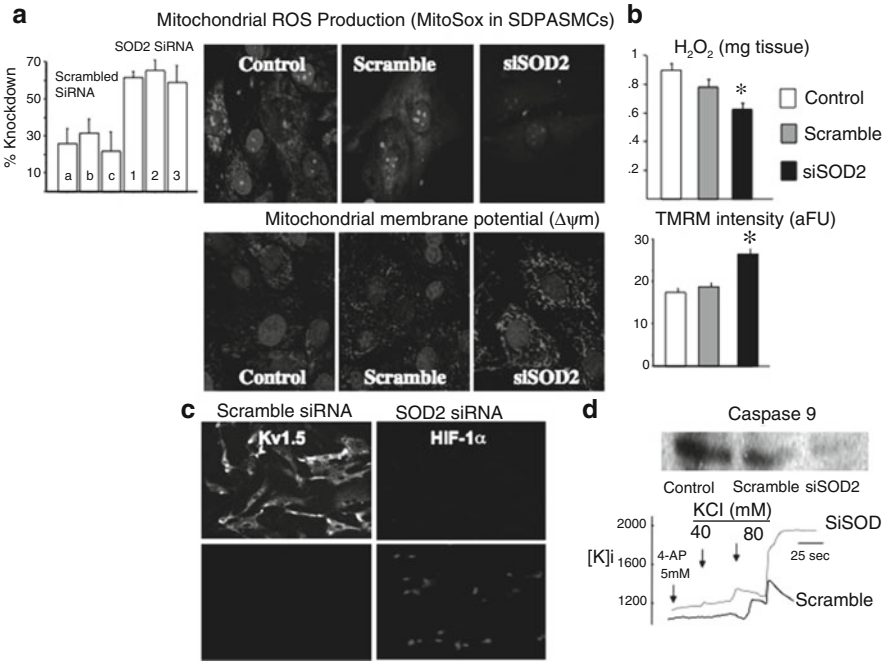

Fig. 3.6 Inhibition of SOD2 expression in normal PASMC recapitulates the PAH phenotype. (**a**, **b**) siRNAs reduce SOD2 mRNA and H2O2 production in normal, Sprague-Dawley PASMC. This causes hyperpolarization of the mitochondrial membrane potential (increased intensity of the TMRM stain) and decreases mitochondrial ROS/H2O2 production. (**c**) Confocal images of cultured Sprague Dawley PASMC showing that SOD2 siRNA activates HIF-1α (note translocation to the nucleus-red in *middle panel*) and decreases Kv1.5 expression (decreased *green*, *left panel*). (**d**) siSOD2 decreases caspase 9. siSOD2 also increased intracellular K+ concentrations (*right panels*). Together these effects would inhibit caspase-mediated apoptosis. Reproduced from Ref. [5] with permission

of the SOD2 mimetic MnTBAP, or 5-aza-2′-deoxycytidine each led to HIF-1α inactivation and restoration of Kv1.5 expression [5]. Although these surrogate endpoints are important, a key therapeutic finding is that MnTBAP treatment regresses PAH in vivo (Fig. 3.9). SOD supplementation reduced right ventricular hypertrophy and improved the exercise capacity of FHR. It should be noted that the endothelium in PAH also has similar abnormalities as the PASMC, including the occurrence of dysmorphic, SOD2-deficient mitochondria and a glycolytic metabolic shift [110].

The benefits of MnTBAP in experimental PAH are consistent with those of tempol (another membrane permeable SOD mimetic) which decreases hypoxic pulmonary hypertension in rats [26] and recombinant SOD1, which ameliorates persistent pulmonary hypertension in newborn lambs [95]. However, the benefit of SOD therapy in these studies was attributed to an antioxidant or nitric oxide-preserving mechanism; whereas our data support restoration of SOD2 as a means of restoring H_2O_2 levels and reestablishing redox signaling to control vascular cell proliferation.

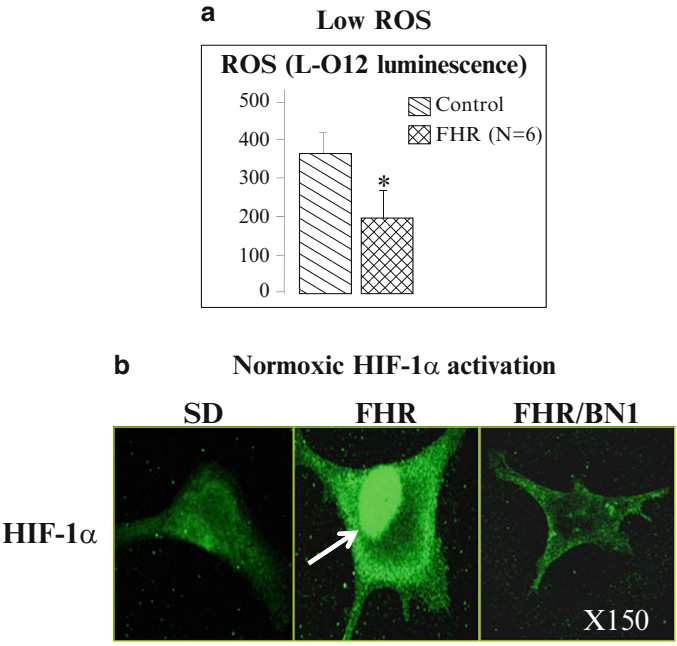

Fig. 3.7 ROS production is low in FHR PASMC and HIF-1α is activated. (**a**) Decreased ROS levels in freshly isolated resistance PAs from FHR versus consomic rats, measured using L-012 chemiluminescence at 37 °C. (**b**) Despite being grown in an incubator at normoxic PO2 (~120 mmHg), HIF-1α is activated (translocated to the nuclei); reproduced from Ref. [12] with permission

3.5.1.1 SOD in Cancer

SOD2 is considered a tumor-suppressor gene [15, 51] and is silenced in several malignancies [37, 40, 41]. Gene methylation has an established role in promoting pathological cell proliferation in cancer. In multiple myeloma and pancreatic carcinoma the epigenetic silencing of SOD2 is caused by hypermethylation of CpG islands within SOD2's promoter [38, 40, 41]. Much as we found in FHR PASMC, demethylation of SOD2 in cancer restores SOD2 activity and increases H_2O_2, which decreases cell proliferation and tumor growth [37, 40, 41]. Likewise, in prostate cancer, SOD2 over-expression increases H_2O_2 and reduces cell proliferation [83]. Although not tested in our PAH study, catalase, which catalyzes the destruction of H_2O_2, prevents SOD2-induced inhibition of cancer cell proliferation [83]. Thus the antiproliferative/proapoptotic effects of SOD2 augmentation in PAH and cancer are mediated by increasing H_2O_2 production from a basal level that is abnormally low. Likewise, 5-aza-2′-deoxycytidine only increases H2O2 production in FHR PASMC, having no effect on H_2O_2 in normal PASMC [5].

SOD2 downregulation in breast cancer also can result from chromatin acetylation, which creates a repressive chromatin structure which impairs binding of transcriptions

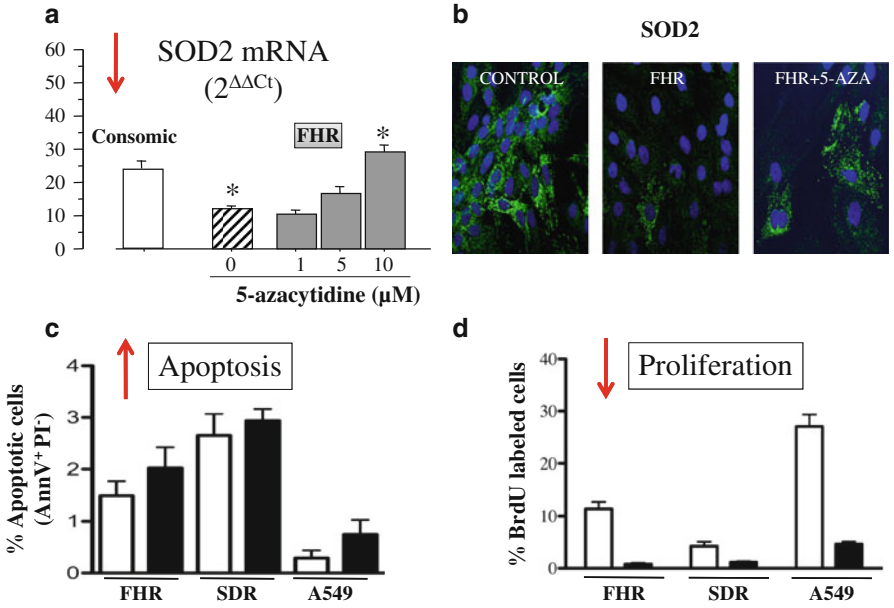

Fig. 3.8 5-Aza-2′-deoxycytidine restores SOD2 expression, reduces proliferation, and enhances apoptosis in FHR PASMC. (**a**, **b**) Representative and mean data showing a dose-dependent increase in SOD2 immunostaining intensity caused by 5-aza-2′-deoxycytidine (5-AZA) in FHR PASMC, $n=4$ per group. * $p<0.05$. (**c**) Percentage apoptotic cells in FHR and Sprague Dawley rat (SDR) PASMC and lung cancer cells (A549) at baseline (white bars) and following treatment with 5-aza-2′-deoxycytidine (black bars), $n=4$–6/group. *, $p<0.05$, statistical significance between baseline and 5-aza-2′-deoxycytidine, † significant difference from SDR PASMC. (**d**) Greater proliferation rates in FHR PASMC and lung cancer cells (A549) vs SDR PASMC ($n=5$–6/group). 5-aza-2′-deoxycytidine inhibits cell proliferation. Reproduced from Ref. [5] with permission

factor, such as SP-1 and AP-1 [37]. Histone deacetylase inhibitors, like trichostatin A [10], restore SOD2 expression in cancer cells [37]. Thus, two epigenetic mechanism of regulating SOD2 transcription can collaborate to control SOD2 expression [27, 28]. The effects of histone deacetylase inhibitors on PAH remains to be determined.

Supporting the central that downregulation of SOD2 is a proliferative, antiapoptotic signal the SOD2 haploinsufficient mouse has an increased risk of cancer [103]. However the net effect on redox state from the loss of SOD2 in these mice is a shift toward an oxidized redox. We suspect the differences in ROS relate to the duration and severity of SOD2 loss (life long and profound) for the knockout mouse versus the modest SOD2 depression acquired adulthood, in FHR. Interestingly, Oberley's group noted that siSOD2 activates HIF-1α, consistent with our findings. However, they found that siSOD2 increased superoxide levels without changing H_2O_2 levels [44]. This again may relate to the magnitude of the SOD2 decrease achieved and cell-specific differences in metabolic activity, prolyl hydroxylase activity, and the status of the many other antioxidant systems.

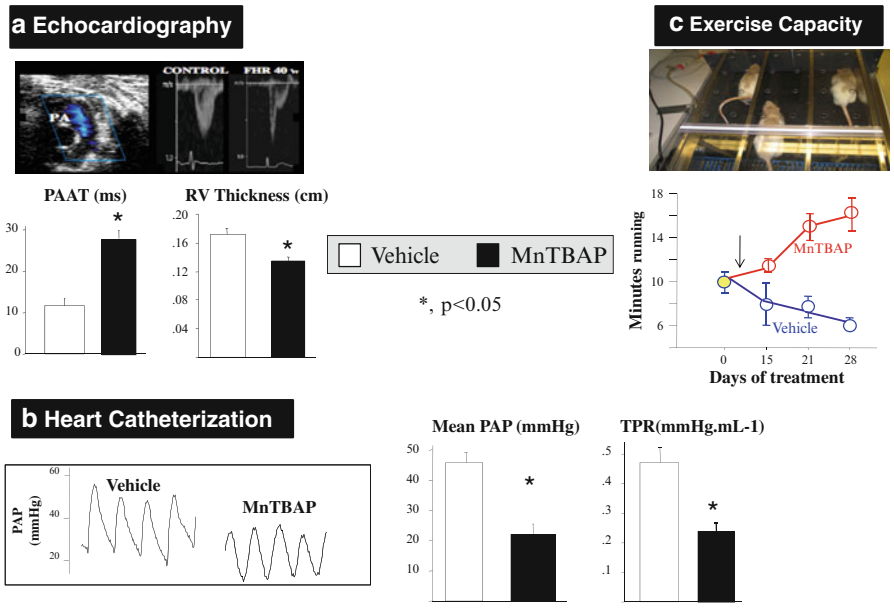

Fig. 3.9 MnTBAP regresses PAH in FHR. (**a**) MnTBAP reduces mean PA pressure measured by Doppler (lengthens PAAT) and decreases right ventricular thickness measured by two-dimensional echocardiography in FHR treated for 4 weeks, $n=5$/group. * $p<0.05$. (**b**) MnTBAP therapy reduces mean PAP and total pulmonary resistance (TPR). (**c**) FHR treated with MnTBAP exercise longer on a graded treadmill versus untreated FHR (*top*), $n=15$/group. Reproduced from Ref. [5] with permission

3.5.2 HIF-1α-Activation

Activated HIF-1α increases expression of glycolytic genes and suppresses oxidative metabolism by transactivating PDK [45], a HIF-induced metabolic inhibitor gene [46, 54]. HIF-1α activation decreases Kv1.5 expression, creating depolarized, calcium-overloaded FHR PASMCs with a proliferative, anti-apoptotic, phenotype [12] and elevated cytosolic Ca^{2+}. Consistent with the hypothesized deleterious effects of HIF-1α in the development of activation in PAH, HIF-1α haploinsufficient mice are resistant to hypoxic pulmonary hypertension and their PASMC retain normal Kv currents [88]. There is evidence supporting the contention that loss of endogenous H_2O_2 activates HIF-1α. Semenza [105] and Bunn [39] have shown that HIF-1α activation is redox regulated. Their findings are consistent with the notion that H_2O_2 inhibits, whereas antioxidants activate, HIF-1α. However, some scientists finding that high ROS (rather than low) activates HIF-1α. Indeed, high calcium levels can, via a calmodulin kinase mechanism, activate HIF-1α [113]. Thus, an alternative possibility is that, loss of SOD2 could activate HIF-1α by increasing superoxide production [10, 44]. In the lung circulation however, the evidence linking low SOD2 and decreased ROS to HIF-1α activation in PAH is clear. Simply lowering SOD2 message levels in normal cells (using siRNA) hyperpolarizes the mitochondria,

decreasing mitochondrial H2O2 production and activating HIF-1α (Fig. 3.6). Conversely, administering SOD2 or a SOD mimetic to PAH PASMC, increases ROS and inactivates HIF-1α [5]. There is a human syndrome (Chuvash pulmonary hypertension) in which activation of HIF-1α leads to pulmonary hypertension. These patients, from the Chuvash region of Russia, have mild pulmonary hypertension and polycythemia due to normoxic HIF-1α activation caused by a prolyl hydroxylase mutation [17, 30, 92].

3.5.3 PDK Activation

There is increased PDK expression in FHR PASMC. PDK phosphorylates and inhibits its pyruvate dehydrogenase (PDH), thereby slowing Krebs' cycle and restricting production of reducing equivalents (NADH, FADH) required to donate electrons to the electron transport chain (ETC). This "inflow" obstruction decreases mitochondrial electron flux and with it the leak of superoxide, which normally occurs in proportion to PO2 [4, 78]. In hypoxia, PDK's inhibition of the ETC is a beneficial, pro-survival mechanism, since ongoing electron transport without oxygen would not generate ATP but would cause ROS formation by autoxidizing the ETC. However, when O_2 is available (as in the FHR) activation of PDK suppress physiological ROS production, reinforcing a proliferative pseudohypoxic signal. The link between SOD2 inhibition and PDK activation is that the former causes HIF-1α activation, which increases PDK expression and activity. Recently, we discovered that PDK inhibition regresses both experimental PAH [12] and human cancers [11]. As in PAH, the effects of PDK inhibition in cancer (whether achieved with PDK siRNA or dichloroacetate) involve reactivation of oxidative metabolism, depolarization of abnormal mitochondria, increased production of hydrogen peroxide, and restoration of Kv1.5 expression [11]. Michelakis et al. showed in an elegant proof of concept, Phase 1, trial that the PDK inhibitor, dichloroacetate, is effective in humans with an intractable form of brain cancer, glioblastoma multiforme [64]. Shrinkage of the glioblastoma resulted from inhibition of cell proliferation and induction of apoptosis. This was achieved with minimal toxicity (a reversible peripheral neuropathy), since the pathologic activation of PDK is largely confined to the cancerous cells [64].

3.5.4 Mitochondrial Fragmentation

Early in our study of the mechanism of PAH we noted fragmentation of mitochondria in both human and FHR PAH PASMC (Fig. 3.10) [12]. Subsequent unpublished data suggests that this fragmentation reflects impaired fusion and increased mitochondrial fission. In both cancer and PAH we find low levels of mitofusin-2, a fusogenic protein in the outer mitochondrial membrane that links mitochondria in chains, promoting the dynamic process of mitochondrial fusion. Mitofusin-2, originally called

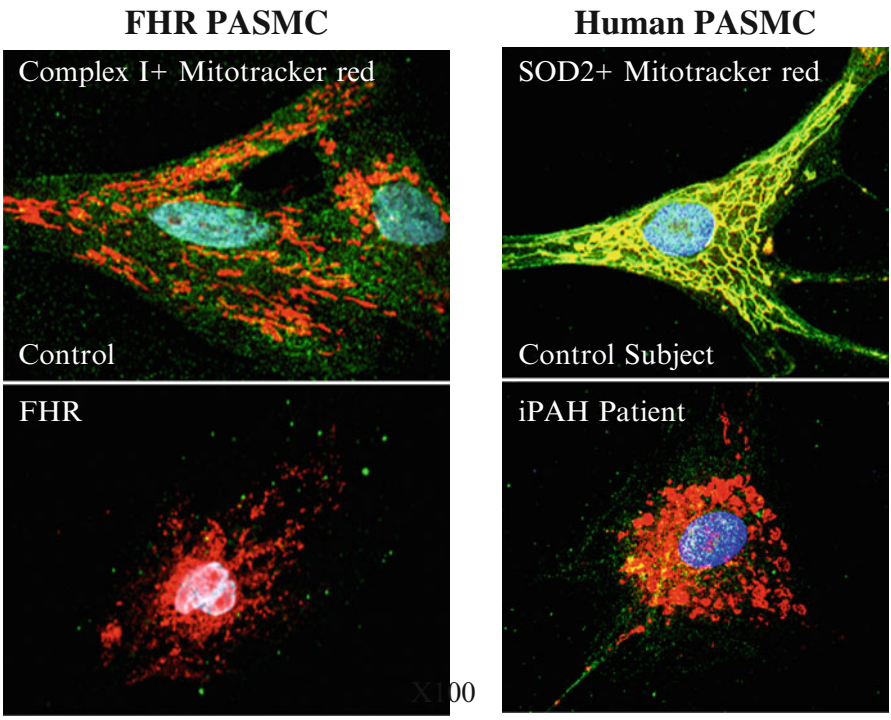

Fig. 3.10 Fragmentation of mitochondria in FHR and human PAH PASMC. Reproduced from Ref. [12] with permission. Cells are stained with antibodies, as labeled

hyperplasia suppressor factor, also suppresses cell proliferation [18, 34, 35, 115]. Unpublished, preliminary data show mitofusin-2 gene therapy slows growth of NSCLC in nude mice. The links between epigenetic silencing of SOD2, HIF-1α activation, and mitochondrial fission and fusion is under investigation in our laboratory. This chapter was written in 2011. Subsequently understanding of the role of mitochondrial dynamics in PAH and cancer have been markedly advanced, see review [116].

3.6 Conclusion

This review summarizes interrelated mitochondrial abnormalities that appear to initiate and maintain PAH, namely impaired expression of SOD2, activation of HIF-1α and PDK and mitochondrial fragmentation. Each of these abnormalities contributes to the abnormal mitochondria-SOD2-ROS-HIF-1α-PDK pathway that is relevant to PAH [12] and cancer [11] (Figs. 3.1 and 3.2). In cancer and PAH it appears that a reversible inhibition of mitochondrial oxidative metabolism and H_2O_2 production contribute to a proliferative and anti-apoptotic phenotype.

3.7 Future Directions

Recognition of the interrelated roles of epigenetic SOD2 deficiency, mediated by CpG methylation, redox-mediated HIF-1α activation, and transcriptional activation of PDK offers many promising therapeutic targets in PAH, including SOD2 replacement therapy, PDK inhibition, and HIF-1α inhibition. The translational potential of this mitochondrial-metabolic hypothesis is strengthened by the availability of drugs that have (to a limited extent) seen clinical use in humans, such as the PDK inhibitor, dichloroacetate (used to treat lactic acidosis related to mitochondrial diseases [1, 93, 94] and glioblastoma) [64]. Other PDK inhibitors are being tested in type II diabetes [55]. The DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (Decitabine) is used to treat myeloproliferative disorders (where it demethylates p15) [89] and sickle cell disease [85]. It is time to consider carefully designed trials of these targets/molecules in humans with PAH and cancer. Moreover, epigenetic mechanisms should be considered as a potential mechanism for transmission of inherited PAH.

Acknowledgements This paper summarizes original science reported in references [5, 11, 12]. The hypotheses have previously been summarized (in part) in review articles, references [3, 7]. Dr. Archer is supported by NIH-RO1-HL071115 and 1RC1HL099462-01, the American Heart Association (AHA).

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Chapter 4
Epigenetics in Cardiovascular Regulation

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Abstract Epidemiological studies have shown an association between pathologic events occurring during early life and the development of cardiovascular and metabolic disease in adulthood. These observations have led to the so-called fetal programming of adult disease hypothesis. In line with this hypothesis, short-term exposure to hypoxia after birth predisposes to exaggerated hypoxic pulmonary vasoconstriction later in life in rats, and transient perinatal hypoxia predisposes to exaggerated pulmonary hypertension during short-term exposure to high altitude in humans. Along the same lines, in recent studies in Bolivian high-altitude dwellers, we found that preeclampsia predisposes the offspring to pulmonary and systemic endothelial dysfunction possibly related to impaired NO bioavailability and augmented oxidative stress. Very recent data from our lab suggest that assisted reproductive technologies may represent another important example consistent with this hypothesis. The mechanisms underpinning the developmental origin of this vascular dysfunction are poorly understood. Increasing evidence suggests that epigenetic alterations, such as DNA methylation or histone acetylation may play a role.

Keywords Epigenetic • Pulmonary hypertension • Endothelial function • Preeclampsia • Hypoxia

4.1 Introduction

4.1.1 The Barker Hypothesis of Fetal Programming

Observations by Barker and colleague [2], that individuals born with a low birth weight present increased cardiovascular mortality during adulthood gave rise to the so called "Barker's hypothesis of fetal programming." This hypothesis postulated that environmental factors acting during the fetal/perinatal period induce alterations

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that predispose to metabolic and/or cardiovascular disease later in life. Since then, many epidemiological studies have confirmed this association and the terms "fetal programming" and "developmental origin of adult diseases" have been coined to describe this problem.

While this concept is well accepted for cardiovascular diseases related to the systemic circulation, it is only very recently that we could provide evidence, in both experimental animal models and in humans, that the pulmonary circulation is also an important target for the developmental programming of adult disease.

4.1.2 Perinatal Hypoxia Predisposes to Exaggerated Hypoxic Pulmonary Hypertension

During the perinatal period, the pulmonary circulation is particularly vulnerable to noxious stimuli because it undergoes important structural and functional changes to allow the sudden transition from gas exchange by the placenta to gas exchange by the lungs [7]. In line with this concept, in normal rats, exposure to hypoxia during the first few days of life induces transient pulmonary hypertension [12] and predisposes to exaggerated pulmonary vaso-constriction in adult life [11].

To investigate whether a similar predisposition exists in humans, we compared pulmonary-artery pressure at high altitude (4559 m) in young healthy adults who had suffered from transient hypoxic pulmonary hypertension during the perinatal period and in control subjects who had had no complications during this period. We chose high-altitude exposure to test our hypothesis because it induces exaggerated hypoxic pulmonary hypertension in patients with pulmonary vascular dysfunction [21, 22].

In line with our hypothesis, we found that the mean increase in pulmonary-artery pressure was roughly 50 % greater in subjects with perinatal hypoxia than in controls, even though the altitude-induced decrease in arterial oxygen saturation was comparable in the two groups [20].

These data represented the first direct demonstration that a perinatal insult causes a defect in pulmonary vascular regulation that predisposes to a pathological response later in life.

4.1.3 Preeclampsia Predisposes to Systemic and Pulmonary Vascular Dysfunction in the Offspring

During a study on the pathogenesis of re-entry pulmonary edema in La Paz (3600–4000 m), Bolivia, we found an unexpectedly large number of offspring of mothers with preeclampsia (the most frequent complication of pregnancy) suffering from this problem. We hypothesized that, as in classical high-altitude pulmonary edema

(HAPE) [3, 23], re-entry HAPE is associated with pulmonary and systemic vascular dysfunction. Moreover, we speculated that in offspring of mothers with preeclampsia this dysfunction could be related to a persistent defect in the fetal circulation caused by vasculotoxic molecules produced by the diseased placenta that cross the placental barrier. We, therefore, measured pulmonary-artery pressure and flow-mediated dilation (FMD, the gold standard for the noninvasive assessment of systemic endothelial function) [8] of the brachial artery in offspring of mothers with preeclampsia and controls subjects who were born and permanently living at high altitude (3600–4000 m).

We found that young normotensive offspring of mothers with preeclampsia display marked vascular dysfunction in the pulmonary and systemic circulation, as evidenced by a 30 % higher pulmonary-artery pressure (Fig. 4.1a) and a 30 % smaller FMD (Fig. 4.1b) than in control subjects [15]. Vascular dysfunction in the pulmonary and the systemic circulation was a robust finding, since we found a close inverse relationship between pulmonary-artery pressure and flow-mediated dilation.

Vascular dysfunction in the offspring of mothers with preeclampsia could be related to preeclampsia per se or to a genetic abnormality that predisposes the mother to preeclampsia and the offspring to vascular dysfunction. To distinguish between these two possibilities, we assessed vascular function in siblings of offspring of mothers with preeclampsia who were born after a normal pregnancy. These siblings had normal pulmonary-artery pressure (Fig. 4.1a) and flow-mediated dilation (Fig. 4.1b) [15]. These findings indicate that preeclampsia per se causes generalized vascular dysfunction in the offspring.

The difference in pulmonary-artery pressure does not appear to be related to a difference in the extent of the pulmonary microcirculation, because carbon monoxide

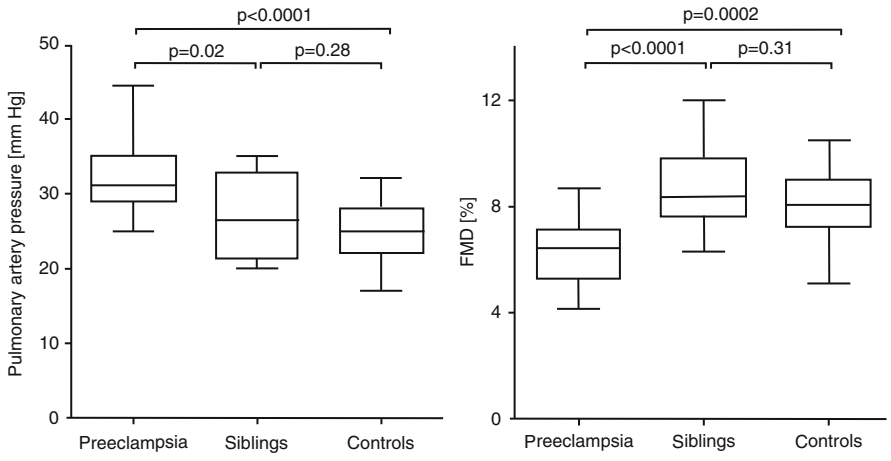

Fig. 4.1 Pulmonary-artery pressure (**a**) and flow-mediated dilation (FMD, **b**) of the brachial artery in young offspring of mothers with preeclampsia, in their siblings who were born after a normal pregnancy, and in control subjects

diffusing capacity was similar in offspring of preeclampsia and controls. Nitric oxide inhalation induced almost twice as large decrease of pulmonary-artery pressure in offspring of mothers with preeclampsia as in controls, indicating that the physiological role of nitric oxide is intact in the offspring and that pulmonary hypertension was related, at least in part, to a functional defect. However, pulmonary-artery pressure during nitric oxide inhalation remained significantly higher in offspring of mothers with preeclampsia than in control subjects, suggesting that a structural defect, possibly related to remodeling of the pulmonary vascular wall, also contributes to pulmonary hypertension.

The preeclampsia-induced vascular dysfunction may have clinical consequences. Due to exaggerated pulmonary vasoconstriction in response to the lack of oxygen, offspring of preeclampsia living at high altitude or suffering from disease states associated with chronic hypoxemia may be at risk for developing sustained pulmonary hypertension, right heart failure and/or pulmonary edema. Defective flow-mediated dilation of the brachial artery in offspring of mothers with preeclampsia was related to endothelial dysfunction, because endothelium-independent dilation was normal in these subjects. Endothelial dysfunction in the systemic circulation represents a very early step in the development of cardiovascular disease. In line with this concept, epidemiological data show that the risk of arterial hypertension ([24, 27] and stroke [16]) is increased in offspring of mothers with preeclampsia.

4.1.4 Underlying Mechanisms of Fetal Programming of Vascular Dysfunction: Role of Oxidative Stress

In both animals and humans fetal insults known to cause vascular dysfunction in the offspring are associated with increased oxidative stress in the circulation of the mother [6]. Oxidative stress may cause endothelial dysfunction and facilitate vascular dysfunction by decreasing NO bioavailability and/or NO-synthase uncoupling. This could be related to oxidative stress-induced epigenetic alterations [30] but the role of this problem in causing circulatory dysfunction in the progeny is not known.

In adult offspring of restrictive diet pregnancy, an experimental mouse model of exaggerated oxidative stress during gestation, we, therefore, examined the role of oxidative stress in the regulation of vascular function in the offspring.

We found that restrictive diet pregnancy caused pulmonary endothelial dysfunction in vitro and exaggerated hypoxia-induced pulmonary hypertension and right ventricular hypertrophy in vivo in the offspring. This vascular dysfunction was induced by increased oxidative stress, because antioxidant administration during restrictive diet pregnancy prevented this problem in the offspring [19].

Several lines of evidence suggest that exaggerated oxidative stress during restrictive diet pregnancy induces vascular dysfunction by an epigenetic mechanism (see below).

We speculate that in humans, pathological events associated with increased oxidative stress early in life could induce vascular dysfunction by a similar mechanism.

Consistent with this concept, in offspring of preeclampsia we found that oxidative stress was increased and related to pulmonary-artery pressure and flow-mediated dilation, suggesting that it may represent an underlying mechanism.

4.2 May Environmental Insults Occurring Earlier in the Development Also Play a Role in the Fetal Programming of Cardiovascular Diseases?

Epigenetic regulatory mechanisms play an important role not only during the fetal/perinatal period but also around the conception during the gametogenesis. We, therefore, wondered whether environmental insults occurring during this period may have similar long-term effects in the offspring as those occurring later during gestation or in the perinatal period.

The steadily increasing use of assisted reproductive technologies (ART) has allowed millions of infertile couples to have children. ART involves the manipulation of early embryos at a time when they may be particularly vulnerable to external disturbances. Environmental influences during the embryonic and fetal development influence the individual's susceptibility to cardiovascular and metabolic disease [1] raising concerns regarding the potential consequences of ART on the long-term health of the offspring [18, 25].

Consistent with this concept, in very recent studies, we found that healthy children conceived by ART display marked pulmonary and systemic vascular dysfunction and signs of early arteriosclerosis. This vascular dysfunction is of similar magnitude as the one observed in offspring of mother with preeclampsia [15] and in children with diabetes [14], a condition known to be associated with an increased risk for premature cardiovascular morbidity and mortality [5].

Interestingly, and similarly to what observed in offspring of mother with preeclampsia, ART-induced vascular dysfunction was associated with increased oxidative stress and antioxidant administration significantly improved vascular function in these children. We propose that antioxidants may allow to prevent/postpone the ART-induced predisposition to premature cardiovascular disease in these individuals.

4.3 ART, An Experimental Animal Model to Study Epigenetic Regulatory Mechanisms of Vascular Function

Owing to the difficulty of studying underlying mechanisms in apparently healthy children, we developed a mouse model of ART. Consistent with observations in humans, we found that in ART mice, endothelium-dependent mesenteric-artery vasodilation in vitro was markedly impaired and translated into arterial hypertension in vivo compared with control mice.

Similarly to what observed in offspring of restrictive diet pregnancies, oxidative stress appeared to contribute to this vascular dysfunction, because addition of the antioxidant Tempol to the organ chambers normalized endothelium-dependent vasodilation in ART animals, whereas it had no detectable effect on this response in controls.

These observations suggested that ART mice might be a novel experimental animal model to study underlying (epigenetic) regulatory mechanisms of vascular dysfunction.

The principal epigenetic modifications that may occur during embryonal-early fetal and during the perinatal period are alterations of DNA methylation on the cytosine of CpG nucleotides.

Acting mainly on promoters, these modifications deregulate the gene expression either by stimulating or repressing it. These changes in gene expression are transmissible throughout life and to the next generation [10, 13, 17] but potentially reversible by the administration of inhibitors of the histone deacetylase [28].

To test for the possible role of epigenetic alterations underpinning the fetal programming of vascular dysfunction in ART mice, we therefore, assessed their meth ylation pattern of several imprinted genes known to be regulated by epigenetic mechanisms. We observed that the global methylation of these imprinted genes was altered in the aortic tissue of ART mice. More importantly, the DNA methylation pattern of the promoter of the endothelial nitric oxide synthase (eNOS, a gene known to be involved in vascular regulation) was altered in arterial tissue of ART mice. This alteration had an important functional importance because it was associated with decreased eNOS expression in the aorta and lower NOx plasma concentration in ART than in control mice.

Vascular dysfunction related to epigenetic changes may be transmitted to the next generation [4, 9, 31]. To test for this possibility, we assessed pulmonary vascular function in the progeny of male ART mice mated to control females. We found that pulmonary vascular dysfunction in the progeny was comparable to the one observed in their fathers.

Epigenetic changes can be reversed by histone deacetylase inhibitors, such as sodium butyrate, that are known to induce replication-independent demethylation of ectopically methylated genes [26, 29, 32]. We therefore examined the effects of Butyrate administration to ART mice on the methylation pattern and vascular function. Butyrate normalized the imprinted genes methylation pattern and vascular function in vitro and in vivo, and prevented the transmission of the vascular dysfunction and dysmethylation to the progeny.

Collectively, these findings indicate that ART induces vascular dysfunction by an epigenetic mechanism.

4.4 Conclusion

Cardiovascular and metabolic diseases were thought to result from the interaction between the behavior of an individual and his genetic inheritance. Recent data indicate, however, that fetal programming also plays an important pathogenic role.

Among the underlying mechanisms by which fetal programming may lead to cardiovascular dysfunction, augmented oxidative stress and/or epigenetic alterations appear to play a major role. Fetal programming may occur at various stages of the development and there is evidence that fetal programming-induced alterations are transmissible from cell to cell throughout life and then also to the next generation.

More importantly from a medical therapeutic standpoint, epigenetic alterations may be reversed by pharmacological interventions opening a window for the potential prevention and treatment of cardiovascular and metabolic diseases associated with the fetal programming.

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Part II
Hypoxia and High Altitude Residents

Chapter 5
Why Are High-Altitude Natives So Strong at Altitude? Maximal Oxygen Transport to the Muscle Cell in Altitude Natives

Carsten Lundby and Jose A.L. Calbet

Abstract In hypoxia aerobic exercise performance of high-altitude natives is suggested to be superior to that of lowlanders; i.e., for a given altitude natives are reported to have higher maximal oxygen uptake (VO_2max). The likely basis for this is a higher pulmonary diffusion capacity, which in turn ensures higher arterial O_2 saturation (SaO_2) and therefore also potentially a higher delivery of O_2 to the exercising muscles. This review focuses on O_2 transport in high-altitude Aymara. We have quantified femoral artery O_2 delivery, arterial O_2 extraction and calculated leg VO_2 in Aymara, and compared their values with that of acclimatizing Danish lowlanders. All subjects were studied at 4100 m. At maximal exercise SaO_2 dropped tremendously in the lowlanders, but did not change in the Aymara. Therefore arterial O_2 content was also higher in the Aymara. At maximal exercise however, fractional O_2 extraction was lower in the Aymara, and the a-vO_2 difference was similar in both populations. The lower extraction levels in the Aymara were associated with lower muscle O_2 conductance (a measure of muscle diffusion capacity). At any given submaximal exercise intensity, leg VO_2 was always of similar magnitude in both groups, but at maximal exercise the lowlanders had higher leg blood flow, and hence also higher maximum leg VO_2. With the induction of acute normoxia fractional arterial O_2 extraction fell in the highlanders, but remained unchanged in the lowlanders. Hence high-altitude natives seem to be more diffusion limited at the muscle level as compared to lowlanders. In conclusion Aymara preserve very high SaO_2 during hypoxic exercise (likely due to a higher lung diffusion capacity), but the effect on VO_2max is reduced by a lower ability to extract O_2 at the muscle level.

Keywords Sherpa • Tibet • Andes • Exercise • Endurance

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5.1 Introduction

Porters carrying huge loads on physically very demanding footpaths on the way to Mount Everest are known by most trekkers and their feats are often said to be the result of "perfect adaptation to high altitude." However, the fact is that most Nepali porters are neither born nor living at high altitude, but reside in the Kathmandu lowlands, or in the Himalayan fore hills (B. Basnyat, personal communication). Nevertheless, the extraordinary carrying capacities of the Nepalese porters have been extensively studied. Incredibly, this group of porters (of mixed ethnicity) is reported to carry loads of on average ~90 % of their body weight for 14 days on the footpaths leading to Mount Everest. Part of the explanation for this, is that they are far more economical when carrying head-supported loads when compared to lowland Caucasians, or even when compared to other tribes renown for their head carrying abilities such as the east African women [5]. In Nepali porters no differences in economy is found between "full time hill porters" and "part time casual porters" [41]. The most recognized porters are of Sherpa ethnicity and for the most part they are born and reside at altitude. Sherpas are also employed to help western mountaineers to summit peaks like Mount Everest, and three Sherpas have climbed to its summit ten or more times. Part of the explanation for the renowned performance of Sherpas at high altitude could be due to altitude-related adaptations (genetic or developmental). Endurance during submaximal exercise depends on a variety of physiological (and psychological) parameters whereof maximal oxygen uptake (VO_2max), the ability to exercise at a given % of VO_2max, and exercise economy are crucial. Only one study has assessed submaximal performance in high-altitude natives [34]. Low- and high-altitude (4540 m) native Peruvians exercised until fatigue at similar workloads, the lowlanders sustained the load for 59.5 min and the highlanders for 34.2 min. The focus of the present review is to describe the oxygen transport cascade, skeletal muscle energy utilization, and exercise economy in high-altitude natives. Oxygen transport and muscle energy utilization data are very limited. This review focuses on studies of the Andean Aymara high-altitude population because these have been studied with invasive techniques. We studied these high-altitude natives at 4100 m (where they reside) and at 5260 m with arterio-venous differences across the exercising leg, assessed leg blood flow, and obtained skeletal muscle biopsies before and during exercise. The maximal exercise data have been published in part, whereas all sub-maximal data are previously unpublished. It is suggested that Andean and Tibetans have adapted differently in regards to altitude exposure [7] and this should be kept in mind before extrapolating these data to other high-altitude ethnic groups. This important point is raised in other reviews on this issue.

5.2 Ventilation, Diffusion Capacity and Arterial O_2 Content

Bulge flow of air into the alveoli is the first step in the oxygen transport cascade, and determines the alveolar PO_2 (P_AO_2) for any given altitude. For a given submaximal metabolic rate the ventilation of high-altitude natives is lower when compared to lowlanders exposed to the same altitude. Brutsaert demonstrated this in 21 high-altitude natives of La Paz at 3600 m who had lower V_E/VO_2 values when compared to European/North American residents at this altitude (minimum duration of acclimatization was 2 months). With maximal exercise at 4380 m, however, V_E/VO_2 is of similar magnitude in subjects born and raised at sea level and in subjects born and raised at altitude. For a given body size, high-altitude natives are reported to have larger lung volumes. High-altitude natives born in Morococha (4540 m) had 38 % larger residual volumes than individuals of the same ethnicity but born and living at sea level. The vital capacity was approximately the same for both groups, and as a result, total lung capacity was higher in the high-altitude natives [34]. In Peruvian highlanders studied at 3850 m forced vital capacity was 5.11 ± 0.64 l, but only 3.73 ± 0.32 in Peruvians born and living at 800 m [7]. At maximal exercise the larger lung volumes do not affect the calculated values for P_AO_2. After 2 and 8 weeks of acclimatization in lowlanders, the values were 69 ± 0.9 and 72 ± 1.4 mmHg, respectively, and 67 ± 0.6 mmHg in the Aymara. Although the had Aymara lower P_AO_2, than the lowlanders, the arterial PO_2 (PaO_2) was lower in the acclimatized lowlanders (52.4 ± 1.3 and 55.7 ± 1.4 mmHg, after 2 and 8 weeks exposure, respectively) than compared to the Aymara (57.9 ± 1.2) (Fig. 5.1a). Therefore, the alveolar-arterial PO_2 difference (A-aDO_2) was 9 ± 1.4 mmHg in Aymara but 17 ± 1.5 and 17 ± 2.1 mmHg in the lowlanders after 2 and 8 weeks of altitude acclimatization. For a given P_AO_2 it is well documented that high-altitude natives have a higher pulmonary diffusing capacity [14, 17, 18, 51], and the smaller A-aDO_2 in Aymara helps to preserve PaO_2 for any given level of ventilation, which is also observed in Tibetans [66]. Theoretical and experimental work has shown that at altitude most of the A-aDO_2 in lowlanders is due to alveolar-capillary diffusion limitations [50, 57], and that the reduced A-aDO_2 of high-altitude Aymara is not the consequence of optimized blood flow distribution within the pulmonary circulation in response to hypoxic pulmonary vasoconstriction [52]. Indeed, the likely reason of the low A-aPO_2 in Aymara is the higher diffusion capacity associated with larger lung volumes [52, 58, 66]. The larger lung volumes may be the result of multiple factors. From animal studies it is known that hypoxic exposure is associated with larger alveolar septal tissue volume and surface areas [35], which are further augmented by smaller alveolar duct volume and smaller mean harmonic diffusion-barrier thickness. In these animals the structural changes induced a higher oxygen diffusing capacity during exercise [46]. For lung adaptations to occur in animals, high altitude exposure has to be initiated during lung maturation whereas altitude exposure in adult (animal) life does not induce structural or volume changes [35]. Once obtained, however, even 2 years of re-exposure to normoxia does not reverse the adaptations [46]. From the above studies it may be concluded that increases in gas exchange

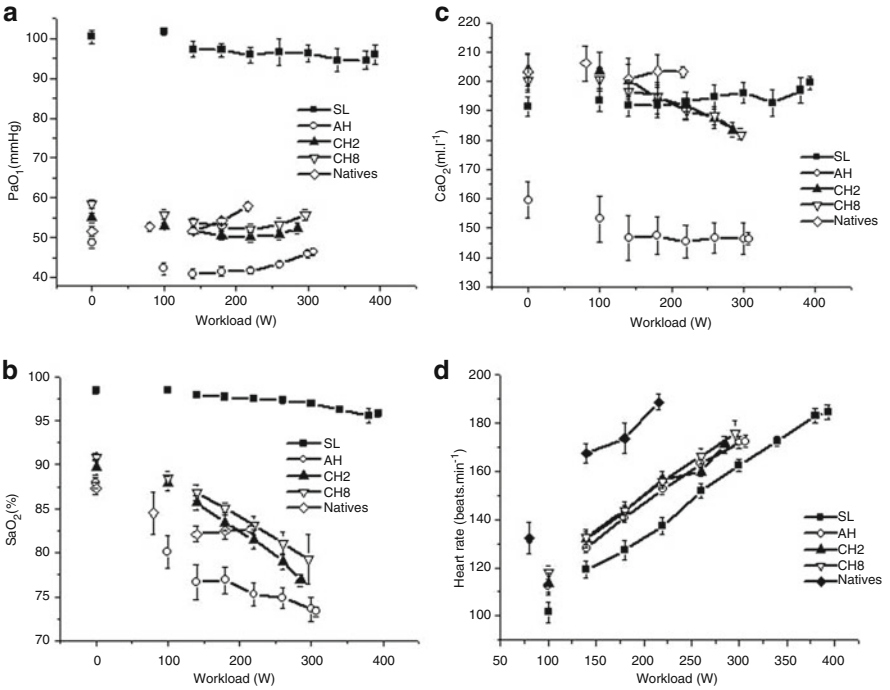

Fig. 5.1 (**a**) Arterial PO_2 (PaO_2, mmHg), (**b**) arterial O_2 saturation (SaO_2, %), (**c**) arterial O_2 content (CaO_2, ml l^{-1}), and (**d**) Heart rate (beats min^{-1}) during incremental exercise in Danish lowlanders at sea level (SL, *black square*), in acute hypoxia (AH, *open circle*) and after 2 (CH2, *black triangle*) and 8 (CH8, *open triangle*) weeks of acclimatization to 4100 m altitude and in high-altitude Aymara (*open diamond*) also investigated at 4100 m altitude

efficiency arises largely from developmental exposure to hypoxia, and is further strengthened by the similar diffusion capacities of natives from Colorado, the Andes, and Tibet. It is tempting to speculate that the adaptations are of genetic nature because this, at least to some extent, could explain why second generation Tibetan lowlanders born at 1300 m and who had never been exposed to high altitude recovered 92 % of their sea level VO_2max after 30 days at 5050 m [44]. It seems unlikely that the superior diffusion capacities of high-altitude natives, which depend on structural adaptations in the lungs, may become available to acclimatizing Caucasians. Other factors, however, may also affect diffusion capacity and include polycythemia, cardiac output, pulmonary arterial hypertension, and pulmonary vascular hyperactivity. Of these the slightly higher haematocrits of Aymara as compared to Danes acclimatized for 8 weeks may account for some of the augmented diffusion capacities. During submaximal exercise cardiac output must be assumed to be similar in both populations, but at maximal exercise the values reported for highlanders (see below), are much lower than those reported from acclimatized Caucasians. The lower Q allow longer mean transit time for the red blood cell in the pulmonary capillaries, and thereby reduces the degree of arterial desaturation at

maximal exercise (see Dempsey et al. [18] for review). During exercise the elevated PaO_2 in Aymara is associated with higher arterial oxyhaemoglobin saturations (SaO_2) (Fig. 5.1b), and is discussed in the following.

The sigmoid shape of the blood-O_2 equilibrium curve (OEC) facilitates blood oxygenation in the lung capillaries as virtually all haemoglobin is loaded with O_2 even at a relatively low arterial PO_2. The ability of haemoglobin to bind O_2 is expressed as the P_{50} value, and represents the PaO_2 at which oxygen saturation is 50 %. In vivo, P_{50} depends on pH, PCO_2, the concentration of 2,3-diphosphoglyceric acid (DPG), Mg^{2+}, ATP and Cl^-, temperature, and the amount of haemoglobin bound to CO (for review see [50]). It is generally believed that a leftward shift in the OEC, i.e. a lowering of the P_{50}, is advantageous for the loading of O_2 in the pulmonary capillaries, whereas a rightward shift is supposed to facilitate the unloading of O_2 at the tissue level [5, 60]. The increasing acidosis and hypercapnia in the capillary blood, combined with the increase in temperature, results in an in vivo rightward shift of OEC (increasing P_{50}), and thereby facilitates O_2 unloading to the exercising muscles [53]. Thus, in theory, if P_{50} is low, pulmonary O_2 uptake is enhanced whereas tissue O_2 unloading is impaired, and vice versa. With acclimatization to 4000–5300 m of altitude, the blood-O_2 affinity of resting humans is decreased, as the standard P_{50} is increased by 2–5 mmHg [36, 58] probably due to increased blood content of DPG [4] and Mg^{2+}, ATP, and Cl^- [39]. In resting conditions there are no differences in the standard P_{50} (i.e., the P_{50} determined at 37°, pH = 7.40, PCO_2 = 40 mmHg) between high and lowlanders [36, 58], and when calculating P_{50} at maximal exercise with actual values for pH, $PaCO_2$, and temperature no differences are observed between acclimatizing lowlanders and Aymara [38]. Thus, for any given PaO_2 low- and high-altitude natives bind the same amount of O_2 to haemoglobin (this may be different in other populations, see Beall [3]). Due to the differences in A-aPO_2 between acclimatizing lowlanders and Aymara, the response of SaO_2 to graded exercise also differs between groups. In the transition from resting conditions to maximal exercise intensity, the exercise induced degree of arterial desaturation in the lowlanders was 12.8 % (89.7–76.9 %) after 2 weeks of acclimatization and 11.6 % (90.9–79.3 %) after 8 weeks of additional exposure. In the Aymara, however, the exercise-induced desaturation was only 4.6 % (from 87.3 to 82.7 %) (Fig. 5.1b), and thereby CaO_2 was also maintained at a higher level than in the lowlanders (see below). After 8 weeks of altitude acclimatization our Danish subjects had increased [Hb] from 139 ± 2 at sea level to 159 ± 3 g l^{-1}, but still did not reach the 167 ± 4 g l^{-1} that we observed in the Aymara. The corresponding haematocrits were 42.4 ± 0.7, 47.6 ± 1.0, and 50.2 ± 1.2, respectively. In lowlanders these changes are accounted for by decreasing plasma volume and by simultaneously enhancing red cell mass. Consequently total blood volume is reduced during the first weeks at altitude. However after about 1–2 months blood volume is similar to that at sea levels and increases further with prolonged sojourn [47]. Compared to lowlanders at sea level, high-altitude natives have high haematocrits as a result of an increased red cell mass. Since plasma volume is similar in low and highlanders, total blood volume is elevated in highlanders [16, 22, 51]. Although Aymara have slightly elevated [Hb], the main reason for Aymara to perform well at altitude is

tightly coupled to their high O_2 lung diffusion capacities, leaving SaO_2 largely unaffected (in comparison to Caucasians) throughout exercise and thereby also maintaining the binding of O_2 to Hb high. This has major effects on the arterial oxygen content (CaO_2) which is kept remarkably constant throughout exercise, the values of the investigated Aymara being 203 ± 6 ml l^{-1} at rest and 203 ± 2 ml l^{-1} at maximum exercise. In contrast CaO_2 was decreased in the lowlanders from 204 ± 4 to 184 ± 2 ml l^{-1} and from 201 ± 4 to 182 ± 2 ml l^{-1} within the transition from rest to maximum effort after 2 and 8 weeks of acclimatization, respectively (Fig. 5.1c). Assuming a total blood volume of 6 l and if using the actual O_2-extraction percentage, the desaturation induced a "loss" in VO_2max corresponding to approximately 110 ml min^{-1} in the Danes. Because arterial desaturation is only very modest in high-altitude natives, this may also in part explain why Aymara do not increase VO_2max to the same extent as lowlanders when breathing hyperoxic oxygen mixtures. Restoring normoxia acutely in Aymara at 3600 and 4100 m increased VO_2max by 7–8 % [21, 36], whereas the VO_2max of acclimatized lowlanders was increased by 10–13 % [36]. When transporting high-altitude Peruvians to sea level, VO_2max increased from 2.97 to 3.25 l min^{-1} (9 %) [56]. At 5260 m breathing 55 % O_2 did not increase maximal workload in Aymara (residing at 3600–4100 m) [58], but enhanced maximal workload and peak leg VO_2 to sea level values in lowlanders acclimatized for 9 weeks at the same altitude [12]. Hence, one clear advantage of Aymara during exercise at altitude is that exercise is not associated with the same degree of arterial desaturation as is known to occur in lowlanders.

5.3 Cardiac Output, Blood Flow, O_2 Delivery, and O_2 Extraction

Data on cardiac output (Q) and stroke volume in high-altitude natives performing exercise are scarce. Using cardiac impedance-based methodology Chen and coworkers recorded Q in Tibetans immediately after maximal exercise, and reported Q to be 12.8 and 11.5 l min at 3417 and 4300 m altitude, respectively. Q measured in Han at the same altitudes was approximately 1.5 l min^{-1} lower in both conditions [15]. Although the subjects were age-matched, the observed differences between groups could be the result of different training backgrounds. Using the dye dilution technique, which is more accurate and valid than cardiac impedance, Vogel and colleagues [56] quantified maximal Q in Peruvians residing at 4350 m. In this study cardiac output was approximately 16.4 l. min^{1} when measured at 4350 m, and 17.4 l min^{-1} when measured at sea level. At rest and during submaximal exercise Q was similar in both situations (lower heart rate, but enhanced stroke volume at sea level). The small increase in Q with normoxic exposure was the consequence of a slightly higher stroke volume and heart rate response. The maximal values obtained by Vogel are not different from those observed in lowlanders brought to altitude. Saltin [49] studied four "fit" sea level residents after 2 weeks at 4300 m and obtained values for maximal Q to range from 15.6 to 19.2 l min^{-1} in these individuals, and

that Q was reduced by 20 % as compared to sea level experiments. From Fig. 5.1d it is seen that the heart rate response to maximal exercise is of similar magnitude in Aymara and lowlanders, and hence stroke volumes must also be assumed to be of similar size. Indeed Vogel [56] observed maximal stroke volumes of almost 100 ml, a value very similar to the 90–115 ml reported by Saltin [49]. For a given VO_2, Q is similar in high and lowlanders [49, 56].

The assessment of leg blood flow during exercise in high-altitude natives has been performed in two studies [38, 46]. In one study [38] blood flow was determined with the thermodilution method in 8 Aymara at 4100 m (Fig. 5.2a). The blood flow response of Aymara to submaximal exercise was similar to that of acclimatizing lowlanders (2 and 8 weeks), although in maximal terms blood flow was lower. Arterial femoral O_2 delivery was essentially the same at submaximal exercise intensities in the two populations (Fig. 5.2b) but peak values were lower in natives. When administrating hyperoxia, leg blood flow was reduced at submaximal exercise intensities in the Aymara as well in the lowlanders in a similar manner. Rådegran [46] quantified leg blood flow in response to submaximal, one-legged, dynamic knee extensor exercise in Danish lowlanders acclimatized for 7–10 weeks at 5260 m altitude. The values were compared to Aymara usually residing between 3800 and 4100 m but investigated at 5260 m altitude. At rest and during the onset of incremental exercise, blood flow was the same in the lowlanders at sea level and altitude, as in the natives at altitude. Acute hypoxia increased blood flow by approximately 55 % during submaximal one-legged exercise in the lowlanders at sea level. Acute hyperoxia decreased blood flow by approximately 22–29 % during exercise in the lowlanders and natives at altitude. Thus, two independent studies clearly demonstrate with invasive techniques that the blood flow response to exercise is similar in Aymara and in Danish lowlanders at the submaximal level, but that at maximal exercise leg blood flow is lower in Aymara as compared to acclimatized Danes. This is in contrast to a study performed by the group of Beall in Tibetans. Following hand grip exercise (5 min of repetitive handgrips consisting on 10 s of contraction followed by 5 s of relaxation) forearm blood flow was measured non-invasively by strain gauge venous occlusion plethysmography in Tibetans at 4200 m and in lowland US residents at sea level. The Tibetans had more than double the forearm blood flow of low-altitude residents [20]. The higher flows correlated with higher values of NO, but the reason for such high flows remains unexplained. These data thus contradict our data obtained in Aymara at rest and during incremental exercise. It should be remembered that blood flow is tightly coupled to CaO_2 and not PaO_2 [48], and when considering the similar CaO_2 in acclimatizing lowlanders and Aymara there is no main additional stimulus to drive blood flow.

The next step in oxygen transport is oxygen extraction. Despite the fact that the driving force for O_2 diffusion, PaO_2, at maximal exercise intensity is slightly higher in the Aymara, fractional O_2 extraction is significantly lower. At maximal intensity the lowlanders extract between 90 and 91 % of arterial O_2 regardless of experimental setting. The Aymara on the other hand extract 83 % (Fig. 5.2c), leaving 37 ml O_2/l in the femoral venous blood (Fig. 5.2d). In comparison the lowlanders had between 14 and 16 ml O_2 left. Thus, despite higher CaO_2 in the Aymara, due to the

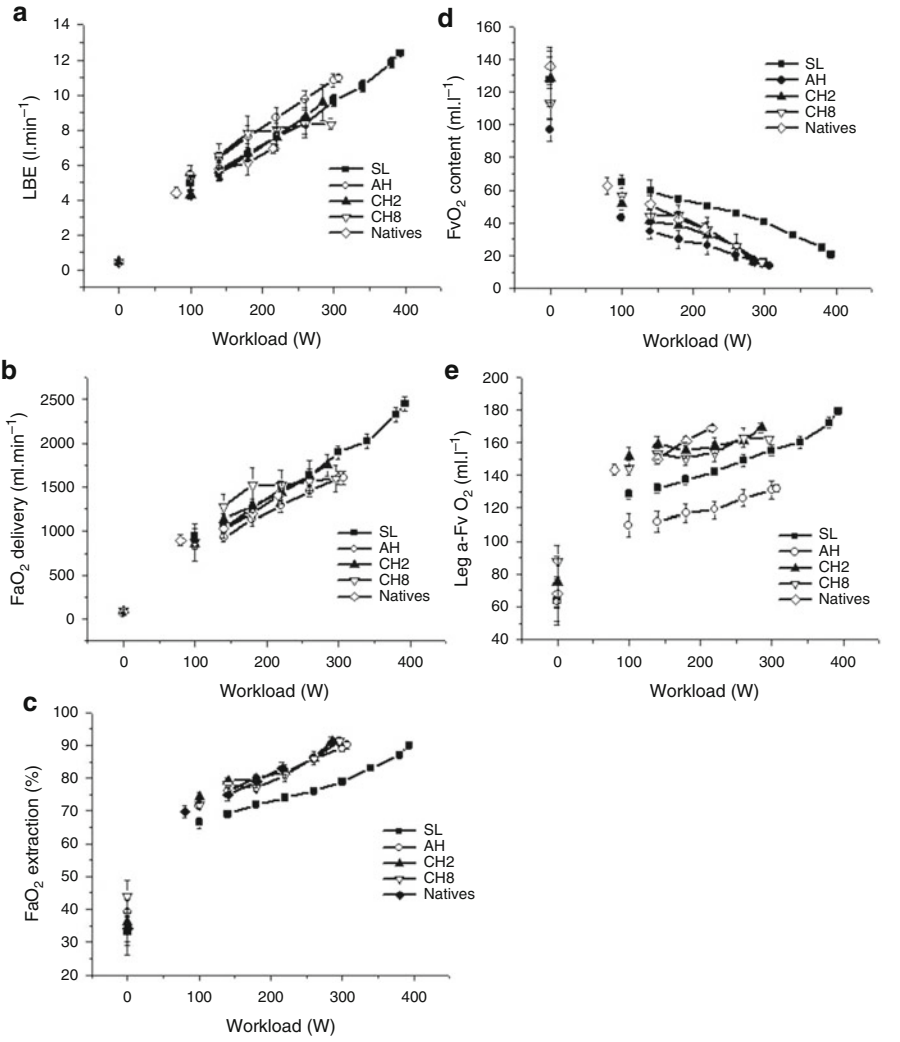

Fig. 5.2 (**a**) Femoral venous blood flow (FBF, l min^{-1}), (**b**) Femoral arterial O_2 delivery (FaO_2 delivery, ml min^{-1}), (**c**) Fractional femoral arterial O_2 extraction (FaO_2 extraction, %), (**d**) Femoral vein O_2 content (FvO_2 content, ml l^{-1}), (**e**) Femoral arterial venous O_2 difference (Leg a-Fv O_2, ml l^{-1}). Abbreviations as for Fig. 5.1

lower extraction rates, the leg a-v O_2 difference (Fig. 5.2e) was similar to the lowlanders after 2 and 8 weeks of acclimatization. Interestingly, when applying acute normoxia in the Aymara fractional O_2 extraction fell to 79 %, leaving 49 ± 7 ml O_2/l in the femoral venous blood at fatigue. In contrast acute induction of normoxia in the Danes did not change fractional extraction, and femoral venous O_2 content was 18 ± 5 and 20 ± 4, after 2 and 8 weeks respectively, which was not much higher than in ambient conditions. The intriguing question is to answer why O_2 extraction is

lower in the Aymara, and why exercise is terminated when relatively large amounts of O_2 are still present in the venous outflow of exercising muscle, especially in the acute normoxic condition. Several factors may account for the observed differences in arterial oxygen extraction at maximal exercise, which depends on the interaction of the following: (1) kinetics of O_2 off-loading from haemoglobin, (2) capillary-muscle O_2 conductance and the degree of mismatch between the metabolic demand and blood flow distribution, (3) muscle oxidative capacity, and (4) exercise intensity. These are discussed in depth in [38], and the main factor responsible for the lower O_2 extraction seems to be related to a gradual reduction in oxygen conductance (leg VO_2/mean capillary PO_2) which is an estimation of muscle diffusing capacity. We subsequently calculated oxygen conductance (in the Danes) using the actual mean capillary PO_2 and blood gasses, but with the blood flows obtained at sea level and then hardly found any differences in oxygen conductance. It would thus seem that in acclimatizing lowlanders the gradual reduction in oxygen conductance is caused by the reduction in maximal blood flow. The leg blood flows of the Aymara as well as the oxygen conductance were lower than in the Danes, but whether the relationship is causal remains unknown.

5.4 Leg VO_2, Whole Body VO_2, and Whole Body Work Efficiency

During submaximal exercise leg VO_2 was similar in all subjects in all conditions. At maximal intensities, however, leg VO_2 was lower in the Aymara (Fig. 5.3a). This was attributed to lower leg blood flow and not to leg a-v O_2 differences since they were of similar magnitude in both groups. With acute induction of normoxia, submaximal leg VO_2 remained unchanged in both groups. At maximal intensities, however, leg VO_2 was increased in the Danes, but not in the Aymara. Leg VO_2max

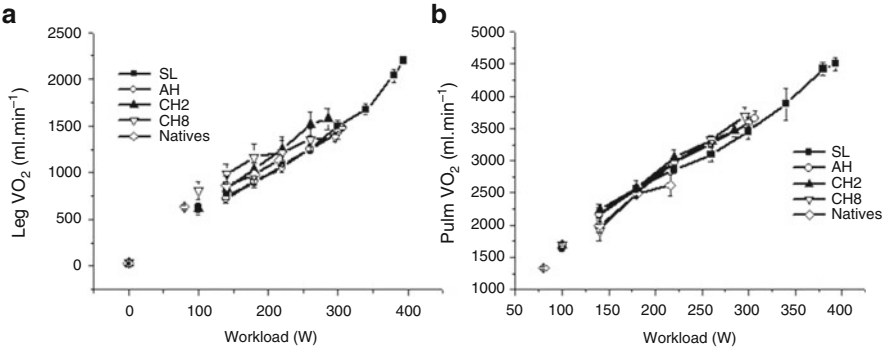

Fig. 5.3 (**a**) Leg VO_2 (ml min^{-1}) and (**b**) Pulmonary VO_2 (Pulm VO_2, ml min^{-1}). Abbreviations as for Fig. 5.1

did not increase in the Aymara because the additional O_2 was not extracted to the same extent as in the lowlanders. The similar leg VO_2 clearly supports the notion of unchanged whole body work efficiency in Aymara [35]. Whole body VO_2 is illustrated in Fig. 5.3b.

Whole body VO_2max was assessed in "elite" (the 6 most acknowledged at the time) Sherpas brought to sea level, the average value being 67 ± 4 ml l^{-1} kg^{-1} [23] and thus somewhat higher than the 60 ± 6 ml l^{-1} kg^{-1} reported in "elite" Caucasian climbers of the 80s [45]. Obviously this comparison is lacking control of multiple factors such as training status. However, VO_2max values obtained from high-altitude natives residing at various altitudes have been commented on as being relatively high as compared to lowlanders [7]. Brutsaert [7] recently combined data from most (but not all) studies reporting VO_2max in Tibetans, Sherpas, and Andean groups to increase statistical power and tested the hypothesis that high-altitude natives have higher values for VO_2max as compared to sea level residents, and indeed found that when combining data these groups have significantly higher mean values for VO_2max. Obviously this analysis relies on the assumption that confounding factors are randomly distributed between the studies and populations. One study that seems to have overcome at least some of theses limitations was completed by Brutsaert and co workers [10] when studying 150 adult males in Bolivia. High-altitude natives ($n = 75$) and low altitude natives ($n = 75$) were studied at high altitude (3600–3850 m) and near sea level. A trend for increased VO_2max with increasing developmental high altitude exposure did not reach statistical significance, and it was concluded that the results did not support the hypothesis that Andean high-altitude natives have been selected to express a greater physical work capacity in hypoxia. To further test this hypothesis, however, 30 men of mixed Spanish and Quechua origins with individual genetic admixture level (%Spanish ancestry) were studied at sea level and with acute exposure to 4338 m. Admixture predicted the decrease in VO_2max with altitude exposure, and it was concluded that Quechua posses a better ability to perform exercise at altitude due to an optimized gas-exchange system [11]. The diminished decrease in VO_2max of high-altitude natives when exposed to higher than residing altitudes was recently also demonstrated in Tibetans. It was shown that second generation Tibetan lowlanders born at 1300 m and who had never been exposed to high altitude recovered 92 % of their sea level VO_2max after 30 days at 5050 m. In comparison untrained and trained Caucasians were able to recover only 70 and 55 % of their sea level VO_2max, respectively [41]. It would thus seem that the VO_2max of high-altitude natives does not decrease as much as that of Caucasians when exposed to altitude, or to altitudes higher than actual residing altitude. This feature could be the consequence of lifelong hypoxic exposure. Quite surprisingly, however, one study demonstrated that lowlanders brought to high altitude for as short as 27 months may achieve VO_2max values very close to those of residing Tibetan natives suggesting that, at least in absolute terms for VO_2max, it is not necessary to have resided at altitude for generations to achieve values close to those observed at sea level [44]. For a long time this was the only scientific report demonstrating that VO_2max may increase with acclimatization in lowlanders, and indeed the general consensus is that VO_2max does not increase with acclimatization to

moderate altitude [12, 38]. Regardless, the ability of high-altitude natives to achieve over 90 % of sea level VO_2max when exposed to high altitude is remarkable.

Besides the importance of a high pulmonary VO_2 in order to achieve a high level of aerobic performance, muscular efficiency is also important. Muscular efficiency is calculated as external + internal work/energy expenditure but is often measured as workload on the cycle-ergometer/pulmonary VO_2 because of the relative difficulties associated with assessing the contribution of glycolytic and phosphate energy production (in the section below we have most of the relevant data) and internal work. The potential effect of hypoxia on work efficiency was recently reviewed by Brutsaert [7], and for in-depth understanding the reader is referred to this publication. In brief, however, one of the better studies addressing the question investigated 186 men and women that were distinguished by ethnicity, place of birth, and testing environment. In this study there was no evidence of higher work efficiencies in Aymara [8]. Also when analyzing multiple data obtained from independent investigations no trend was observed toward higher work efficiencies in high-altitude natives from around the world [7]. This is in contrast to recent work performed by Marconi and co-workers [42]. They reported that Tibetan migrants born and living between 3500 and 4500 m had a better treadmill assessed locomotor economy (assessed at 1300 m) as compared to Nepali born and living in the Kathmandu valley [42]. It was concluded that the increased economy in Tibetans was due to chronic hypoxia induced metabolic adaptations, although these still need to be elucidated. It should be mentioned, however, that at least some of the differences may be attributed to differences in body mass between subject groups.

5.5 Skeletal Muscle Morphology and Energy Utilization

Although of hypothetical advantage, Aymara do not possess a higher fraction of oxidative skeletal muscle fibres, and also capillarization is not increased when compared to Danish lowlanders. At sea level, the Danish lowlanders had 4.0 ± 0.6 capillaries fibre^{-1}, and this ratio was not significantly changed with acclimatization. By comparison, the high-altitude natives had 2.4 ± 0.3 capillaries fibre^{-1}. Despite a smaller mean fibre area in the high-altitude natives, the capillary density tended to be smaller in high-altitude natives than in lowlanders, and the difference reached significance when comparing with the 8-week value for the lowlanders [37]. This is in agreement with other published data from a similar experimental population [19] and similar muscle fibre areas have been observed in high-altitude Sherpas [34]. Altogether, these findings indicate that, at 4100 m, angiogenesis is not necessary in order to preserve oxygen delivery to skeletal muscle. However, the reduced capillary density in Aymara could explain their lower oxygen conductance and O_2 extraction values.

A hypothesis presented by Hochachka [27] suggested a reduced glycolytic potential and tighter coupling between ATP production and utilization in Quechuas (another Andean native population). Hochachka and coworkers subsequently

observed that Quechuas accumulated less lactate in blood for a given amount of work when studied at sea level and that they had lower muscle LDH activity [28, 29]. However these observations have been questioned because the subjects were anaemic, and it was suggested that the metabolic adaptations observed were linked to anaemia rather than to genetic or developmental hypoxia-induced adaptations [21].

We have compared data obtained from skeletal muscle (*vastus lateralis*) biopsies at rest, during steady state exercise, and a few seconds after termination of VO_2max test in acclimatizing Danish lowlanders and Aymara high-altitude natives [55]. The general observation from this study was that the Aymara have higher glycolytic capacities than the Danes. After acclimatization to high altitude the Aymara and Danes arterial lactate and leg net lactate release were very similar at sub-maximal and maximal relative workloads. However, muscle lactate accumulation was nearly doubled in Aymara as compared to Danes. The density of skeletal muscle transporters involved in lactate and proton transport was similar in Aymara and Danes [33], which might explain the similar leg lactate release despite the much higher muscle lactate content. The Aymara total lactate dehydrogenase (LDH) activity and isoform pattern were not significantly different from Danes. However, this is not in disagreement with the higher muscle lactate accumulation in Aymara since the rate of lactate formation is not dependent on total activity or isoform of the equilibrium enzyme LDH but on the local intracellular pyruvate and NADH concentration [54].

5.6 Recovery from Exercise

The ability to recover from a given physical challenge affects subsequent performance. Analysis of ATP regeneration after fatiguing exercise has demonstrated that re-synthesis of ATP may be significantly slowed in hypoxia [24–26]. One could speculate that lifelong adaptation to high altitude would result in a faster recovery from a given exercise challenge. In Fig. 5.4 data are shown which were obtained at termination of a VO_2max test, and at minutes 3, 6, 10, 15, and 30 into passive recovery (sitting on a chair). The heart rate response to recovery was lowest in the Danish subjects at sea level and with acute hypoxic exposure (also breathing the hypoxic gas in recovery). After 2 and 8 weeks of acclimatization in the lowlanders, the heart rate response was elevated by approximately 15 beats min^{-1}, and was at this time point similar to the values found in the Aymara (Fig. 5.4a). Arterial values for typical variables used to describe fatigue such as lactate (Fig. 5.4b), pH (Fig. 5.4c), and K^+ (Fig. 5.4d) also show no deviation form the values obtained in the lowlanders. If anything, the arterial lactate concentrations are higher in the Aymara after 15 and 30 min of recovery as compared to the lowlanders. Thus, in regards to recovery following maximal aerobic exercise, Aymara are not superior as compared to Danish subjects.

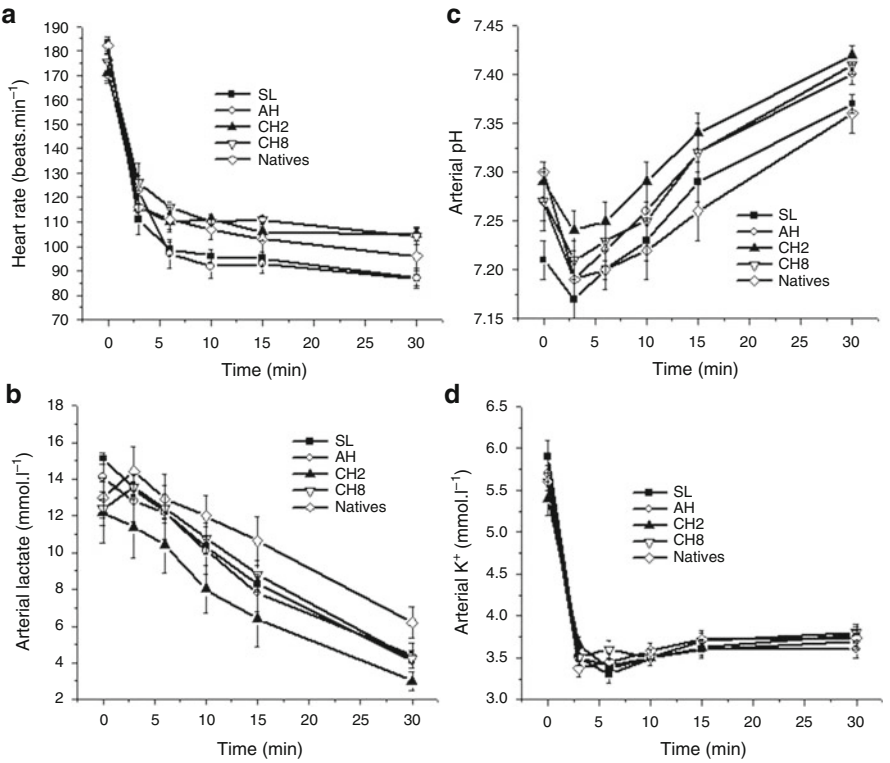

Fig. 5.4 Values obtained for (**a**) heart rate (beats min^{-1}), (**b**) arterial lactate (mmol l^{-1}), (**c**) Arterial pH (AU), and (**d**) Arterial K^+ (mmol l^{-1}) at termination of maximal incremental exercise test, and then after 3, 6, 10, 15, and 30 min of seated recovery. Abbreviations as for Fig. 5.1

5.7 Real Life Performance

The anecdotal reports of superior working abilities of high-altitude natives are many. However, they usually describe differences between full time porters (Sherpas) or miners (Aymara, Quechuas) with Caucasian scientists who likely are not remotely as physically active, and these reports should be regarded as nothing more than what they are—"anecdotal." It should also be remembered that the strong climbing Sherpas engaged by western tourist to climb Mount Everest do so because it's their occupation. Nonetheless it is a great accomplishment of the three Sherpas who have summited Everest ten times. Interestingly, however, Peter Athans and Dave Hahn have summited Everest seven times, but turned back on four occasions on technically difficult routes. Even more surprising perhaps, is that the speed record for climbing both the south and north face of Mount Everest without supplemental O_2 belongs to Europeans. There are obviously numerous accounts of astonishing accomplishments by high-altitude natives and by acclimatized lowlanders,

but whether one is "stronger" than the other is presently unsettled. It is, however, very clear that high-altitude natives posses unique physiological characteristics that need further investigation in the future.

5.8 Conclusion

If assuming an equal degree of training in high- and lowlanders (i.e. similar cardiac output) and if normalizing to body mass, it seems likely that high-altitude natives could have higher VO_2max values as compared to acclimatized lowlanders due to less desaturation at maximal exercise. At maximal exercise, however, fractional O_2 extraction was lower in the Aymara, and the a-vO_2 difference was similar in both populations. The lower extraction levels in the Aymara were associated with lower muscle O_2 conductance (a measure of muscle diffusion capacity). At any given submaximal exercise intensity, leg VO_2 was always of similar magnitude in both groups, but at maximal exercise the Danes had higher leg blood flow, and hence also higher maximum leg VO_2. With the induction of acute normoxia fractional arterial O_2 extraction fell in the highlanders, but remained unchanged in the Danes. Hence high-altitude Aymara seem to be more diffusion limited at the muscle level as compared to lowlanders. In conclusion, Aymara preserve SaO_2 very high during hypoxic exercise (likely due to a higher lung diffusion capacity), but the effect on VO_2max is reduced by a lower ability to extract O_2 at the muscle level.

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Chapter 6
Novel Insights into Cardiovascular Regulation in Patients with Chronic Mountain Sickness

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Abstract Studies of high-altitude populations, and in particular of maladapted subgroups, may provide important insight into underlying mechanisms involved in the pathogenesis of hypoxemia-related disease in general. Chronic mountain sickness (CMS) is a major public health problem in mountainous regions of the world affecting many millions of high-altitude dwellers. It is characterized by exaggerated chronic hypoxemia, erythrocytosis, and mild pulmonary hypertension. In later stages these patients often present with right heart failure and are predisposed to systemic cardiovascular disease, but the underlying mechanisms are poorly understood. Here, we present recent new data providing insight into underlying mechanisms that may cause these complications.

Keywords Chronic hypoxemia • Endothelial function • Arterial stiffness • Intima-media thickness • Oxidative stress • Nitric oxide

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6.1 Introduction

Due to its critical role in energy production, oxygen is essential for the survival of the cells. A reduction in tissue oxygen availability stimulates a complex series of adjustments, both at the cellular and at the systemic level. As adaptation to hypoxia proceeds, these responses are generally limited by inhibitory feedback mechanisms. There exist, however, situations in which, for unknown reasons, these feedback mechanisms are impaired, leading to exaggerated compensatory responses to hypoxia with detrimental consequences for the organism. The underlying mechanisms regulating the delicate balance between positive (self-limited) and negative (exaggerated) adjustments to hypoxia are incompletely understood.

Studies of populations permanently living at high-altitude, and in particular of maladapted subgroups, may provide important insight into adaptation/maladaptation to hypoxia and, even more importantly, into underlying mechanisms involved in the pathogenesis of hypoxemia-related disease states in general.

Pulmonary artery vasoconstriction and erythrocytosis are two hallmarks of the adaptation to hypoxia [1] and represent important defense mechanisms in high-altitude dwellers chronically exposed to ambient lack of oxygen. If exaggerated, however, these defense mechanisms may lead to pulmonary hypertension and erythrocytosis, two conditions characterizing chronic mountain sickness (CMS or Monge disease), a disease associated with high morbidity and mortality in high-altitude dwellers [2].

Studies of CMS patients, may, therefore, provide important new insight into underlying mechanisms involved in the pathogenesis of hypoxemia-related disease states in general. In the following we will focus on cardiovascular adaptation in healthy high-altitude dwellers and patients with CMS.

6.2 Short-Term Cardiovascular Adaptation to Hypoxia

Gain of altitude is associated with a decrease of barometric pressure. As a consequence, partial pressure of oxygen is also reduced and oxygen availability progressively decreases with increasing elevation. High altitude is defined as the terrestrial elevation at which the oxygen hemoglobin saturation decreases below 90 %. At moderate altitude this corresponds to an altitude of about 2500 m. Starting at this altitude, mainly via chemoreflexes involving the sympatho-adrenal system [3–5], hypoxemia triggers a series of pulmonary and cardiovascular adjustments intended to maintain an adequate oxygenation of the different organ systems.

In the heart, the major adjustments are an increase in heart rate, cardiac contractility, and cardiac output [6–8]. As a direct consequence of these adjustments, myocardial workload and oxygen demand increase. To respond to this increased

demand, the myocardium has to rely almost exclusively on coronary vasodilation and stimulation of coronary blood flow [9] because the coronary oxygen extraction is already submaximal at low altitude.

At the vascular level, the main initial adaptive mechanisms to altitude-induced hypoxemia are pulmonary-artery vasoconstriction and peripheral- and cerebral-artery vasodilation. Very rapidly, however, for yet unknown reasons, the direct hypoxia-induced vasodilation and the adrenal medullary response decrease, and systemic vascular resistance and blood pressure tend to increase [10, 11].

The hypoxia-mediated stimulation of the cardiovascular system reaches its maximum effects during the first few days of high-altitude exposure. Thereafter, probably related to the beneficial effects of subsequent vascular, respiratory, hematological, and muscular adaptation mechanisms, this stimulation tends to decrease to attain a new steady state.

During the initial phase of high-altitude adaptation, several additional phenomena may have important pathophysiological and clinical consequences [12]. First, while there is little intra-individual variability of the magnitude of the cardiovascular response during repeated high-altitude exposure, there is a large inter-individual variability of this response. Second, progressive stimulation of high-altitude adaptation mechanisms is not invariably associated with increasing benefits. Indeed, once these adjustments have reached their optimal effect, any further stimulation may have detrimental effects and induce specific high-altitude-related diseases such as high-altitude pulmonary edema (exaggerated pulmonary hypertension) and/or high-altitude cerebral edema (exaggerated cerebral vasodilation).

Pulmonary vasoconstriction, which occurs very rapidly after exposure to hypoxia, is intended to reduce blood flow through poorly ventilated alveoli. When self-limited, this vasoconstriction helps to match alveolar perfusion to ventilation. It thereby decreases the shunt effect and attenuates systemic hypoxemia. When sustained, however, hypoxic pulmonary vasoconstriction may have detrimental consequences, such as exaggerated pulmonary hypertension, right ventricular hypertrophy, and right heart failure, diseases which are associated with a high morbidity and mortality [1].

Studies in HAPE-prone subjects. Over the past decade our research focused on cardiopulmonary adjustments to short-term hypoxia in patients who are susceptible to high-altitude pulmonary edema (HAPE) and in subjects who had suffered from pathological events during the fetal/perinatal period, paradigms of exaggerated hypoxic pulmonary hypertension in otherwise healthy subjects [13, 14]. Several mechanisms contribute to exaggerated hypoxic pulmonary hypertension in these subjects.

Role of nitric oxide. There is abundant evidence that *endothelial dysfunction* related to defective endothelial NO synthesis is involved in the pathogenesis of hypoxic pulmonary hypertension in both animals and humans [15, 16]. Accordingly, *maneuvers intended to increase the vascular bioavailability of NO* are expected to have beneficial effects in this setting.

For example in humans, NO inhalation was significantly more effective in reducing pulmonary artery pressure both in young adults with a history of transient perinatal hypoxic pulmonary hypertension and in HAPE-prone subjects, than in control subjects

[16]. In line with this observation in humans, in rats sildenafil, an inhibitor of the phosphodiesterase 5 (the enzyme that hydrolyzes cGMP, the mediator of nitric oxide activity), prevents hypoxia-induced pulmonary hypertension, and decreases pulmonary artery pressure when given after chronic exposure to hypoxia [17].

There is evidence in humans, that in addition to pulmonary endothelial, respiratory epithelial NO also regulates pulmonary artery pressure [18]. In line with this hypothesis, we and others found that alveolar epithelial NO synthesis (as evidenced by lower respiratory tract exhaled NO) is defective in HAPE-prone subjects [19, 20]. These observations suggest that *defective pulmonary endothelial and epithelial NO synthesis contribute to exaggerated pulmonary hypertension during short-term hypoxia in humans*.

6.3 Role of Vasoconstrictor Mechanisms

In addition to relaxing factors, the endothelium also synthesizes vasoconstrictor factors. *Endothelin-1* (ET-1) is the most potent among them. To examine whether ET-1 may contribute to exaggerated pulmonary vasoconstriction in HAPE-prone subjects, we measured ET-1 plasma levels and pulmonary-artery pressure at low (580 m) and high altitude (4559 m), in HAPE-prone and HAPE-resistant mountaineers. We found that, at high altitude, ET-1 plasma levels were roughly 33 % higher in mountaineers prone to pulmonary edema than in those resistant to edema. Moreover, there was a direct relationship between the changes, from low to high altitude, in ET-1 plasma levels and systolic pulmonary artery pressure [21]. Thus, an *augmented release, or a reduced pulmonary clearance of ET-1, could also contribute to exaggerated hypoxic pulmonary hypertension in humans*. Interestingly, in human endothelial cells, NO inhibits hypoxia-induced stimulation of ET-1 gene expression and synthesis, suggesting that defective NO-synthesis and augmented ET-1 synthesis could be causally related.

Hypoxic pulmonary vasoconstriction may also be mediated by increased *neuronal sympathetic outflow*. We therefore measured muscle sympathetic nerve activity (MSNA) in HAPE-prone and -resistant subjects at high altitude. *MSNA was much higher in HAPE-prone subjects* than in controls. Moreover, there existed a positive correlation between pulmonary artery pressure and MSNA measured at 4559 m [3]. Noteworthy, we showed that inhibition of NO synthesis increases sympathetic nerve activity in humans [22] suggesting that defective NO-synthesis may not only contribute to augmented ET-1 synthesis but also to sympathetic activation.

6.4 Long-Term Adaptation/Maladaptation to Hypoxia

While the abovementioned studies provided important insight regarding the cardiopulmonary adjustments to short-term hypoxia, for the clinician, the long-term adjustments to chronic hypoxia are even more important. To this end, in collaboration

with Bolivian researchers at the Instituto Boliviano de Biologia de Altura, we have studied cardiopulmonary adaptation in high-altitude dwellers living in La Paz, Bolivia (3600–4000 m).

High-altitude populations are generally thought to be better protected from hypoxic insults than low-altitude natives [23, 24]. The mechanisms conferring such protection are incompletely understood and may differ from one high-altitude population to another [25]. Hypoxic pulmonary vasoconstriction is one of the major adjustments to ambient hypoxia. It is thought to match the pulmonary perfusion to the altitude-induced alteration of the alveolar oxygenation [26], and if exaggerated, it may lead to disease [27]. Compared to European or North-American low-altitude natives, hypoxic pulmonary hypertension has been reported to be attenuated in high-altitude populations [23, 24], but the underlying mechanism is not clear.

6.5 Role of Endothelial and Epithelial Nitric Oxide

Pulmonary respiratory nitric oxide (NO) may represent a candidate mechanism.

Recently, pulmonary exhaled NO was found to be higher in high-altitude dwellers than in low-altitude residents in the United States [28]. Moreover, exhaled NO explained part of the variation of pulmonary-artery pressure at high altitude in Tibetans [29]. Based on these data, it has been speculated that increased pulmonary respiratory NO synthesis may represent a specific high-altitude adaptation pattern which helps to maintain pulmonary-artery pressure in high-altitude populations within normal limits, but direct evidence for this speculation is lacking. We, therefore, measured the systolic right ventricular to right atrial pressure gradient and exhaled NO in healthy Bolivian high-altitude dwellers and Caucasian low-altitude natives living at high altitude (3600 m).

We found that in contrast to what might have been expected, pulmonary-artery pressure in Bolivian high-altitude natives was similar, and not lower than in well-adapted Caucasian low-altitude natives living at high altitude [30]. Furthermore, exhaled respiratory NO was also comparable in the two groups, and did not appear to be a determinant of pulmonary-artery pressure, because there existed no detectable relationship between these two variables in this relatively large group of healthy people living at high altitude. These findings provide no evidence that Bolivian high-altitude natives are better protected from hypoxic pulmonary hypertension than Caucasian low-altitude natives, and provide no evidence for a differential role of respiratory NO in the regulation of pulmonary-artery pressure at high altitude in these two populations.

The present findings differ from recent data reported in Tibetans, in whom exhaled NO explained a small fraction of the variation of pulmonary-artery pressure and cardiac index [29]. It is possible that this difference in the role of exhaled NO in cardiovascular regulation between Tibetans and Bolivians could be related to ethnicity, and thereby represent an additional example of a different adaptive

mechanism to high altitude among high-altitude populations [25]. Interestingly, when measured at the same altitude, pulmonary-artery pressure is lower in Tibetan than in Andean high-altitude residents [24], and it appears possible that respiratory NO may represent an underlying mechanism.

While these findings do not provide evidence for a role of respiratory epithelial NO in the regulation of pulmonary-artery pressure during long-term exposure to hypoxia in Bolivians and Caucasians, they do not exclude the possibility that pulmonary vascular endothelial NO may represent an important determinant of the long-term regulation of pulmonary-artery pressure in high-altitude dwellers. Exhaled NO does not reflect pulmonary vascular endothelial NO synthesis [31, 32], and there is evidence in humans that endothelial nitric oxide synthase (eNOS) polymorphisms associated with augmented vascular NO synthesis are related with attenuated hypoxic pulmonary hypertension [33], whereas eNOS polymorphisms associated with defective NO synthesis predispose to exaggerated hypoxic pulmonary vasoconstriction [34]. Consistent with this concept, recent data show increased plasma concentration of bioactive NO products associated with a higher systemic blood flow in Tibetans [35].

Finally, the present findings in chronically hypoxic Caucasian high-altitude dwellers contrast with observations made during short-term exposure to hypoxia, where low levels of exhaled NO were associated with exaggerated hypoxic pulmonary hypertension [19, 20]. Taken together, these earlier and the present observations could be consistent with the hypothesis that in Caucasians, respiratory epithelial NO may be a determinant of the pulmonary-artery pressure during short-term, but not during long-term exposure to hypoxia.

Epidemiological studies suggest that adverse events in utero may predispose to cardiovascular disease in adulthood [36], but the underlying mechanisms are unknown, and there is no information with regard to the pulmonary circulation. Preeclampsia is the most frequent complication of pregnancy. It is associated with endothelial dysfunction in the mother, which is related to the release of circulating vasculotoxic factors and the induction of augmented oxidative stress by the diseased placenta [37]. We speculated that these circulating factors may pass the placental barrier and leave a persistent defect in the circulation of the offspring that may predispose to a pathological response later in life. The hypoxia associated with high-altitude exposure is expected to facilitate the detection of this problem because it induces exaggerated pulmonary hypertension in persons displaying endothelial dysfunction [38]. In experimental animal models, fetal insults are associated with a persistent increase in oxidative stress in the offspring [39]. Here, we found that offspring of women with preeclampsia display exaggerated hypoxic pulmonary hypertension and *increased oxidative stress* related to pulmonary artery pressure, suggesting that it may represent an underlying mechanism [40]. In line with this concept, exaggerated oxidative stress induced by restrictive diet during pregnancy in mice predisposes the offspring to exaggerated hypoxic pulmonary hypertension and right ventricular hypertrophy [41].

6.6 Exaggerated Exercise-Induced Pulmonary Hypertension in CMS

Pulmonary hypertension in CMS is relatively mild [40, 41]. It is, however, a leading cause of morbidity and mortality and its underlying mechanisms are not known [42]. Exercise is known to increase pulmonary-artery pressure [43, 44]. The exercise-induced increase in pulmonary artery pressure is exaggerated by pre-existing pulmonary hypertension [45]. Moreover, hypoxemia, which is universally associated with high-altitude exposure, is also known to increase exercise-induced pulmonary hypertension [46]. We speculated that light-to-moderate exercise associated with daily activities induces a much larger increase in pulmonary-artery pressure in patients with CMS than in control subjects.

To test this hypothesis, we measured pulmonary-artery pressure at rest and during mild semi-supine bicycle exercise in patients suffering from CMS and normal control subjects who were all born and permanently living at 3600–4000 m [47]. To discriminate between anatomical and functional defects, we measured the carbon monoxide diffusion capacity (a proxy of the extent of the pulmonary microcirculation) and we tested the effects of nitric oxide inhalation on pulmonary-artery pressure in the two groups.

As expected, we observed that at rest, pulmonary artery pressure was slightly, but significantly higher in patients with CMS than in controls (Fig. 6.1a) [47].

However, during light bicycle exercise at 50 W, the increase in pulmonary-artery pressure was almost twice as large in patients than in controls. Thus, during mild exercise, pulmonary-artery pressure was much higher in patients with CMS than in controls (Fig. 6.1b) and the pressure gradient difference between the 2 groups was more than three times greater than at rest [47].

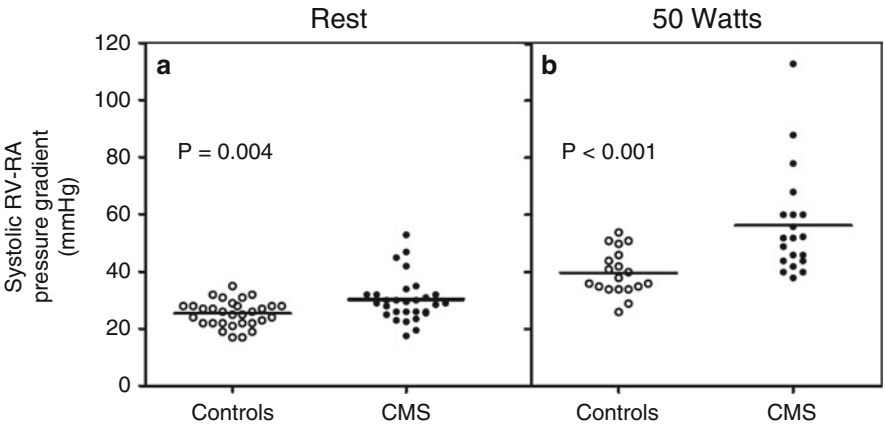

Fig. 6.1 Systolic right ventricular (RV) to right atrial (RA) pressure gradient at rest (**a**) and during mild exercise (50 W, **b**) in patients with chronic mountain sickness (CMS) and controls at 3600 m (adapted form ref. [49])

During exercise, the arterial oxygen saturation decreased significantly in both groups, but the exercise-induced decrease tended to be greater in patients than in controls. During nitric oxide inhalation, pulmonary-artery pressure decreased significantly and comparably in the 2 groups, but remained significantly higher in patients than in controls.

These data represent the first direct comparisons of pulmonary-artery pressure at rest and during mild exercise between Andean high-altitude dwellers suffering from CMS and healthy control subjects born and living at the same altitude. They indicate that in patients with CMS, measurements at rest greatly underestimate pulmonary artery pressure during daily activity and may explain the clinical observation that right ventricular failure is a leading cause of morbidity and mortality in these patients [42].

Structural vascular defects appear to contribute to exaggerated pulmonary hypertension in CMS as evidenced by the persistence of significantly higher pulmonary artery pressure during NO-inhalation in patients with CMS. In line with this hypothesis, after descent to low altitude, pulmonary artery pressure takes several years to normalize in patients with CMS [42]. The mechanism causing this structural defect is not known. Exaggerated chronic hypoxemia, possibly related to a blunted hypoxic ventilatory response [42, 48] may play a significant role (see below).

6.7 Exercise-Induced Pulmonary Interstitial Fluid Accumulation in CMS Patients

Circumstantial evidence suggests that in healthy subjects exaggerated pulmonary hypertension may cause lung fluid accumulation [49]. Chest sonography allows quantifying extra-vascular lung water by assessing ultrasound lung comets (ULCs) originating from water-thickened interlobular septa [50–52]. We speculated that in patients with CMS exercise intolerance is caused by pulmonary fluid accumulation related to pulmonary hypertension-induced capillary stress failure and/or left ventricular dysfunction. To test for this hypothesis, we assessed ULCs, pulmonary artery pressure, and left ventricular function in patients with CMS and control subjects at rest and during exercise at 3600 m. To evaluate the role of hypoxemia in this setting we repeated these measurements during a 1-h inhalation of 100 % oxygen.

Our data show that the exaggerated exercise-induced increase in pulmonary artery pressure is associated with pulmonary interstitial fluid accumulation in patients with CMS [53]. There existed a direct relationship between pulmonary artery pressure and ULCs during exercise and pulmonary interstitial fluid accumulation was associated with marked hypoxemia. Oxygen inhalation attenuated the exercise-induced pulmonary hypertension and interstitial lung fluid accumulation in patients with CMS [53].

These data show for the first time that exercise in pulmonary hypertension may induce rapid interstitial lung fluid accumulation and hypoxemia in patients with CMS. We speculate that this problem contributes to exercise intolerance in patients with CMS.

6.8 Systemic Vascular Function in CMS

While in the more advanced stages, patients with CMS often present functional and structural changes of the pulmonary circulation, pulmonary hypertension, and right heart failure, there is little information on the systemic circulation. In patients suffering from diseases associated with chronic hypoxemia at low altitude systemic vascular function is altered [54] and cardiovascular morbidity and mortality increased [55, 56]. We hypothesized that patients with CMS, in addition to pulmonary vascular dysfunction, also display systemic vascular dysfunction that may predispose them to increased cardiovascular morbidity. To test this hypothesis, we assessed systemic vascular function in patients with CMS and control subjects living at high altitude using the following techniques.

6.9 Assessment of Subclinical Atherosclerosis in the Systemic Circulation

There is abundant evidence that atherosclerosis starts many years before the first clinical events (Fig. 6.2).

Several noninvasive techniques have been developed to evaluate the (subclinical) atherosclerotic burden before its clinical manifestation. Here we will very

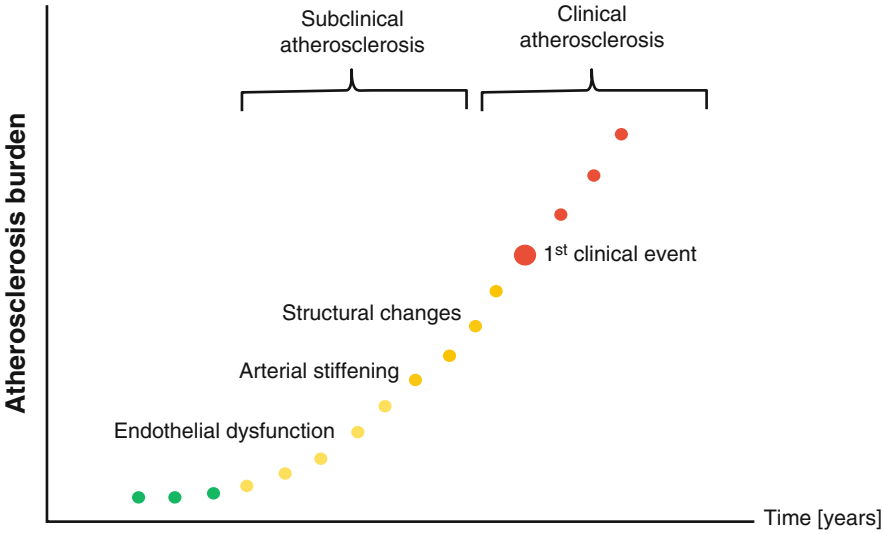

Fig. 6.2 Endothelial dysfunction, arterial stiffening, and structural alterations are progressive subclinical atherosclerotic changes involving the systemic circulation

briefly describe three methods, which are considered the "gold standard" of non-invasive assessment of subclinical atherosclerosis.

6.9.1 Flow-Mediated Vasodilation

Alterations of the endothelium play a central role in the development of atherosclerosis and precede the structural and morphological changes of the vessel [57, 58]. Systemic conduit artery endothelial function can be assessed by determining the increase of the brachial artery diameter evoked by reactive hyperemia (flow-mediated vasodilation, FMD) using high-resolution ultrasound and automatic wall tracking software. The brachial artery is identified above the antecubital fossa with a high-resolution ultrasound device and a high frequency (7–10 MHz) linear array probe. The ultrasound probe is then fixed in a stereotactic clamp with micrometer movement capabilities (Fig. 6.3, Panel a) and Doppler flow is recorded continuously throughout the study (Fig. 6.3, Panel b). After 1 min of baseline measurements, a pressure cuff placed around the forearm is inflated to 250 mmHg for 5 min. After deflation of the cuff, the hyperemia-induced changes of brachial artery diameter and flow are continuously measured (Fig. 6.3, Panel c). FMD is expressed as the maximal percentage change in vessel diameter from baseline.

Endothelial function assessed by FMD has been shown to be negatively affected by cardiovascular risk factors [59], to be related to structural arterial disease [60], and to cardiovascular outcome [61, 62, 63, 64].

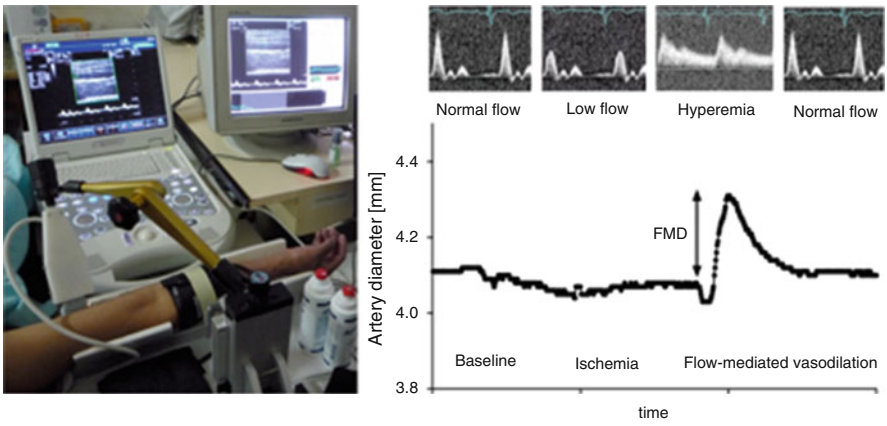

Fig. 6.3 Setup for the assessment of flow-mediated dilation (FMD, panel **a**). After placing a cuff pressure around the forearm, the ultrasound probe is fixed in a stereotactic clamp with micrometer movement capabilities. Doppler flow (Panel **b**) and artery diameter (Panel **c**) are continuously recorded during the data acquisition

6.9.2 Pulse Wave Velocity

In addition to endothelial function, stiffening of the vasculature also occurs during the development of atherosclerosis and represents an independent predictor of cardiovascular risk. Carotid-femoral pulse wave velocity (PWV) is considered the gold standard measurement of arterial stiffness and very recently carotid-femoral PWV has been demonstrated to be a strong predictor of future CV events and all-cause mortality [65]. The independent predictive value of aortic stiffness has been demonstrated after adjustment to classical CV risk factors, including arterial blood pressure. In line with this concept, aortic stiffness retains its predictive value for cardiovascular events after adjustment to the Framingham risk score, suggesting that aortic stiffness has an added value to a combination of CV risk factors [66].

6.9.3 Carotid Intima-Media Thickness

Finally, ultrasound measurement of carotid intima-media thickness (IMT) can be considered the most widely noninvasive method, used to assess structural changes of the systemic circulation. IMT is considered as a surrogate marker for subclinical atherosclerosis. Increased IMT is associated with coronary artery disease and is predictive of future cardiovascular events, including stroke and myocardial infarction [67]. IMT has proved sufficiently robust and reproducible in the evaluation of changes over time to serve as an end point in clinical trials assessing the impact of antihypertensive and lipid-lowering medications on cardiovascular risk [68, 69].

6.10 Patients with CMS and Chronically Hypoxemic High-Altitude Dwellers Without CMS Display Systemic Vascular Dysfunction

In addition to altered function of the pulmonary circulation, in a very recent study, we found that *patients with CMS without additional cardiovascular risk factors displayed marked systemic vascular dysfunction* [70], as evidenced by impaired flow-mediated dilation, increased vascular stiffness, and carotid intima-media thickness (Fig. 6.4).

The impairment of FMD in patients with CMS was related to endothelial dysfunction (Fig. 6.4a), since endothelial-independent vasodilation evoked by nitroglycerine was similar in the patients and control subjects (Fig. 6.4b).

In addition to endothelial dysfunction, stiffening of the vasculature also occurs during development of atherosclerosis and represents an independent predictor of cardiovascular risk [71]. We found that in patients with CMS several proxies of arterial stiffness were significantly increased compared to controls (Fig. 6.4c). Moreover

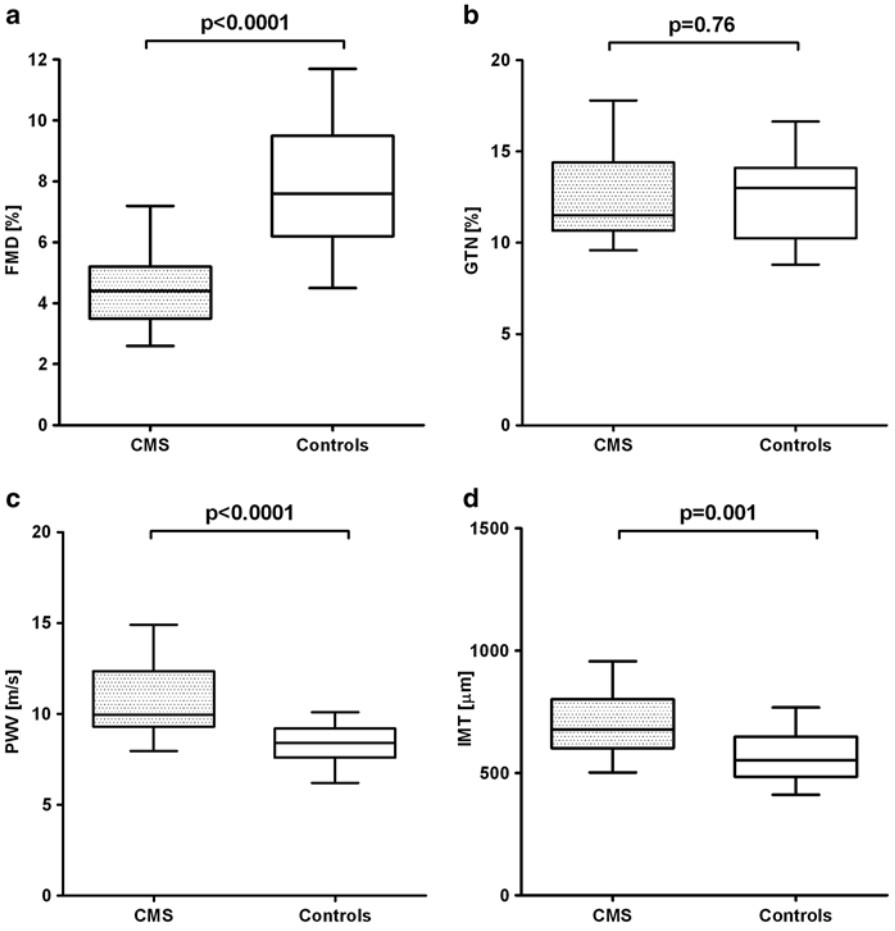

Fig. 6.4 Flow-mediated dilation (FMD, Panel **a**), nitroglycerine-induced endothelium-independent dilation (GTN, Panel **b**), carotid-femoral pulse wave velocity (PWV, Panel **c**) and carotid intima-media thickness (IMT, Panel **d**) in 23 patients with chronic mountain sickness (CMS) and 27 control subjects at 3600 m. Horizontal lines represent the median; boxes, 25th to 75th percentiles; and T bars min to max. (Adapted from Rimoldi et al. [70])

carotid IMT was also significantly increased in patients with CMS (Fig. 6.4d) directly demonstrating structural alterations of the systemic circulation in these patients. These vascular alterations in CMS patients were of similar magnitude as those reported in asymptomatic subjects presenting with two to three classical cardiovascular risk factors [61]. In line with these observations, there is evidence from uncontrolled studies that patients with CMS are at increased risk for systemic cardiovascular disease [72].

An interesting finding of our study was that *vascular dysfunction was not limited to patients with CMS but also present in chronically hypoxemic control subjects* (SaO_2<90 %) living at high altitude. In contrast, in normoxemic control subjects

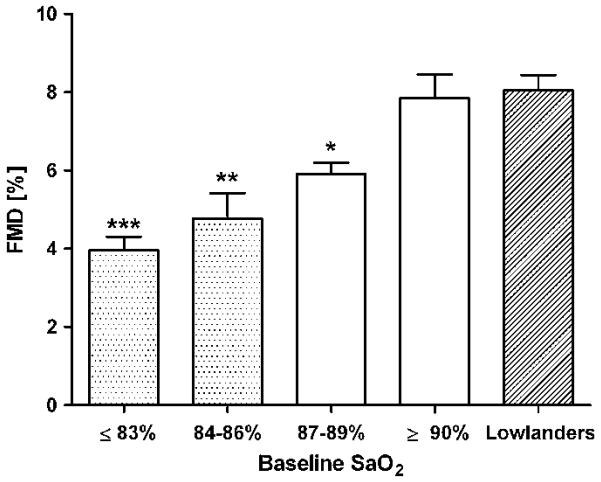

Fig. 6.5 Flow-mediated dilation (FMD) depending on baseline arterial oxygen saturation (SaO_2) in 16 patients with chronic mountain sickness (CMS), in 17 control subjects living at high altitude and in 22 healthy control subjects living at low altitude. Subjects were classified in quartiles according to SaO_2. The two lower quartiles included all subjects with CMS and four control subjects whereas the two higher quartiles included only controls. FMD in the subgroup of the highest quartile was significantly greater ($P<0.0001$) than in the 3 other subgroups (Dunnett's post-hoc test: $*P<0.05$, $**P<0.01$, $***P<0.001$). FMD in the highest quartile ($SaO_2 \geq 90\,\%$) was similar to FMD in healthy control subjects living at low altitude. Data are mean ± SEM. (Adapted from Rimoldi et al.[70])

($SaO_2 \geq 90\,\%$), FMD was normal and comparable to age-matched control subjects living at low altitude (Fig. 6.5), suggesting that there exists a cutoff value for the detrimental vascular effects of chronic hypoxemia.

Interestingly, oxygen inhalation improved FMD in both patients with CMS and hypoxemic control subjects, but had no detectable effect in normoxemic ($SaO_2 \geq 90\,\%$) control subjects suggesting that *vascular dysfunction in CMS patients and hypoxemic controls was, at least in part related to hypoxemia*. In line with this speculation, there existed a direct relationship between FMD and arterial oxygen saturation (Fig. 6.6).

Finally, we found that NO_2-plasma concentration was lower in CMS patients than in controls and that there was a significant relationship between nitrite plasma concentration and FMD, suggesting that *impaired nitric oxide bioavailability contributes to vascular dysfunction in chronically hypoxemic humans* [73, 74]. Interestingly and in line with this observation, very recently we showed that oxidative-nitrosative stress underpin systemic vascular dysfunction in CMS patients [75]

In conclusion, these findings provide the first evidence that patients with CMS without additional cardiovascular risk factors display functional and morphological alterations of the systemic circulation. Moreover, since vascular alterations were also found in hypoxemic high-altitude dwellers who did not suffer from CMS and were partially reversible during oxygen inhalation, our findings suggest that chronic hypoxemia may represent an underlying mechanism.

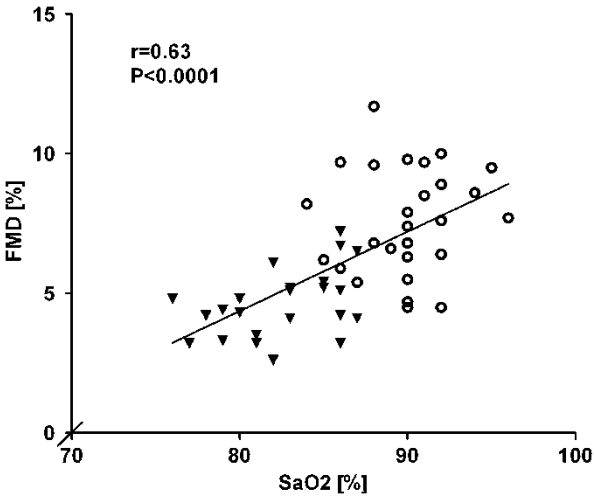

Fig. 6.6 Relationship between arterial oxygen saturation (SaO_2) and flow-mediated dilation (FMD) in 23 patients with chronic mountain sickness (*inverted filled triangle*) and 27 control subjects (*open circle*) at 3600 m. (Adapted from Rimoldi et al. [70])

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Chapter 7
Why Are High Altitude Natives So Strong at High Altitude? Nature vs. Nurture: Genetic Factors vs. Growth and Development

Tom Brutsaert

Abstract Among high-altitude natives there is evidence of a general hypoxia tolerance leading to enhanced performance and/or increased capacity in several important domains. These domains likely include an enhanced physical work capacity, an enhanced reproductive capacity, and an ability to resist several common pathologies of chronic high-altitude exposure. The "strength" of the high-altitude native in this regard may have both a developmental and a genetic basis, although there is better evidence for the former (developmental effects) than for the latter. For example, early-life hypoxia exposure clearly results in lung growth and remodeling leading to an increased O_2 diffusing capacity in adulthood. Genetic research has yet to reveal a population genetic basis for enhanced capacity in high-altitude natives, but several traits are clearly under genetic control in Andean and Tibetan populations e.g., resting and exercise arterial O_2 saturation (SaO_2). This chapter reviews the effects of nature and nurture on traits that are relevant to the process of gas exchange, including pulmonary volumes and diffusion capacity, the maximal oxygen consumption (VO_2max), the SaO_2, and the alveolar-arterial oxygen partial pressure difference ($A\text{-}aDO_2$) during exercise.

Keywords Hypoxia • Developmental response • Genetic adaptation • Gas exchange • Exercise • Pulmonary • Andes • Himalayas

7.1 Introduction

In a 1571 chronicle by the Spanish functionary, Juan Polo De Ondergardo, there is an early and clear reference to the idea of high-altitude native "strength." Regarding the physical work capacity of the Cusco (i.e., Inca) high-altitude native, De Ondergardo remarked that: "It is a thing worthy of the greatest wonderment that

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[…] they grow up to be very strong, ready for any work and tireless in walking, not only on level ground but also over the roughest and steepest roads" (quoted in [60]).

The question of strength still provokes some wonderment, not just for physical work capacity but also for the apparent strength of high-altitude natives in other functional domains. For example, Andeans and Tibetans may have enhanced reproductive capacity [61, 75, 82] and the ability to resist chronic or subacute altitude illnesses [63, 67]. Underlying these strengths (in part) may be an enhanced capacity for pulmonary gas exchange [55, 68, 77, 83]. This paper considers the origin of enhanced pulmonary gas exchange, including the evidence for and against developmental and genetic effects. A high altitude developmental effect is defined here as an irreversible trait acquired during the process of growth and development that reflects cellular or organ system plasticity in response to early-life hypoxia exposure. As will be described, developmental effects of this type are ubiquitous in hypoxia and have been especially well documented for the pulmonary system [16, 33, 46, 47, 49] and the ventilatory control system [4, 21, 34]. A genetic effect is defined here as a trait under at least partial genetic control that reflects a unique aspect of the population genetic structure of an indigenous high-altitude native population. A broad literature will be reviewed focusing on traits that are relevant to the process of gas exchange, including pulmonary volumes, the maximal oxygen consumption (VO_2max), and direct or indirect measures of gas exchange efficiency especially during exercise.

7.2 Pulmonary Volumes

High-altitude resident populations worldwide have larger mean pulmonary volumes compared to sea-level controls, including the total lung volume, the forced vital capacity (FVC), and the residual volume (RV). Also, diffusion capacities are higher in high-altitude resident populations [22, 25, 26, 48, 68]. These differences are apparent in both developmentally exposed individuals of lowland ancestry and in indigenous altitude groups [16, 30, 72]. Recent studies also show large differences in pulmonary volumes between Andean highland residents (above 3500 m) compared to genetically matched controls born and raised in lowland South American cities [10, 16]. Thus, lung growth during gestation or early post-natal development must be an important factor. The clearest line of evidence to support this hypothesis comes from controlled animal studies. Rodents raised in hypoxia show enhanced alveolar growth and remodeling compared to sea-level raised animals [3, 18, 19, 46, 69, 70]. Similarly, dogs raised in hypoxia show increased pulmonary volume and increased resting diffusion capacity for carbon monoxide (DLCO) [43, 49]. These effects can only be induced by exposure of dog pups during the developmental period and they have been attributed to larger alveolar tissue volume and increased surface area for diffusion. In contrast, long-term exposure of adult dogs to hypoxia (>3 years) does not affect the morphology or physiology of

the pulmonary system [43, 49]. The recent study by Hsia et al. [47] in fox-hounds raised at 3800 m assessed pulmonary gas exchange during exercise using the multiple inert gas elimination technique. Compared to littermates raised at sea-level, altitude-raised dogs had enhanced gas exchange efficiency at altitude. That is, during exercise, the proportion of the alveolar-arterial oxygen partial pressure difference ($A\text{-}aDO_2$) attributable to diffusion limitation was smaller and the altitude-raised dogs had 12–15 % larger lung diffusion capacity for O_2. This study is important because it links developmental effects on lung volume with gas exchange effects that plausibly explain the high work capacity of the high-altitude native. The idea that larger lungs lead to enhanced work capacity has been somewhat controversial. For example, one study in the Himalayas reported a positive correlation between forced vital capacity and VO_2max [72], while another study in the Andes failed to find this association within study groups [13, 17].

Whether the large lungs of indigenous high-altitude natives have a population genetic basis is unknown. Several studies have argued that this is the case [30, 38], but the direct lines of evidence are weak. Andean studies using a migrant study design report significantly larger lung volumes normalized for body size in Andeans vs. European controls born and raised at high altitude (see Fig. 7.1). However, the group differences are small and must be interpreted with reference to the large body size differences that exist between Andeans and Europeans, as Fig. 7.1 shows for FVC-by-stature. A reasonable conclusion is that the putative genetic effect on pulmonary volumes (if it exists at all) is small and is dwarfed by the aforementioned developmental effect.

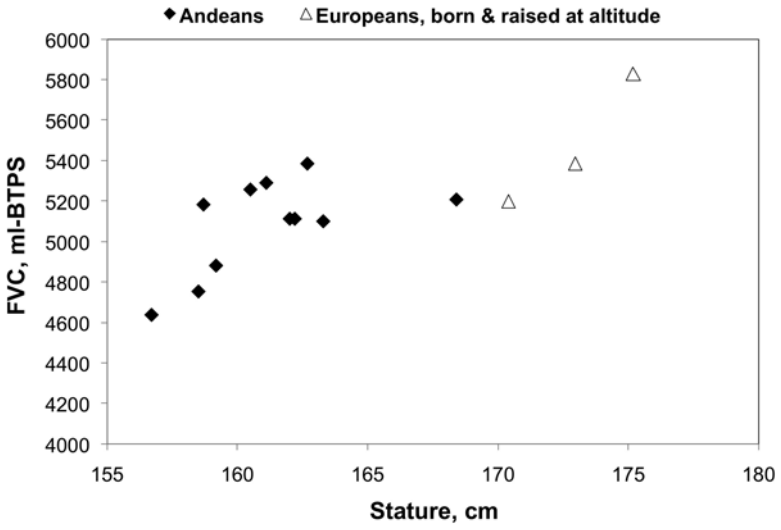

Fig. 7.1 Mean values of forced vital capacity (FVC) by stature from studies conducted in the Andes. Andean high-altitude natives are compared with Europeans who were born and raised at similar altitudes. Data are from the following sources [9, 16, 25, 30, 41, 45, 48, 50, 68, 80]

A few studies have directly addressed the genetic hypothesis by using skin-pigmentation as a proxy for Andean ancestry. The assumption of this approach is that darker skin at the level of the individual reflects a higher proportion of Native American ancestors. Interestingly, three studies to date show significant positive associations between skin pigmentation and pulmonary volumes. In the study by Frisancho et al. [30], the residual lung volume (RV) and total lung volume (both expressed as ml/m^2 surface area) were negatively correlated to skin reflectance in male high altitude rural ($R=-0.52$ for RV) and urban Bolivian Aymara (males, $R=-0.32$ for RV; females, $R=-0.42$ for RV). Skin reflectance is a measure of the light reflected or absorbed by the skin, and low reflectance indicates dark pigmentation. In the study by Greksa et al. [38], the first principle component of 6 skin reflectance measures was negatively correlated to TLV ($R=-0.21$), FVC ($R=-0.12$), and RV ($R=-0.21$), adjusting for stature, in males of Aymara ancestry residing in La Paz, Bolivia. In a study by Brutsaert et al. [10], the partial correlation between a measure of melanin content and FVC in highland born Peruvians was $R=0.27$, adjusting for age and stature. In the latter study, FVCs were 5–10 % higher in subjects with the darkest vs. lightest skin color. Unfortunately, the same study showed no association of pulmonary volumes with a molecular genetic estimate of Native American ancestry proportion. The newer molecular genetic estimates of ancestry use a panel of molecular markers that are highly informative for individual ancestry when parental populations are clearly defined vis-à-vis a documented history of population admixture [71]. These methods must be considered superior to the proxy method of using skin pigmentation. Thus, the association of skin pigmentation to pulmonary volume in the Andes may have little to do with ancestry, but rather may reflect a correlated response of both traits to an unknown environmental or genetic factor/mechanism.

7.3 Aerobic Capacity (VO_2max)

There is little doubt that high-altitude natives have high average aerobic capacity. A number of studies have documented the relatively high VO_2max (ml min^{-1} kg^{-1}) in both Andean [2, 28, 32, 52, 58, 59] and Himalayan natives [35, 36, 72, 83]. These data are presented in Fig. 7.2 as study mean values (VO_2max, ml min^{-1} kg^{-1}) by altitude compared to a sample of VO_2max mean values from sea-level natives who were measured in hypoxia. The lowland native values are updated from Buskirk et al. [20] who originally described the VO_2max decrement with increasing hypoxia. VO_2max values for Andean males are significantly higher than the lowland reference values at altitudes between 2500 and 5000 m ($P=0.002$). Unfortunately, for natives of the Himalaya, a small sample size precludes an adequate meta-analysis. It should also be noted that high-altitude natives experience small decreases in VO_2max when exposed to increasing hypoxia with decrements typically at ~30–80 % of the decrement seen lowland comparison groups [1, 11, 28, 32, 44, 57, 73, 76, 78].

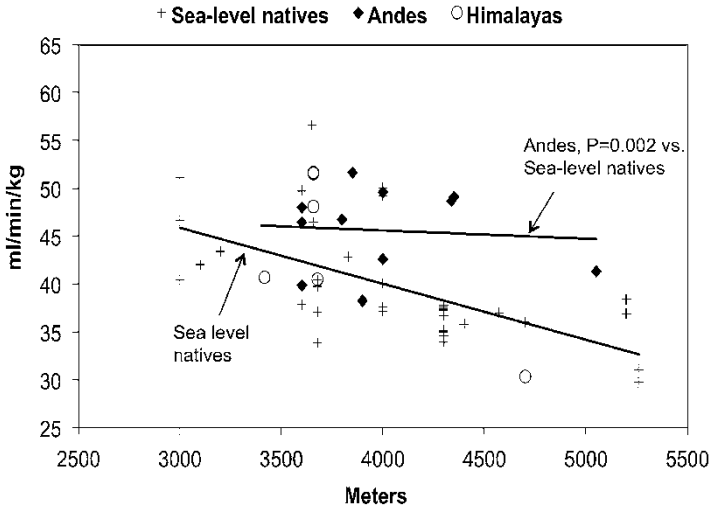

Fig. 7.2 Mean values of VO_2max by altitude for native Andean and Himalayan males, and for sea-level natives exposed to hypobaric hypoxia. Sea-level data are updated from Buskirk et al. [20] (See Brutsaert et al. [12]). Andean and Himalayan mean values are from the literature [10, 17, 24, 31, 32, 35, 36, 40, 52, 57–59, 62, 68, 76]

The origin of high VO_2max in highland natives is difficult to ascertain given the possibility of both genetic and developmental effects, as well as the confounding influence of training effects on aerobic capacity. Thus, the literature is conflicted. For example, in the Andes, several previous studies have argued for a genetic basis [11, 31], while others have emphasized developmental and/or covariate effects over genetic effects [17, 39, 42]. In support of the developmental hypothesis, Frisancho et al. studies of European migrants show that an early age of migration to altitude during childhood results in a higher VO_2max [31, 32]. In support of the genetic hypothesis, Brutsaert et al. [11, 31] showed a negative correlation between the VO_2max decrement and the proportion of Native American ancestry in an admixed sample of Peruvian males who were transiently exposed to hypoxia. Unfortunately, the association detected by Brutsaert et al. was relatively weak and the problem of physical activity level was not addressed. The latter has been a recurrent problem in the Andes where most Native American study populations are very physically active due to a subsistence agricultural lifestyle [51].

7.4 Gas Exchange Efficiency

Three studies have measured arterial gas partial pressures during exercise in highland natives. Two of these were conducted with Aymara [55, 77] and the other with Tibetans [83]. In general all show a relative exercise hypoventilation (i.e., higher

$PaCO_2$) and enhanced gas exchange efficiency (lower A-aDO_2) in high-altitude natives compared to acclimatized lowlanders. The study by Zhuang et al. [83] showed that Tibetans compared to Han Chinese at 3658 m had lower exercise ventilation, lower ventilatory equivalent for O_2 (V_E/VO_2), and higher $PaCO_2$ (2–4 mmHg). Also, the A-aDO_2 during exercise was about half the value in acclimatized Han Chinese with the difference increasing between groups as VO_2 or power output increased. The study by Wagner et al. [77], at the relatively extreme altitude of 5260 m, showed Aymara males to have markedly lower ventilation, higher $PaCO_2$ (~8 mmHg), and lower (A-a)DO_2, especially as exercise intensity increased i.e., 5.3 vs. 10.5 mmHg at maximal exercise. These differences were all the more surprising as the Aymara subjects were only transiently exposed to 5260 m (up from their place of residence at ~4000 m), and thus were not acclimatized to the higher altitude. Wagner et al. also calculated that the O_2 diffusing capacity during maximal exercise was 40% higher in Aymara, consistent with the aforementioned studies that document higher diffusion capacities for highland natives at rest [65, 74]. The recent study by Lundby et al. [55] at 4100 m showed remarkably low A-aDO_2 (1–2 mmHg) at rest and during exercise to maximum in Aymara compared to Europeans with 8 weeks of acclimatization. An earlier study by Dempsey et al. [26] makes clear the importance of developmental effects to explain the Andean and Tibetan advantage. Dempsey et al. compared sojourners to high altitude with residents of Leadville, Colorado at 3094 m. The Leadville residents maintained $PaCO_2$ at or above resting levels except during the highest level of work, and had smaller A-aDO_2 especially as exercise intensity increased. Although the sample sizes in the Dempsey study were relatively small, the effect of developmental exposure on the A-aDO_2 was relatively large. Indeed, the difference in A-aDO_2 between the Leadville natives and the lowland controls was about the same as the difference between the Tibetans vs. Han Chinese [83] and the Aymara vs. lowland Europeans [77].

7.5 The Ventilatory Equivalent (V_E/VO_2) and Arterial Oxygen Saturation (SAO_2)

The VE/VO_2 may be a useful proxy measure of gas exchange efficiency in studies where blood gases were not measured [55]. Most studies of high-altitude natives report lower absolute ventilation (V_E, l/min) and/or lower ventilatory equivalent (V_E/VO_2) during exercise, consistent with the lower A-aDO_2 described above. These include nearly all of the studies conducted in the Andes [13, 52, 68, 77], and most [27, 35, 53, 54, 83] but not all [72] studies in the Himalaya. One of these, a migrant study by Brutsaert et al. [13], provides evidence that developmental factors determine the VE/VO_2 during submaximal exercise. In that study, Aymara males and females had significantly lower V_E/VO_2 during exercise compared to acclimatized European sojourners at 3600 m. However, the V_E/VO_2 was similar between Andeans and Europeans who were born and raised at altitude showing the importance of growth and development in hypoxia. It should be noted that evidence for a developmental

effect does not disqualify the possibility of a genetic effect. Indeed, in Peruvians, a high proportion of Quechua ancestry is strongly associated with lower exercise V_E, V_E/VO_2, and V_E/VCO_2 during exercise in subjects born and raised at sea-level and acutely exposed to 4380 m [15]. Thus, the enhanced gas exchange efficiency of the Andean native could be the result of both a developmental response to hypoxia and a unique population genetic background.

The ability to maintain SaO_2 during exercise may also be a useful proxy measure of gas exchange efficiency, although clearly other factors impact SaO_2 including the hemoglobin (Hb) oxygen affinity. An early study by Winslow et al. [81] reported no differences in the pH adjusted Hb-O_2 affinity of Andeans, and a more recent study by Lundby et al. [56] reported no differences in the standard arterial P_{50} or in vivo (muscle) P_{50} of Andeans.)Nevertheless, a large number of studies report higher or comparable SaO_2 despite lower ventilation at rest and during exercise in Andean and/or Himalayan high-altitude natives compared to acclimatized lowland controls [13, 23, 29, 35, 36, 56, 57, 68, 72, 77, 83]. Of these, two migrant studies showed higher exercise SaO_2s in Aymara compared to Europeans born and raised at 3600 m, suggesting a genetic effect [13, 31]. Interestingly, in both studies there were no significant differences in SaO_2 between European sojourners and Europeans born and raised at altitude. Similarly, the study by Dempsey et al. [26] shows no difference in SaO_2 by developmental exposure to hypoxia.

Quantitative genetic and candidate gene work directly supports the hypothesis that resting and exercise SaO_2s are under strong genetic control in high-altitude natives. In Tibetans, quantitative trait analysis suggests the presence of a major gene conferring a large (5–6 % point) increase in resting SaO_2 [6, 7]. Further, Tibetan women with a high likelihood of possessing one to two of the putative alleles for the high SaO_2 phenotype have more surviving children [5]. The latter provides evidence that hypoxia is acting as selective agent on the locus for SaO_2 by the mechanism of higher infant survival in Tibetan women with the "high" SaO_2 genotypes. In the Andes, a recent candidate gene study now shows the association of the angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism with exercise SaO_2 at 4380 m [8]. In that study, the ACE I-allele predicted a 1–2 % point higher submaximal exercise SaO_2 at altitude in both sea-level and altitude born Peruvians of mostly Quechua ancestry.

While the studies referenced above clearly show that SaO_2 has a genetic basis, it should be emphasized that these studies do not provide direct evidence of genetic adaptation in high-altitude natives. In the case of Tibetans, quantitative trait analysis (by itself) cannot reveal specific genes, genomic regions, or gene products. Furthermore, recent large-scale hematology surveys from Qinghai China report no differences in resting SaO_2 between large cohorts of Tibetans vs. Han Chinese who were born and raised at altitude [79]. In the case of the Andes, the critical point is that the)ACE I-allele is a common genetic variant which is likely present in all human populations. The frequency of the I-allele in Quechua is approximately 0.72 [8, 66]. This is slightly higher than the mean European, African, or Asian I-allele frequencies in the literature [14], but not particularly high compared to other Native American populations [66]. For example, the I-allele ranges from a low in Alaskan

Natives (Eskimos, Native Amerindians, and Aleuts) of 0.45 to a high of 1.0 in the Ache of eastern Paraguay [8]. Interestingly, in the Himalaya, the I-allele frequency is also relatively high (0.67 in highland native Ladakhis from Northern India [64], and ranging from 0.51 to 0.64 in native Tibetans from Lhasa depending on whether subjects were hypertensive [37]). Perhaps the I-allele is a part of a haplotype (i.e., a collection of alleles) that confers evolutionary benefit at altitude, but this remains to be elucidated.

7.6 Summary

Indigenous high-altitude natives compared to lowland controls have impressive work abilities, large pulmonary volumes and increased lung diffusion capacities, and an enhanced efficiency of gas exchange at rest and during exercise. In general, direct evidence that these trait differences have a genetic basis between populations is lacking. In contrast, depending on the specific trait, there is abundant evidence that developmental effects are important. This is especially true for pulmonary volumes and the reduced A-aDO_2 (and lower VE/VO_2) during exercise. For other traits, including the VO_2max and the SaO_2, the evidence for developmental effects is less convincing. In the case of VO_2max, the problem of physical activity continues to confound study interpretation. In the case SaO_2, this trait may be under strong genetic control and thus insensitive to early life hypoxia exposure.

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Chapter 8
Functional Genomic Insights into Regulatory Mechanisms of High-Altitude Adaptation

Jay F. Storz and Zachary A. Cheviron

Abstract Recent studies of indigenous human populations at high altitude have provided proof-of-principle that genome scans of DNA polymorphism can be used to identify candidate loci for hypoxia adaptation. When integrated with experimental analyses of physiological phenotypes, genome-wide surveys of DNA polymorphism and tissue-specific transcriptional profiles can provide insights into actual mechanisms of adaptation. It has been suggested that adaptive phenotypic evolution is largely mediated by *cis*-regulatory changes in genes that are located at integrative control points in regulatory networks. This hypothesis can be tested by conducting transcriptomic analyses of hypoxic signaling pathways in conjunction with experimental measures of vascular oxygen supply and metabolic pathway flux. Such studies may reveal whether the architecture of gene regulatory networks can be used to predict which loci (and which types of loci) are likely to be "hot spots" for adaptive physiological evolution. Functional genomic studies of deer mice (*Peromyscus maniculatus*) demonstrate how the integrated analysis of variation in tissue-specific transcriptomes, whole-animal physiological performance, and various subordinate traits can yield insights into the mechanistic underpinnings of high-altitude adaptation.

Keywords Functional genomics • Hypoxia • *Peromyscus* • Population genomics • Systems genetics • Transcriptomics

8.1 Introduction

There is currently a growing interest in conducting genome scans of DNA polymorphism to identify loci that have contributed to high-altitude adaptation in humans [1, 2, 5–7, 25, 45, 55, 82–85]. This population genomics approach is premised on the idea that the locus-specific effects of positive selection can be detected against the genome-wide backdrop of stochastic variation. Consider a genome-wide survey of DNA polymorphism in individuals sampled from a pair of high- and low-altitude populations. If a given trait is subject to directional selection only at high altitude,

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then the underlying loci are expected to undergo shifts in allele frequency in the highland population relative to the lowland population. In principle, it is possible to identify chromosomal regions that harbor such loci by exploiting theoretical predictions about the effects of positive selection on patterns of DNA polymorphism at linked neutral sites. However, the effects of selection on patterns of variation at or near causative loci depend strongly on the genetic architecture of the selected trait and numerous other factors [13, 28, 31, 32, 46, 60, 68, 73, 74].

The population genomics approach can be used as a means of "outlier detection" to nominate candidate loci as putative targets of positive selection, or it can be used to assess evidence for selection on previously identified candidate loci by assessing whether such loci emerge as outliers against a backdrop of genome-wide variation. Both approaches have been used successfully to identify loci involved in adaptation to high-altitude environments. In humans, for example, genome-wide surveys of DNA polymorphism in Tibetan highlanders revealed evidence for strong and recent positive selection on several genes that function as upstream regulators of the hypoxia inducible-factor (HIF) oxygen signaling pathway [5–7, 45, 55, 82–85]. The HIF family of transcription factors plays a key role in regulating oxygen homeostasis by coordinating the transcriptional response to hypoxia. One of the HIF genes that exhibited an especially clear signal of positive selection in the Tibetan population was the *EPAS1* gene (*endothelial PAS domain protein 1*) (Fig. 8.1), also known as *HIF2α*, which encodes the oxygen-sensitive α subunit of the HIF-2 transcription factor. The product of *EPAS1* is known to play an especially important role in

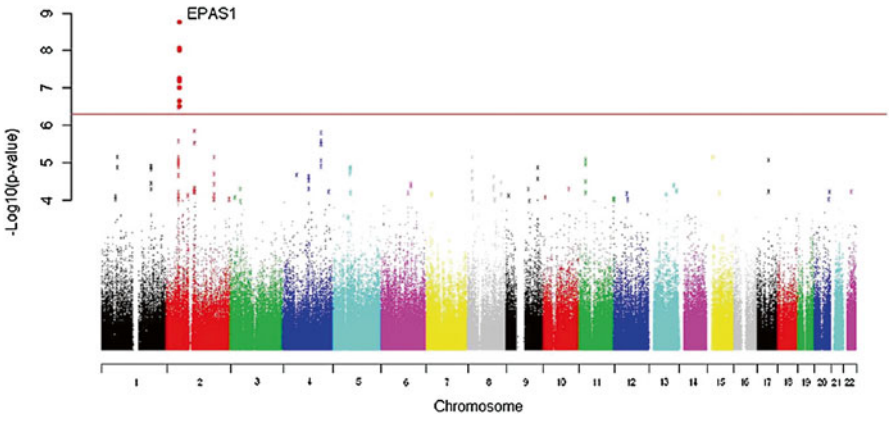

Fig. 8.1 A genome-wide scan of allelic differentiation between population samples of Tibetans (resident at 3200–3500 m in Yunnan Province, China) and Han Chinese. The vertical axis of the graph shows the negative log of site-specific *P*-values for allele frequency differences between the Tibetan and Han Chinese population samples (low *P*-values denote allele frequency differences that are too large to explain by genetic drift). The horizontal axis of the graph shows the genomic positions of each assayed nucleotide site, arranged by chromosome number. The red line indicates the threshold for genome-wide statistical significance ($P = 5 \times 10^{-7}$). Values are shown after correction for background population stratification using an intragenomic control. Several noncoding variants flanking the EPAS1 gene are highly significant outliers. Reprinted from [5]

regulating the erythropoietic response to hypoxia [47]. Another HIF-regulatory gene that exhibited strong evidence for selection in both Tibetan and Andean populations was *EGLN1* (*Egl Nine homolog 1*) [1, 6, 7, 45, 55, 82, 84, 85], which encodes the prolyl hydroxlase isozyme (PHD2) that is responsible for hydroxylating the α subunit of the HIF1 transcription factor. Results of these studies provide proof-of-principle that the genome scan approach can successfully identify targets of recent positive selection, and the integration of such analyses with functional studies can provide additional insights into possible phenotypic targets of selection [34].

An example of how the population genomics approach can be combined with the functional analysis of individual candidate genes is provided by an integrative analysis of hemoglobin polymorphism in natural populations of North American deer mice (*Peromyscus maniculatus*). Multilocus surveys of nucleotide polymorphism in high- and low-altitude populations revealed evidence for a history of spatially varying selection at two α-globin gene duplicates and two β-globin gene duplicates [40, 62–66], and site-directed mutagenesis experiments involving recombinant hemoglobins quantified the additive and nonadditive effects of the causative mutations [41]. The population genetic evidence for spatially varying selection and the experimental measures of mutational effects corroborated previous research on wild-derived strains of deer mice, which had demonstrated that allelic variation in hemoglobin-oxygen affinity contributes to adaptive variation in whole-animal aerobic performance under hypoxia [11, 12, 59]. Similarly, genome-wide surveys of nucleotide variation in a number of Andean birds species have provided insights into the evolutionary forces that have shaped altitudinal patterns of hemoglobin polymorphism [17, 21, 42]. In each of these studies, population-genomic inferences about the adaptive significance of observed amino acid polymorphisms were tested by conducting functional analyses of native hemoglobin variants and engineered recombinant hemoglobin mutants that quantified the phenotypic effects of individual mutations.

In addition to identifying particular candidate loci for high-altitude adaptation, genome scans for signatures of positive selection can also be used to gain more general insights into the nature of adaptation to different environments. For example, in comparisons between high- and low-altitude populations of a given species, it is possible to assess whether certain classes of loci make disproportionate contributions to adaptive phenotypic evolution. We can assess whether genes that occupy particular positions in metabolic pathways or regulatory networks make disproportionate contributions to adaptation, and we can assess the relative importance of structural mutations (e.g., amino acid mutations that alter the catalytic efficiency of an enzyme) and regulatory mutations (e.g., *cis*- or *trans*-acting mutations that alter the expression of the enzyme-encoding gene).

8.2 The Relative Importance of Structural vs. Regulatory Changes in Physiological Adaptatation

It has been suggested that *cis*-acting regulatory mutations may make a disproportionate contribution to adaptive evolution because such changes generally have fewer pleiotropic effects relative to changes in coding sequence [10, 57, 58]. This is

because *cis*-regulatory elements (e.g., promoters, enhancers, and 5′ and 3′ untranslated regions [UTRs]) are often functionally modular—distinct sequence motifs control discrete temporal phases and/or spatial patterns of gene expression [10, 56, 81]. Each *cis*-regulatory module represents a collection of transcription factor binding sites that encodes a particular transcriptional output, and mutational changes in a single module will typically alter a small part of the gene's total transcriptional pattern. For example, a *cis*-regulatory mutation may affect transcription in one particular tissue or cell type, and will therefore have minimal pleiotropic effects on the regulatory network as a whole. By contrast, structural changes in the coding sequence of a given gene would be manifest in every tissue or cell type in which the affected protein is expressed. Likewise, mutations in the coding sequence of a transcription factor that affect DNA binding affinity could potentially affect the transcriptional control of myriad downstream regulatory targets. For these reasons, coding mutations are generally expected to have more far-reaching pleiotropic effects than *cis*-regulatory mutations, and may therefore have smaller net fitness benefits.

A number of recent studies have documented evolutionary changes in phenotype that were caused by mutations in modular *cis*-regulatory elements [26, 48, 75]. In humans, persistence of lactase expression into adulthood has evolved independently in several different ethnic groups, and in all cases the ontogenetic changes in the expression of the *Lct* gene were attributable to point mutations in *cis*-regulatory elements [75]. Another good example involves the evolution of reduced abdominal pigmentation in *Drosophila santomea*, which is caused by several distinct inactivating mutations in an abdomen-specific *cis*-regulatory element of the *tan* gene [26].

There are good reasons to expect that a disproportionate number of the mutations that contribute to phenotypic evolution are concentrated in the *cis*-regulatory regions of transcription factor genes that serve as central control points in regulatory networks [57, 58]. This expectation is based on the observation that pleiotropic effects are determined by how regulatory networks shape the phenotype. For example, among species in the genus *Drosophila*, evolutionary changes in larval trichome patterning are mediated by *cis*-regulatory substitutions in a transcription factor called *shavenbaby/ovo* (*svb*) [38, 71, 72]. The *svb* transcription factor integrates a vast array of cellular signals and produces an on/off transcriptional output that determines cellular differentiation into trichomes or naked cuticle. It appears that anatomically localized changes in trichome patterning can be achieved most efficiently through mutations in specific *cis*-regulatory enhancers of *svb* because such mutations have minimal pleiotropic effects. By contrast, coding mutations in *svb* would produce changes in trichome patterning in every spatial domain of the cuticle in which the protein is expressed, and changes in upstream regulators of *svb* would alter the development of other epidermal structures besides trichomes. Thus, genes located at integrative control points in a regulatory network can accumulate mutations with specific, minimally pleiotropic effects, and these mutations are predicted to be especially common in *cis*-regulatory regions of "control point" genes [57, 58].

In analogy with the role of *svb* in the development of trichome patterning in *Drosophila* embryos, the *EPAS1* gene may occupy an analogous position in the

regulatory network that governs the transcriptional response to hypoxia. It may be that the physiological response to hypoxia is most efficiently accomplished by modulating the transcription of *EPAS1*, which then causes a coordinated change in the expression of all downstream target genes.

Future research should reveal whether *cis*-regulatory mutations in *EPAS1* and other upstream regulators of the HIF signaling pathway have made disproportionate contributions to hypoxia adaptation in other animal species that are native to high-altitude environments. Beyond the important goal of identifying convergent mechanisms of hypoxia adaptation in different species, such research could also contribute to the more general goal of discovering whether the architecture of gene regulatory networks can be used to predict which genes are likely to be "hot spots" for adaptive physiological evolution.

8.3 Integrating the Analysis of Coding Sequence Variation and Transcriptional Variation

Gene expression profiles represent an important source of phenotypic data at the molecular level, and detailed studies of transcriptional variation may help to identify mechanisms of genetic adaptation and/or physiological acclimatization [61, 67, 79]. Plasticity in most physiological traits is probably mediated to a large extent by environment-specific changes in the transcriptional activity of multiple underlying genes. As stated by Hochachka and Somero [24]: "The evolution of phenotypic plasticity requires development of a complex set of tightly integrated environmental sensing and gene regulation mechanisms that allow the organism to sense and then respond appropriately to an environmental change".

In principle, genomic technologies that permit the simultaneous analysis of sequence variation and expression profiles for a set of genes in the same pathway can be used to identify both structural and regulatory mechanisms of adaptation. For example, RNA-seq technology can be used to characterize sequence polymorphism and transcript abundance in thousands of expressed mRNAs from specific tissue types [27, 43, 78]. The integrated analysis of DNA sequence variation, genome-wide variation in transcriptional profiles, and variation in organismal phenotypes in a linkage or association mapping population can yield important insights into how regulatory networks shape variation in complex traits [3, 36, 49]. This approach is most powerful when implemented in a common garden or reciprocal-transplant experimental design to quantify the environmental and genetic components of gene expression differences between populations.

To appreciate the types of evolutionary inferences that can be drawn from a typical RNA-seq analysis, consider a common garden experiment involving individuals sampled from a pair of high- and low-altitude populations. Using animals that have experienced uniform acclimation histories to control for environmentally induced variation in gene expression, tissue specific cDNA libraries are then constructed for transcriptome sequencing. For each protein-coding gene, it is possible to measure

nucleotide differentiation between high- and low-altitude populations and the corresponding differentiation in expression levels that is attributable to additive genetic effects. If adaptive genetic differentiation in the expression of a given gene is attributable to changes in proximal *cis*-regulatory elements (i.e., mutations in the promoter region), then the gene in question may exhibit an elevated level of nucleotide differentiation due to divergent selection at the immediately adjacent noncoding sites. When adaptive differentiation in the expression of a given gene is attributable to *trans*-acting mutations, then we would not expect a correlated increase in nucleotide differentiation at or near the gene itself because flanking regulatory mutations would not have contributed to the selection response.

For the purpose of identifying candidate loci for environmental adaptation, one of the disadvantages of the RNA-seq approach is that the assayed variation is exclusively restricted to the transcripts of protein-coding genes. Thus, adaptive changes in noncoding DNA may go undetected if the causal mutations are distant from genic regions. Population genomic analyses of spatially varying selection in *D. melanogaster* provide suggestive evidence that noncoding DNA polymorphisms may make an unexpectedly large contribution to environmental adaptation. For example, in genomic comparisons between temperate and tropical Australian populations of *D. melanogaster*, chromosomal regions that exhibited the highest levels of differentiation contained an over-representation of unannotated noncoding sequence [30]. Secondly, RNA-seq analysis can only identify expression differences that are mediated by changes in the rate of transcription or mRNA stability—regulatory changes that are mediated by posttranslational modifications would remain undetected.

8.4 Dissecting the Genetic Architecture of Adaptive Regulatory Variation

It is possible to further assess the contributions of *cis*- and *trans*-acting regulatory mutations to variation in the expression of a given gene by quantifying the relative abundance of allele-specific transcripts (provided that the transcripts are distinguished by at least one diagnostic nucleotide change). In a diploid cell, *trans*-acting regulatory mutations affect the expression of both alleles of a given target gene, whereas *cis*-acting regulatory mutations exert effects that are allele-specific [19, 39, 80]. For this reason, it is possible to distinguish the effects of *cis*- and *trans*-acting regulatory mutations in modulating the expression of a given gene by comparing the relative expression of alternative alleles in heterozygotes. Assuming that the expression of each allele is independent of the other, a marked asymmetry in allele-specific transcript abundance is typically attributable to *cis*-regulatory changes because both alleles are transcribed in the same *trans*-regulatory cellular environment [19, 39]. RNA-seq allows for high-throughput assessment of allele-specific gene expression because it provides a means of quantifying transcript abundance on an allele-specific basis. Although analyses designed to characterize allele-specific

expression patterns are most powerful when applied to controlled crosses between inbred strains [22, 29, 39, 70], powerful evolutionary inferences can still be made in RNA-seq analyses of samples from outbred, natural populations.

Ideally, inferences derived from genomic or transcriptomic analyses can help guide the design of detailed follow-up experiments to obtain insights into the mechanistic basis of adaptive trait variation [68, 69]. For example, at the biochemical level, variation in metabolic traits may stem from allelic variation in enzyme concentration, enzyme kinetics, or a combination of the two. The combined effects of enzyme concentration and enzyme kinetics are measured by the maximal velocity of the enzyme-catalyzed reaction, V_{max}, which is defined as the product $[E] \times k_{cat}$, where $[E]$ is the enzyme concentration and k_{cat} is the kinetic constant. In RNA-seq analyses of samples from high- and low-altitude populations, metabolic genes that exhibit extreme differences in expression or allele frequency can be targeted for functional studies of enzyme kinetics. For example, cases where V_{max} and gene expression vary in the same direction suggest that differences in V_{max} may be attributable to differences in enzyme concentration (regulatory mutations). Conversely, cases in which differences in V_{max} are not accompanied by changes in gene expression would implicate mutations that alter enzyme catalytic efficiency (structural mutations). Similarly, traditional expression QTL (eQTL) mapping approaches can be used to confirm inferences about the genetic architecture of the observed regulatory variation. Thus, results of RNA-seq analyses can generate novel hypotheses that can be experimentally tested.

8.5 Integrating Functional Genomics and Experimental Analyses of Whole-Animal Physiology

Integrating analyses of transcriptomic variation with measures of physiological traits can reveal how variation in regulatory networks gives rise to phenotypic variation at different hierarchical levels of organization. This is the goal of the "systems genetics" approach—to achieve a mechanistic understanding of the mapping function that relates genotype to phenotype by examining how genetic variation in intermediate molecular phenotypes such as transcript abundance is transduced into variation in higher-level traits [3, 18, 33, 36, 49, 54].

In analyses of tissue-specific transcriptome profiles, statistical associations between particular traits and levels of transcript abundance can reveal trait-specific modules of co-regulated genes. These transcriptional modules typically comprise sets of genes that interact in the same pathway or regulatory network. For this reason, correlations in transcript abundance among genes in the same module provide a means of constructing genetic interaction networks, and can suggest hypotheses about causal effects [49].

Integrated analyses of transcriptomic variation and physiological phenotypes have been successfully applied in studies of high-altitude adaptation in North American deer mice. Relative to mice that are native to lowland environments,

high-altitude deer mice have evolved enhanced capacities for aerobic performance under hypoxia [14–16, 23, 35], and these population differences in whole-organism performance stem from genetically based physiological changes at several hierarchical levels of biological organization. For example, the elevated thermogenic capacity of high-altitude deer mice is associated with an increased capacity to oxidize lipids as a primary fuel source during aerobic thermogenesis [14]. This difference in lipid catabolic capacities is associated with differences in the activities of enzymes that influence flux through fatty acid oxidation and oxidative phosphorylation pathways, and with concerted changes in gene expression in these same pathways [14, 16]. For example, high-altitude mice that were acclimated to low-altitude conditions for 6 weeks exhibited wholesale upregulation of genes in the β-oxidation and oxidative phosphorylation pathways compared to their lowland counterparts that were acclimated to the same laboratory conditions [14]. Follow-up experiments on mice with different acclimation histories revealed that the regulatory changes associated with enhanced performance are highly plastic. Specifically, four of five transcriptional modules that were significantly associated with whole-organism thermogenic performance comprised a set of genes that exhibited significant effects of rearing environment (native elevation vs. common garden) that were independent of population-of-origin (highland vs. lowland), suggesting that a large fraction of the transcriptional variation is environmentally induced [16]. These environmentally sensitive transcriptional modules were enriched for genes involved in hematopoiesis, angiogenesis, muscle development, and immune response (Fig. 8.2). Interestingly, the single transcriptional module that exhibited a significant population-of-origin effect (expression differences persisted in the F1 generation) was enriched for genes involved in lipid oxidation, and this module was expressed at higher levels in high-altitude natives. Again, there was generally good correspondence between these transcriptional patterns and enzyme activities that serve as biomarkers for the flux capacity of the β-oxidation pathway and other core metabolic pathways, lending further support to the hypothesis that the enhanced aerobic performance of high-altitude deer mice under hypoxia is partly attributable to their enhanced capacities for lipid oxidation.

Taken together, these studies suggest that metabolic adaptation to high-altitude in deer mice involves the maintenance of a highly aerobic phenotype in the face of reduced oxygen availability, and this aerobic phenotype is achieved through both genetically based, constitutive differences in gene expression and transcriptional plasticity. Elite endurance athletes and highly aerobic nonhuman mammals are also characterized by an enhanced capacity for fatty acid oxidation under normoxic conditions [4, 8, 37]. Under conditions of chronic oxygen deprivation at high-altitude, a similar enhancement of fatty acid oxidation capacity could promote enhanced thermogenic performance, but it would require additional physiological changes to ensure adequate oxygen and fuel flux through oxidative pathways, suggesting that modifications of upstream steps in the oxygen-transport cascade may be necessary to support the increased lipid oxidation rate of high-altitude deer mice.

Indeed, the enhanced aerobic capacity of high-altitude deer mice under hypoxia is also partly attributable to increases in capillary density, oxidative fiber abundance, and oxidative enzyme activity in skeletal muscle [14, 16, 35]. These changes

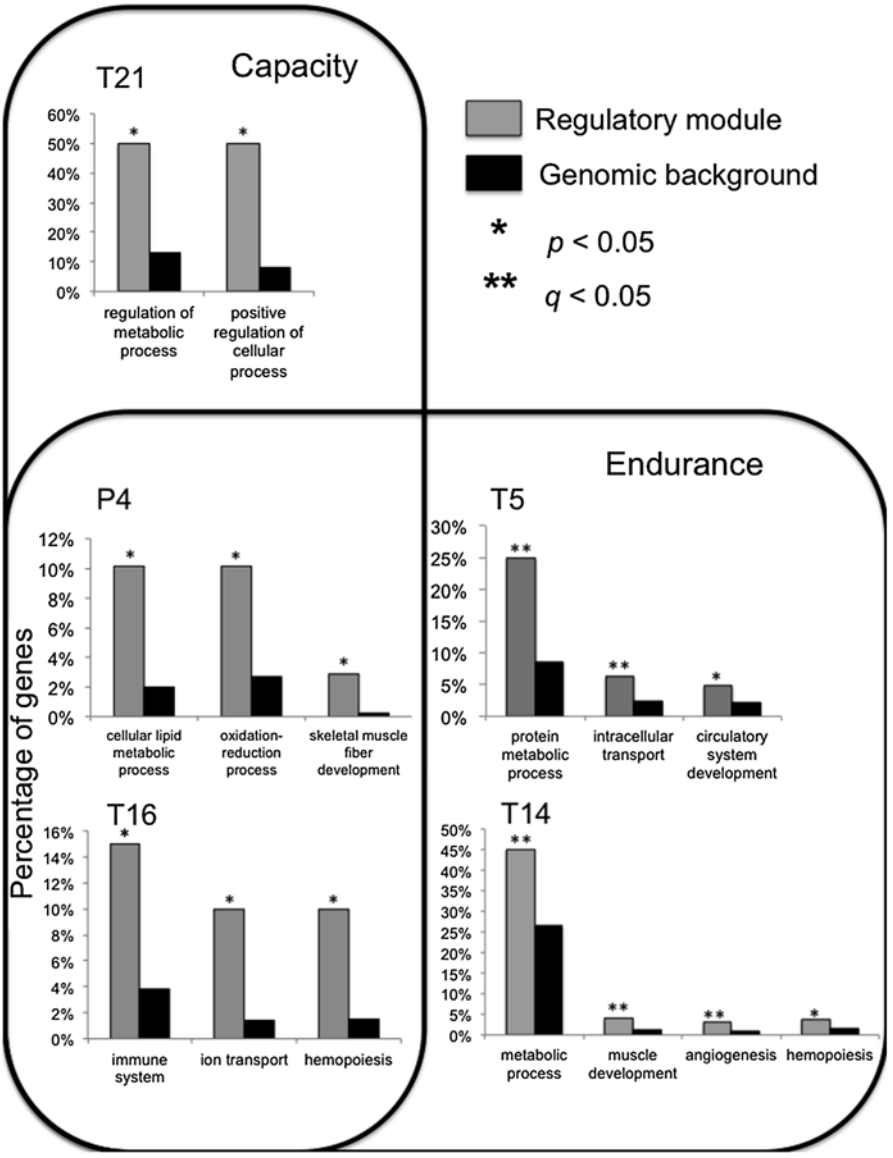

Fig. 8.2 Functional enrichment of transcriptional modules that are associated with thermogenic performance in deer mice. Categorical enrichments are shown for five separate modules that are associated with thermogenic capacity (cold-induced VO_2 max) (T21), thermogenic endurance (the length of time individuals can maintain >90 % of VO_2 max (T5 and T14), or both measures of performance (P4 and T16). Modules T5, T14, T16, and T21 are comprised of genes that differ in expression across rearing environments independent of population of origin, whereas module P4 is comprised of genes differ in expression between highland and lowland mice across rearing environments. For each performance-associated transcriptional module, the proportional representation of genes in different gene ontology categories is compared between the transcriptional module (*gray bars*) and the genome as a whole (*black bars*). Asterisks denote gene ontology categories that are significantly enriched within the transcriptional module (*uncorrected $p<0.05$, **FDR corrected $q<0.05$). Reprinted from [16]

enhance the oxidative capacity and oxygen-diffusion capacity of working muscle, which could compensate for the diminished tissue oxygen supply under hypoxia [9, 51, 53, 77]. The transcriptional underpinnings of these phenotypic changes were identified in a common-garden experiment involving wild-caught high- and low-altitude mice as well as laboratory-reared F1 progeny of wild-caught mice [35, 52]. Expression analysis of a panel of genes that regulate angiogenesis and energy metabolism revealed that the increased capillarity and oxidative capacity of skeletal muscle in high-altitude mice was associated with an increased transcript abundance and protein abundance of peroxisome proliferator-activated receptor γ (PPARγ) (Fig. 8.3), a transcription factor that regulates mitochondrial biogenesis. PPARγ protein expression also increased during acclimation to chronic hypoxia [35]. Intriguingly, the underlying gene (*pparg*) exhibits a strong signature of recent positive selection in indigenous Tibetan and Mongolian highlanders [83].

Expression patterns of the deer mouse *pparg* gene proved to be somewhat anomalous, however, as most other genes involved in angiogenesis were actually downregulated during acclimation to hypoxia [35] and were either not differentially expressed between hypoxia-acclimated high- and low-altitude mice or were downregulated in the high-altitude mice [52]. Transcriptomic analysis of gastrocnemius muscle revealed a small set of transcripts with genetically based expression differences between high- and low-altitude mice (i.e., nonplastic differences that persisted in the F1 progeny of mice with different populations-of-origin), and these transcripts clustered into two discrete modules. Some genes involved in regulating

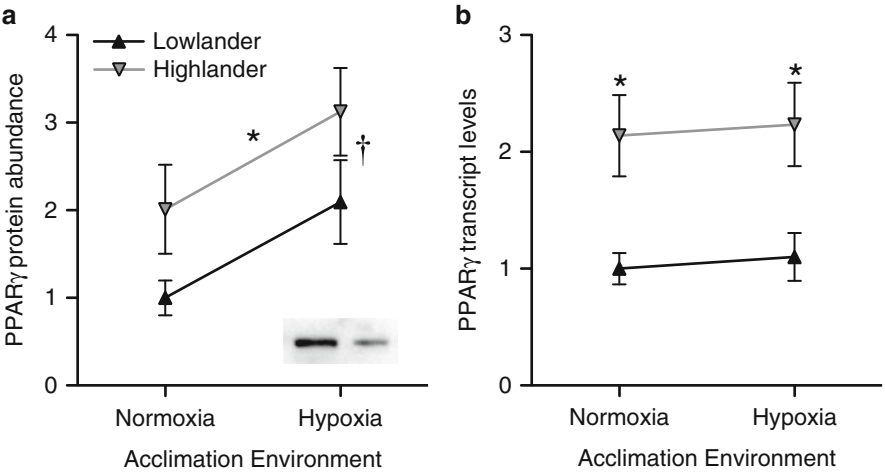

Fig. 8.3 Relative to low-altitude deer mice, high-altitude mice have higher expression of PPARγ protein (**a**) and mRNA (**b**) in the gastrocnemius muscle. Population-of-origin (i.e., high- or low-altitude) had significant main effects on PPARγ protein abundance and transcript abundance, but acclimation environment only had a significant main effect on protein abundance. There was no significant interaction between population-of-origin and acclimation treatment in either case [35]. The inset image in panel A shows representative immunoreactive bands for a high-altitude mouse (*left*) and low-altitude mouse (*right*) after acclimation to hypoxia. Modified from [35]

angiogenesis (*Cadherin-7* and *Notch-4*) were significantly upregulated in high-altitude mice, but most genes within these modules were actually expressed at a lower level in high-altitude mice and expression levels were negatively correlated with measures of muscle capillarity and oxidative capacity. A possible explanation for this seemingly paradoxical result is that particular genes like *Notch-4* may be responsible for maintaining the high muscle capillarity of high-altitude mice, and the associated increase in cellular oxygen tension simply dampens the stimulus for the expression of other genes that respond to oxygen limitation [35, 52]. As stated by Scott et al. (Ref. [52]: 1972): "A negative association between muscle phenotypes and expression could thus result for genes that do not cause the differences in muscle phenotype but are sensitive to its effects." This highlights the difficulty of distinguishing the effects of genes that cause a given phenotype vs. effects that are simply consequences of the induced changes.

These integrative studies of deer mouse physiology revealed that genetically based regulatory changes and acclimation responses both contribute to improvement in whole-animal aerobic performance under hypoxia via changes in oxygen-transport capacity and oxygen utilization [14–16, 35, 52, 76]. Thus, variation in whole-organism aerobic performance seems to stem from changes at several hierarchical levels of biological organization, and changes at each level appear to stem from concerted expression changes in co-regulated sets of genes.

8.6 Future Outlook

Whole-genome and transcriptome sequencing will continue to play a central role in studies of hypoxia adaptation in humans and other animals. Analyses of genomic/transcriptomic data and gene ontology databases can provide lists of candidate loci for hypoxia adaptation, but such analyses need to be integrated with experimental measures of whole-animal performance and other subordinate traits to obtain insights into actual mechanisms of physiological adaptation. Gene ontology analyses can be useful for generating hypotheses but, in the absence of experimental validation, in silico approaches lend themselves to overinterpretation and storytelling [44, 61]. Surveys of genomic/transcriptomic variation can suggest hypotheses about the adaptive significance of particular polymorphisms or expression changes, but functional experiments are required to test such hypotheses [17, 20, 50, 68].

In analyses of transcriptomic variation, it is also critical to use common-garden and/or reciprocal transplant experimental designs to disentangle genetic and environmental components of variation in gene expression, otherwise it is not possible to distinguish between evolved changes and plastic changes. Studies of high-altitude physiology in humans face obvious constraints with regard to experimental manipulations. However, recent work by Lorenzo et al. [34] provides an example of how reverse-genetics approaches and in vitro experiments can be used to follow up on results of population genomic studies to gain insights into mechanisms of hypoxia adaptation.

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Part III
Hypoxia and the Brain

Chapter 9
Influence of Hypoxia on Cerebral Blood Flow Regulation in Humans

Craig D. Steinback and Marc J. Poulin

Abstract The brain is a vital organ that relies on a constant and adequate supply of blood to match oxygen and glucose delivery with the local metabolic demands of active neurones. It is well established that cerebral blood flow is altered in response to both neural activity and humoral stimuli. Thus, augmented neural activation (e.g. visual stimulation) leads to locally increased cerebral blood flow via functional hyperaemia, whereas humoral stimuli (i.e. alterations in arterial PO_2 and PCO_2) produce global increases in cerebral blood flow. Perhaps not surprisingly, cerebrovascular responses to neural activity and humoral stimuli may not be highly correlated because they reflect different physiological mechanisms for vasodilation. Exquisite regulation of cerebral blood flow is particularly important under hypoxic conditions when cerebral PO_2 can be reduced substantially. Indeed, cerebrovascular reactivity to hypoxia determines the capacity of cerebral vessels to respond and compensate for a reduced oxygen supply. This reactivity is dynamic, changing with prolonged exposure to hypoxic environments, and in patients and healthy individuals exposed to chronic intermittent periods of hypoxia. More recently, a number of animal studies have provided evidence that glial cells (i.e. astrocytes) play an important role in regulating cerebral blood flow under normoxic and hypoxic conditions. This review aims to summarize our current understanding of cerebral blood flow control during hypoxia in humans and put into context the underlying neurovascular mechanisms that may contribute to this regulation.

Keywords Cerebrovascular reactivity • Intermittent hypoxia • Acclimatization • Neurovascular coupling

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9.1 Historical Perspective

Our current understanding of the cerebral circulation and its regulation during hypoxia is based on the pioneering work of a number of scientists. The genesis of this line of research can be traced to the anatomical and neurological descriptions of Sir Thomas Willis and his student, Sir Christopher Wren, in the mid-1600s. Based on numerous postmortem dissections, Willis produced the first anatomically correct description of the cerebral circulation. Including the Circle of Willis, these descriptions were sketched in detail by Sir Christopher Wren. Willis also correctly described the function of this collateral circulation noting that it would allow perfusion of both hemispheres in the event of occlusion to one carotid artery (he also noted the vertebral arteries would provide circulation in the event of bilateral carotid occlusion) [54].

In 1932, William Lennox and Erna Gibbs used a clever adaptation of the Fick principle to investigate differences in the cerebral and peripheral (femoral) blood flow responses to variations in O_2 and CO_2 in humans [41]. By assuming a constant metabolic rate, alterations in a-vO_2 difference were inferred to be proportional to changes in blood flow velocity, and in turn representative of either vasodilation or vasoconstriction. The authors' comprehensive data clearly demonstrate cerebral vasodilation in response to decreased O_2 and elevated CO_2, thus providing the basis of our fundamental understanding of cerebrovascular responsiveness to altered arterial gas tensions. The authors also contrasted the responses of the cerebral circulation with that of the leg, further demonstrating the preferential redirection of blood to the brain during alterations in blood gases.

In the mid-1940s Seymour Kety and Carl Schmidt also applied the Fick principle to the cerebral circulation to derive the first quantitative measurements of cerebral blood flow in humans [34, 35]. By determining the time-course of arteriovenous equilibrium of nitrous oxide, the authors were able to derive a normative value for total cerebral blood flow (~54 cc/100 g/min). The same principles used in these early studies have been subsequently adapted to allow for the noninvasive determination of regional cerebral blood flows using radiolabelled tracers (i.e., ^{133}Xe, ^{85}Kr, etc) and CT scanning [40].

With the ability to quantify cerebral blood flow, researchers quickly adapted these early techniques to examine the cerebrovascular responses to various stimuli. Of interest in the context of the present review is the work of Severinghaus et al., which first quantified the time-course of cerebral blood flow adaptation to altitude [58]. These data, collected at the Barcroft Laboratory on White Mountain, California (3810 m), demonstrated an early rise in blood flow (+24 % within 6–12 h) which declined towards baseline (+13 %) over the subsequent 3–5 days. The authors were also the first to highlight the important role of cerebrospinal fluid pH in regulating cerebral blood flow during acclimatization.

In the current review we aim to expand upon the current state of understanding of cerebral blood flow control, with an emphasis on cerebrovascular responses and adaptations to hypoxia.

9.2 Continuum of Cerebrovascular Responses to Oxygen

In the absence of continuous perfusion and supply of oxygen, the brain is prone to irreversible damage. The delivery of oxygen may be compromised in many clinical (e.g. sleep apnoea, stroke, and traumatic brain injury) or environmental settings (e.g. exposure to carbon monoxide and altitude). Hence, an understanding of how the brain responds to decreases in oxygen is important for delivering better health care to patients as well as preventing serious outcomes in those exposed to low-oxygen environments.

9.2.1 Acute Exposure to Hypoxia

The regulation of cerebral blood flow is quite robust in its response and adaptation to alterations in blood gases. Acute exposure to hypoxia causes an immediate increase in cerebral blood flow which is curvilinear with respect to arterial PO_2 and linear with respect to hemoglobin saturation (Fig. 9.1). In humans, middle cerebral artery (MCA) blood flow velocity increases 0.35–0.75 cm/s for every % decrease in blood oxygen saturation [5, 28, 61, 65].

Importantly, the cerebral vasculature is also highly sensitive to changes in the partial pressure of arterial CO_2 ($PaCO_2$) [27], which critically affects the magnitude of the cerebrovascular response to hypoxia. As such, it is necessary to consider the influence of changes in $PaCO_2$ when studying the cerebrovascular effects of hypoxia. The concomitant ventilatory response during hypoxia typically results in hypocapnia

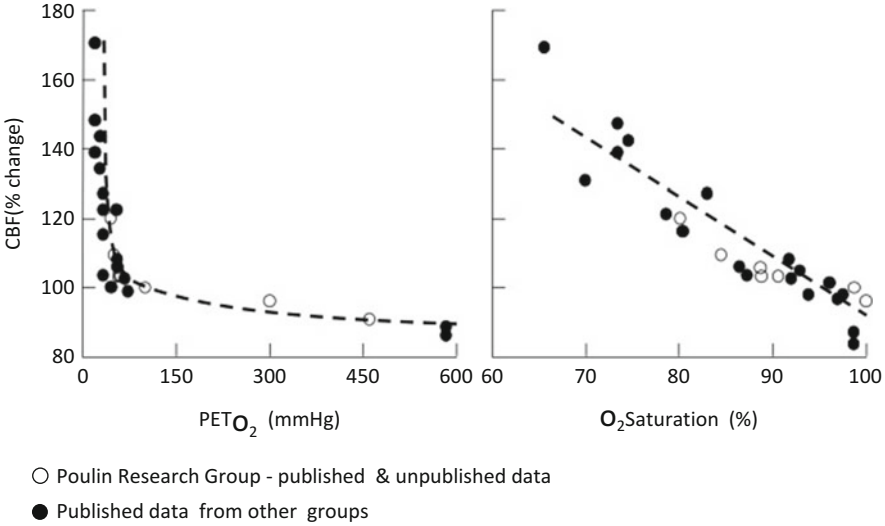

Fig. 9.1 The cerebral blood flow (CBF) response to hypoxia is curvilinear with respect to the measured end-tidal partial pressure of O_2 ($PETO_2$) (*left*), but can be linearized when expressed in relation to hemoglobin oxygen saturation (*right*). Data generated from [50]

under normal conditions. This ventilatory induced hypocapnia has been previously shown to blunt the increase in cerebral blood flow in response to hypoxia [5, 59, 61]. Hypocapnia also alters the time-course of the cerebrovascular response to acute hypoxia, causing an initial decrease in blood flow followed by a gradual increase to levels similar to those observed during isocapnic conditions [61] (Fig. 9.2). Conversely, the cerebral blood flow response to hypoxia is augmented under hypercapnic conditions [5, 51].

The acute increase in cerebral blood flow in response to hypoxia can be defined by both the magnitude of change as well as the rate at which this change occurs (i.e. time-course). The cerebral blood flow response to a step change into hypoxia occurs with an on-transient time constant of ~80s [51, 61]. However, the subsequent off-transient response occurs with a time constant of ~30s [51] (Fig. 9.3). Despite this difference in rate, Ainslie and Poulin demonstrated a similar cerebrovascular gain in response to hypoxia regardless of whether O_2 was rising or falling and independent of prevailing CO_2 [5]. Further, a difference in on- and off-transients is also observed during hyperoxia. The on-transient response for a step into isocapnic hyperoxia occurs with a time constant of ~30s whereas the off-transient response occurs with a time-constant of ~80s [70]. Together, these data suggest that the cerebral vasculature is more responsive to increases in O_2 than to decreases in O_2, regardless of where along the O_2 spectrum the stimulus occurs. The mechanisms responsible for the differences in on- and off-transient time-constants have not been defined. However, it is likely that differences in the molecular signalling pathways resulting in Ca^{2+} sequestering and release within vascular smooth muscle play a role. It remains unknown whether cerebral blood flow kinetics in response to acute changes in O_2 (either hypoxic or hyperoxic) adapt to prolonged exposure to hypoxia.

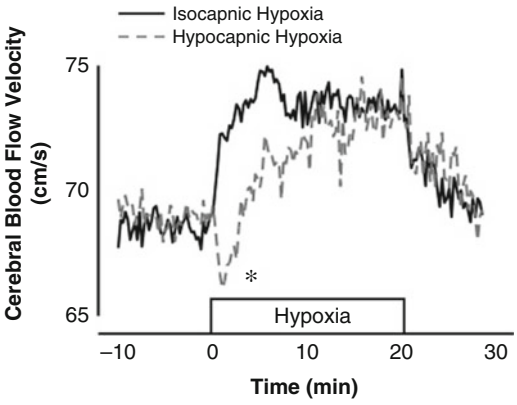

Fig. 9.2 The cerebral blood flow response to acute hypoxia. Concurrent hypocapnia (*gray dashed line*) causes an initial decrease in cerebral blood flow (indicated by the *asterisk*) followed by a gradual increase to similar levels as during isocapnic hypoxia (*solid black line*). Figure adapted from Ref. [61] (Steinback and Poulin, J Appl Physiol, 2008, Am Physiol Soc, used with permission)

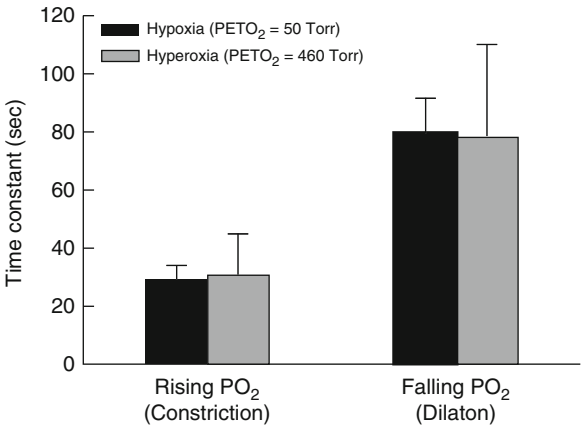

Fig. 9.3 Time constants for the cerebral blood flow responses to hypoxia (*black bars*) and hyperoxia (*grey bars*). Time constants were similar when O_2 was falling (dilation) and rising (constriction) irrespective of the prevailing PO_2. Data adapted from Refs. [51, 70]

9.2.2 *Cerebrovascular Adaptation to Prolonged Exposure to Hypoxia*

Similar to the response to acute hypoxia, the adaptation of cerebral blood flow during prolonged hypoxia is highly dependant on the prevailing $PaCO_2$. During poikilocapnic conditions (as observed at altitude) the concurrent hypoxic ventilatory response acts to reduce $PaCO_2$. Thus, the magnitude of cerebral blood flow over time is dependant on both the cerebrovascular and ventilatory reactivity to hypoxia, as well as the ventilatory and cerebrovascular reactivity to $PaCO_2$ (for further review of this complex interaction see [4]). Based on these interactions, cerebral blood flow follows a dynamic time-course during prolonged periods of poikilocapnic hypoxia [4, 11] (Fig. 9.4). Following ascent to altitude, resting cerebral blood flow [24, 58] and reactivity to hypoxia [32] progressively increase over the first few days. This is followed by a gradual fall in resting cerebral blood flow to values similar to those measured at sea level [24, 45, 58]. Whether or not cerebrovascular reactivity to hypoxia follows a similar time-course is not known.

The mechanisms contributing to the dynamic pattern of cerebrovascular acclimatization to prolonged hypoxia include changes in cerebral spinal fluid pH, angiogenesis, and increased hematocrit [9, 21, 55, 57]. Over the first few days to weeks of altitude exposure it is hypothesized that a resetting of the relationship between $PaCO_2$ and pH occurs in part due to a decrease in cerebral spinal fluid HCO_3^- [57]. This resetting is thought to result in an increase in cerebral spinal fluid pH and a reduced cerebral blood flow [57]. Severinghaus et al. demonstrated indirect evidence of this relationship by measuring $PaCO_2$ and cerebral spinal fluid pH and calculating cerebral spinal HCO_3^- during an examination of the adaptation of cerebral blood flow to altitude [58]. However, direct experimental evidence of this relationship is still required.

With extended stays at altitude (weeks-to-years) there is an increase in hematocrit [21, 55], while rodent data also demonstrate an increase and brain capillary density [9]. Both of these adaptations can be expected to improve both convective oxygen

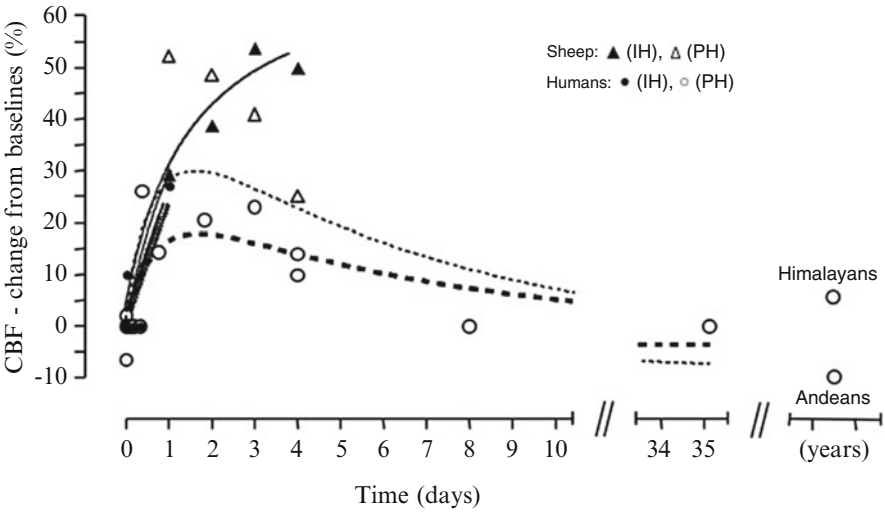

Fig. 9.4 Cerebral blood flow (CBF) acclimatization in response to prolonged hypoxia. Figure and data adapted from Refs. [11, 29]. (Reprinted from Respir Physiol Neurobiol 158, Brugniaux JV, Hodges AN, Hanly PJ and Poulin MJ. Cerebrovascular responses to altitude, Pages 212–223, Copyright (2007), with permission from Elsevier)

delivery and cerebral blood volume. This increase in oxygen delivery in turn acts to decrease the need for an increased blood flow to match metabolic demand. Similar physiological adaptations are evident in various populations of high-altitude natives suggesting a long-term genetic adaptation to hypoxia which may favor cerebral oxygenation. However, there is evidence that even long-term genetic adaptation may vary over extended periods of time. A recent meta-analysis of data from high altitude Andean and Himalayan populations indicates a higher cerebral blood flow in Himalayans and a higher hematocrit in Andeans [29]. Once differences in hematocrit were corrected for, cerebral blood flow in Himalayan residents remained higher than their Andean counterparts (~ +20 %). However, even after generational adaptation to altitude Andeans still exhibit a decreased cerebral reactivity to CO_2 and nitric oxide [7]. Further, Jansen et al report an impaired cerebral autoregulation at altitude in both Nepalese sherpas and sojourners to altitude [30, 31]. Further investigation of the mechanisms leading to specific genetic adaptations in cerebrovascular function is still needed.

9.2.3 Effects of Chronic Intermittent Hypoxia of Cerebrovascular Function

Chronic intermittent periods of hypoxia, as experienced with sleep disordered breathing, are associated with negative affects on cerebrovascular function. Although basal cerebral blood flow appears unaffected, the cerebrovascular response to changes in

blood gases is impaired in obstructive sleep apnoea patients [18, 19, 53] as well as in normal subjects exposed to prolonged periods of intermittent hypoxia [17, 20]. This impairment can be subsequently improved with the removal of the intermittent hypoxia stimulus in healthy subjects [17, 20] or continuous positive airway pressure treatment in sleep apnoea patients [19]. The brain's ability to buffer oscillations in blood pressure and maintain constant perfusion (autoregulation) is also impaired in OSA patients and may increase the risk of ischemia during drops in blood pressure [47, 68]. Although the specific mechanism contributing to decreased cerebral function with exposure to chronic intermittent hypoxia remain unknown, there is good evidence to suggest that augmented sympathetic vasomotor activity [46], decreased endothelial function [39], and increased arterial stiffness [39] are contributing factors.

9.2.4 The Influence of Sleep on Cerebrovascular Reactivity to Hypoxia

During sleep there is a downregulation of cardiovascular function as metabolism slows, brain centers associated with wakeful drives become quiescent, and perturbations to homeostasis are minimized. In general, non-REM sleep is associated with a concurrent decrease in heart rate, blood pressure, and importantly, alterations in cerebral blood flow regulation. In healthy subjects, cerebral blood flow is reduced during sleep [13, 73]. Further, cerebrovascular sensitivity to hypoxia (and hypercapnia) is not only reduced during sleep in normal subjects [43, 44], but there is an absence of a response to arterial oxygen desaturation [44]. These data suggest that sleep, even in healthy subjects, is associated with a state-dependant vulnerability to decreases in blood-oxygen. The mechanisms responsible for blunted cerebrovascular responsiveness during sleep are not known. It is also unknown if this decreased responsiveness plays a role in the genesis of sleep disordered breathing or contributes to the higher risk of ischemic events in these patients.

9.3 Neurovascular Coupling and Cerebral Blood Flow

9.3.1 Intrinsic Innervation of Cerebral Vessels

Intracerebral vessels penetrating the brain and supplying cortical and subcortical regions are coupled with glial and neural processes to form neurovascular units. The glia surround and support the vascular smooth muscle and endothelium to form the blood brain barrier. Subsequently, the end-feet of cortical and subcortical neurones

and interneurones synapse with glial cells and other neighboring interneurones. The interconnectiveness of neurones, interneurones, astrocytes, and cerebral vessels form a complex network of communication which propagates vasoconstrictor and vasodilator signals. In this arrangement astrocytes may act as important intermediaries. Several recent reviews have highlighted the emerging importance of these cells as signal integrators in regulating vascular tone [8, 12, 25, 38]. Depending on the neural inputs from surrounding neurones, substrates linked to either constriction or dilation may be released from astrocytes. Further, cerebral metabolic state is a key regulator of astrocyte function, with increased metabolism or low O_2 availability promoting the production of vasodilatory mediators [8, 12, 22]. Despite recent advances in our understanding of intrinsic control of cerebral blood flow, a number of areas require further investigation. First, despite the inter-connectiveness between astrocytes, interneurones, neurones, and microvessels, it remains unclear how dilatory and constrictor signals are propagated upstream to affect conduit artery inflow. Second, emerging evidence suggests that astrocytes may be functionally different depending on their localization within the brain [12, 38]. Characterization of this apparent heterogeneity is required. Third, a greater understanding of the vasoactive signalling pathways and communication between components of the neurovascular unit is needed.

9.3.2 Sympathetic Innervation of Cerebral Vessels

The "extrinsic" perivascular sympathetic innervation of superficial cerebral vessels has been well described [23]. Sympathetic neurons originating from the superior cervical ganglion with varicosities of norepinephrine and neuropeptide Y surround the internal carotid and pial arteries [2, 14] and cause vasoconstriction when stimulated [14]. However, the extent to which sympathetic innervation contributes to the normal regulation of cerebral blood flow remains controversial [62, 69]. While there is evidence to suggest that sympathetic nerves play a role in cerebral blood flow regulation in humans [26, 67, 72], including during hypoxia [6, 36], this control is likely much less than that observed in the peripheral vasculature and may be limited to regulating flow during extremes of pressure (hypo- or hypertension) [42]. Further, sympathetic influence may be primarily limited to extra-cranial vessels, in turn affecting only superficial blood flows, or cerebral inflow [33]; whereas deeper tissue may receive neural input from "intrinsic" neurones and astrocytes [23]. Alternatively, it has been suggested that the true nature of sympathetic involvement in cerebral blood flow regulation may be to support autoregulatory mechanisms, by shifting the autoregulation curve to higher pressures when needed [42]. Due to the complex interaction between sympathetic activation, peripheral blood pressure, autoregulation, and cerebral blood flow, determination of a definitive, functional role for sympathetic innervation of cerebral vessels remains elusive.

9.3.3 Neural Versus Humoral Regulation of Cerebral Blood Flow

Cerebral blood flow is altered by both neural activity and humoral stimuli. Augmented neural activation leads to local increases in cerebral blood flow via intrinsic neurovascular signalling (see Intrinsic Innervation of Cerebral Vessels) whereas humoral stimuli produce a more global increase in cerebral blood flow (due to the vascular responsiveness to hypoxia). Neurally mediated increases in cerebral blood flow can be observed during a number of visual stimulation paradigms [1, 60, 63]. These types of tasks evoke increases in blood flow in the posterior cerebral artery, which feed the occipital lobes and visual cortex, but not the middle cerebral artery, which supplies a broader range of cerebral tissue [1, 60]. Alternatively, humoral stimulation by either decreased O_2 or increased CO_2 results in increased cerebral blood flow in both the posterior and middle cerebral arteries. Although a direct comparison of the cerebrovascular responses to neural stimulation and hypoxia has not been performed, recent data from our laboratory demonstrate a heterogeneous response to visual stimulation when compared to a more global blood flow response elicited by hypercapnia (unpublished data). It would be expected that exposure to hypoxia would demonstrate a similar pattern of response. Importantly, there are also interactions between neural and humoral mechanisms. As highlighted above (see Intrinsic Innervation of Cerebral Vessels), decreased O_2 availability may facilitate a greater tendency towards neurally mediated dilation. Therefore, under hypoxic conditions there may be an augmented blood flow response to neural activation. Any potential synergy between hypoxia and neural activation on cerebral blood flow has yet to be investigated.

9.3.4 The Role of Cerebral Blood Flow in Neurocognitive Disorders

There is increasing evidence that cerebrovascular dysregulation is linked to decreased neurocognitive function [10, 16]. Sleep disordered breathing, including obstructive sleep apnoea (OSA), is characterized by repeated bouts of nocturnal hypoxia and is commonly associated with decreased cerebrovascular responsiveness [18] and neuropsychological function [3, 15]. Although the relationship between repeated bouts of hypoxia and decreased cognition remains correlative, and the mechanisms are still unknown, there is evidence to suggest that reduced cerebrovascular responsiveness [10, 16] and/or oxidative stress [56] may play a role. Treatment of OSA with continuous positive airway pressure (CPAP) restores cerebrovascular responsiveness to hypoxia [18] and improves cognitive function [15]. Similarly, the cognitive decline observed in chronic obstructive pulmonary disease (COPD) and small vessel disease patients is

related to hypoxia [66] and cerebral hypoperfusion [37]. Under these varied circumstances, micro-strokes (or transient ischemic attacks, TIAs), focal ischemia, and oxidative stress may cause neural degradation leading to cognitive impairment. However, these previous data also demonstrate a neural plasticity wherein the correction of hypoxia, through CPAP or oxygen therapy, leads to a restoration of neuropsychological function.

9.3.5 The Effect of Cerebral Blood Flow on Ventilatory Control

Just as the ventilatory response to hypoxia may influence cerebral blood flow by altering arterial PCO_2, it has been speculated that the ventilatory response to hypoxia (and hypercapnia) may, in turn, be influenced by the magnitude of the concurrent cerebral blood flow response. The mechanisms contributing to this relationship are hypothesized to be a decrease in brain PCO_2, and downregulation of the central chemoreceptors, subsequent to the rise in cerebral blood flow [48]. Evidence in support of this hypothesis demonstrates a role for cerebral blood flow in modulating the ventilatory response to hypoxia [49], as well as hypercapnia [71]. However, other studies have failed to find a linkage between changes in cerebral blood flow and the ventilatory decline observed during longer durations of hypoxia [52, 64], indicating other contributing mechanisms.

Based on the above understanding of neurovascular coupling, and the influence of hypoxia on cell signalling, it is also possible that cellular signalling within the medulla (i.e. respiratory nuclei) is altered due to hypoxia per se, secondary to changes in cerebral blood flow and PCO_2.

9.4 Future Directions

In this review we attempted to highlight our current understanding of cerebral blood flow regulation during periods of hypoxia and altitude in humans. We have also tried to emphasis some of the neurovascular interactions which may underlie cerebrovascular responses and adaptations to hypoxia and linkages to disease states. As highlighted, there remain a number of areas for further research. The cellular processes regulating the cerebral responses to changes in oxygen are not completely understood, nor are the specific mechanisms leading to short and long term adaptation and maladaptation to hypoxia. Further, the emergence of genetic technologies offers the opportunity to intricately examine the basis for variability in cerebrovascular responses between individuals and populations. Finally, translational research is required to verify and integrate data from animal, reduced preparation, and cellular models of hypoxia.

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Chapter 10
Imaging the Respiratory Effects of Opioids in the Human Brain

Kyle T.S. Pattinson and Richard G. Wise

Abstract Opioid analgesia is limited by the potentially fatal side effect of respiratory depression. In humans the brain mechanisms of opioid-induced respiratory depression are poorly understood. Investigating pharmacological influences upon breathing helps us to understand better the brain's respiratory control networks. Blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (FMRI) maps neuronal activity in the brain, and is therefore a potentially useful, noninvasive technique to investigate the functional neuroanatomy of respiratory control in humans. Contrast in FMRI is derived from the vascular response to brain activity (neurovascular coupling). Therefore, FMRI studies of the neuronal effects of opioids are rendered more complex by the non-neuronal effects of opioids including those on systemic physiology, cerebral blood flow, and direct effects on the cerebral vasculature such as altered vascular reactivity. Here we review our series of studies that dissect the vascular and neuronal breathing-related effects of opioids in the brain. These methodological considerations have enabled successful FMRI studies revealing the brain networks responsible for opioid effects upon respiratory awareness. Similar considerations would be necessary for FMRI studies in hypoxia or in disease states that affect the physiological state of the brain.

Keywords BOLD • Functional MRI • Respiration • CBF • Carbon dioxide

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10.1 Introduction

This chapter describes the challenges with functional magnetic resonance imaging (FMRI) of respiratory control and its modulation by opioid analgesics. Opioid drugs such as morphine are a mainstay for the treatment of severe pain. Respiratory depression is their most feared side effect, as it is potentially fatal [36, 39]. In humans the functional neuroanatomy of opioid-induced respiratory depression is poorly understood, because inferences from animal models may not always be applicable and invasive studies are generally not practicable. We expect that the noninvasive study of opioid-induced respiratory depression in humans will improve our understanding of the mechanisms of this effect as well as the central breathing control networks and may also suggest targets for treating respiratory depression. Respiration and opioids have nonneuronal effects that can affect interpretation of FMRI contrast, the blood oxygen level-dependent (BOLD) signal. The considerations described in this paper would be equally relevant to FMRI studies in hypoxia, drug studies, or in disease states affecting the physiological state of the brain [18, 56]. In such studies we need to consider the effects of the intervention on blood gases (O_2 and CO_2), regional baseline cerebral blood flow and regional cerebrovascular reactivity as defined from our haemodynamically sensitive FMRI signal.

10.2 Image Contrast in FMRI

FMRI noninvasively visualizes brain function, and thus is a potentially useful tool to investigate the neural control of breathing in humans, in vivo. The basis of the most commonly employed image contrast in FMRI is the BOLD response. With neural activity, there is a localized increase in cerebral blood flow (CBF), volume (CBV), and venous oxygen saturations (SvO_2) [37, 38]. As deoxyhaemoglobin is paramagnetic compared with oxyhaemoglobin, the increased SvO_2 associated with neural activity causes an increase in the T2*-weighted MR signal in the region of neural activation. A typical FMRI experiment involves repeatedly acquiring rapid T2*-weighted images and alternating periods of stimulation with “off” periods (Fig. 10.1). FMRI analysis typically uses specialized modeling software (e.g. FSL http://www.fmrib.ox.ac.uk/fsl) to examine the BOLD response to a stimulus across the brain. A recent review of the strengths and weaknesses of FMRI for studying brain function has recently been provided by Logothetis [31]. The brainstem is of particular interest in the control of breathing. However, it is unfortunately susceptible to sources of physiological noise in FMRI including breathing and cardiac cycle related signal changes. By separately recording the timing of these processes we have optimized a linear regression approach for cleaning up these noise components, increasing the sensitivity of detecting breathing related neuronal activity [21].

Arterial spin labelling (ASL) is an MRI method for the quantitative measurement of CBF that utilizes magnetically labelled water molecules as a contrast agent [7,

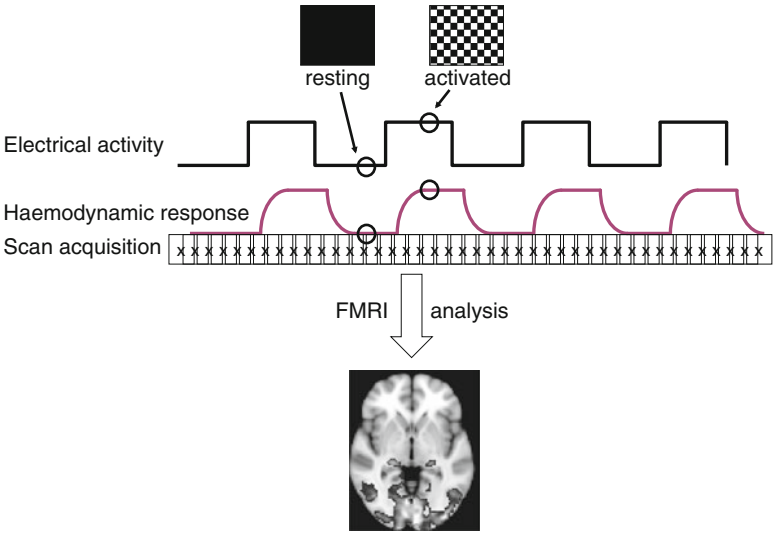

Fig. 10.1 Basic explanation of a BOLD FMRI experiment. In this visual stimulation study, a flashing chequerboard was alternated with a dark screen, for 15 s each (*top boxes*), that caused alternating periods of neuronal activity in the visual cortex. Multiple scans are acquired during this experimental sequence. The hemodynamic (BOLD) response to this electrical activity occurs a few seconds later than the neuronal activity, and this "hemodynamic" delay is incorporated into the analysis of the MR scans, to give images of "activation" in the visual cortex, in the lower part of the figure

30]. Water in arterial blood is magnetically labelled in the neck. After a delay this magnetically labelled blood arrives in the area of brain that is being imaged and a tag image is acquired. The time between labelling and acquisition of the tag image is called the inversion time. In addition to the tag image, a control image is also taken without labelling. The difference between the tag and the control image is proportional to the CBF. To obtain accurate measurements of CBF, multiple images can be taken with different inversion times, and a kinetic curve can be calculated that represents the time course of the magnetically labelled blood in the area imaged. ASL is a quantitative CBF measure, whereas BOLD depends on a complex physiological response to generate contrast (Fig. 10.2). The main disadvantage of ASL is that it suffers poor signal-to-noise.

10.3 Neurovascular Coupling

Signalling between neurones and capillaries is generally known as neurovascular coupling, and is considered to be the precursor to the increase in capillary blood flow that occurs with neuronal activity (Fig. 10.2a). Its exact mechanism remains to be fully elucidated, not least because of the wide range of potential vasoactive neurotransmitters and other species that have been identified in recent years [2, 16, 29,

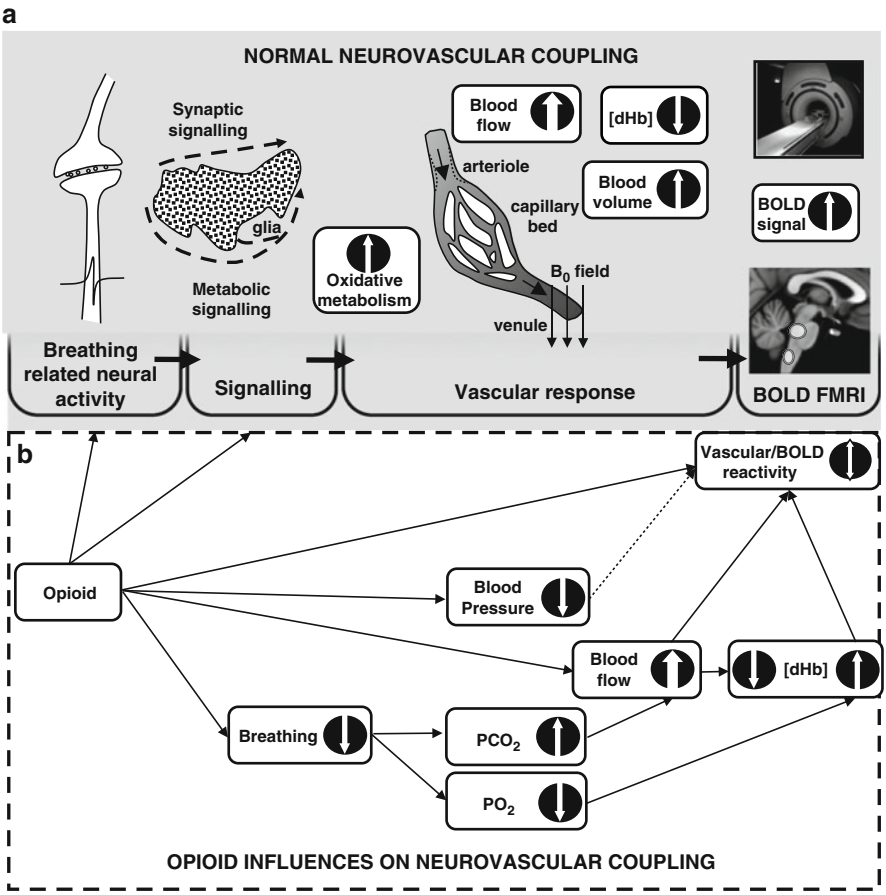

Fig. 10.2 Neurovascular coupling. The *top panel* (**a**) illustrates the pathway from neural activity, to the hemodynamic response that is the basis for the BOLD signal. Drugs, disease, and altered physiology may affect any point in this pathway, and confound interpretation of BOLD as neuronal in origin. The *lower panel* (**b**) illustrates the potential effects of opioids upon this pathway that need to be considered before interpreting BOLD changes as neuronal in origin

46, 58, 65] and the potential role of neuronal control of the vasculature [52]. Because BOLD is an indirect measure of neuronal activity, any factors that affect neurovascular coupling need to be considered before interpreting BOLD changes as neuronal in origin [24, 59] (Fig. 10.2b). This is a particularly important consideration when investigating neural effects of processes that may cause physiological derangement, (e.g. opioid drugs, hypoxia, disease states) and may be:

1. Systematic physiological effects, including
   (a) Arterial partial pressure of carbon dioxide ($PaCO_2$),
   (b) Arterial oxygen saturation (SaO_2), and
   (c) Systemic blood pressure.

2. Direct effects upon the cerebral vasculature. For example, binding to a capillary wall may affect its responsiveness to metabolic signalling or to systemic physiological changes. Drugs may also change the resting tone of the cerebral vasculature and thereby affect the extent of vasodilatation response to a neural stimulus.
3. Effects on local transmitter release or metabolic rate affecting the coupling relation ship between neuronal activity and the blood flow response. Although potentially important, there are currently no MRI methods to measure this.

10.4 Systematic Physiological Effects on Bold FMRI Signal

10.4.1 Carbon Dioxide

Opioids cause hypercapnia (raise $PaCO_2$) by depressing respiration. Hypercapnia has a profound effect upon the BOLD response (Fig. 10.2). The increased cerebral blood flow of hypercapnia leads to higher SvO_2 than in the normocapnic state because CBF and CBV increase to a much greater extent than does metabolism. With neuronal activation during hypercapnia, the neuronal-induced vasodilatation causes a lesser increase in BOLD signal than during normocapnia because of the already elevated SvO_2 and comparatively reduced flow reserve, thus dampening the stimulus-evoked BOLD response [3, 11, 12, 23, 44] (vascular reactivity in Fig. 10.2). This effect is more profound at higher $PaCO_2$ levels. Nonlinearities in the relationship between $PaCO_2$, CBF, and BOLD further complicate the interpretation of activation studies during hyper- or hypocapnia and may vary between different brain regions [9, 10, 17, 25, 35, 48].

Approaches to dealing with CO_2 related issues in BOLD imaging have traditionally aimed at maintaining end-tidal CO_2 ($P_{ET}CO_2$) constant throughout an experimental run. Various techniques including computerized [45, 53, 61], mechanical [4] or manual [36, 42] adjustment of gas mixtures have been used to maintain blood gasses constant during spontaneous ventilation. Mechanical ventilation is another excellent way to have complete control over blood gas tensions [15] although makes a study considerably more complex to perform. Such CO_2 control measures have allowed identification of brain networks associated with voluntary respiration [15, 34] and air hunger [14].

There are situations, however, where it is not possible to maintain $PaCO_2$ constant. There is currently no simple and quantitative calibration model available without severely limiting experimental design by simultaneously performing CBF measurements [23]. We have therefore employed other strategies to help to account for systematic CO_2 effects on BOLD:

1. Measuring the neuronal response to CO_2 stimulation is one such situation where by definition $PaCO_2$ cannot remain constant. In this situation, the method we used to disentangle the neuronal from vascular effects of CO_2 [41], was to compare the BOLD response to baseline "resting" fluctuations in $P_{ET}CO_2$ [60] with

the BOLD response to intermittent CO_2 challenges. The brain areas with the greatest increase in BOLD signal responsiveness to CO_2 were determined to represent an increase in neuronal activity in response to CO_2 increases.

2. FMRI investigations of the brain mechanisms of breath holding [33, 40] controlled for the CO_2 rises during the breath hold by delivering intermittent externally delivered CO_2 challenges between the breath hold tasks. During the analysis we were then able to dissociate the time course of the CO_2 changes from that of breath holding which was assumed to consist of CO_2 increases and neuronal activity associated with breath holding.
3. Our final approach to dealing with hypercapnia was to use a whole-brain arterial spin labelling technique to obtain quantitative measures of CBF prior to and during opioid administration [32]. The voxel-wise maps of CBF were incorporated into the BOLD FMRI analysis as a covariate [40]. The performance of this approach was assessed by using visual stimulation as a control measure that is thought to be unaffected by opioids (Fig. 10.3). Although nonlinearities in the BOLD-CO_2 relationship are not fully modelled, we have found this to be a useful technique in explaining and therefore accounting for the baseline-flow dependence of the neuronal BOLD response amplitude, thus permitting a fairer comparison between breathing-related neuronally induced BOLD responses arising during different states of baseline CBF.

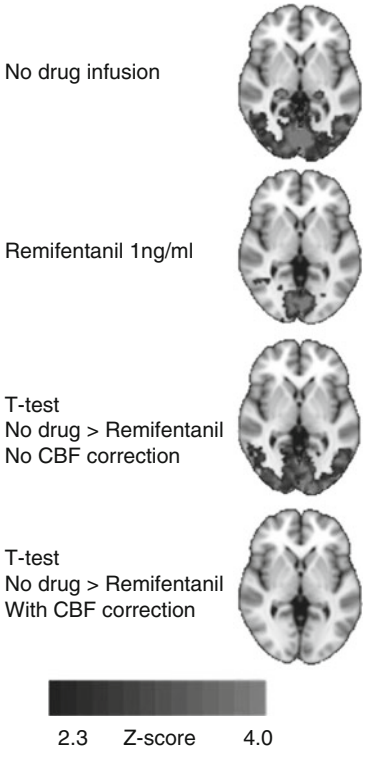

Fig. 10.3 Comparison of BOLD response to visual stimulation before and during infusion of remifentanil in a group of 11 healthy volunteers [40]. $P_{ET}CO_2$ was 5.3 ± 0.67 kPa at baseline and rose to 6.4 ± 0.8 kPa with remifentanil infusion, leading to an increase in global CBF of approximately 25 % [32]. The apparent strong difference in activation between no drug and remifentanil conditions is explained by the change in baseline cerebral blood flow that was measured on a voxel by voxel basis and incorporated into the FMRI analysis

10.4.2 Oxygen

Changes in arterial O_2 pressure (PaO_2) affect the BOLD signal, through changes in SvO_2 and through effects on CBF [19, 27, 49, 51]. The effects of hyperoxia on BOLD are discussed in more detail in the Chapter by Bulte, in this issue [8]. Our approach with imaging opioid effects in the brain was to maintain constant mild hyperoxia to avoid stimulus-correlated changes in PaO_2 [61] and also to avoid swings in oxygen saturations during periods of hypoventilation.

10.4.3 Blood Pressure

Blood pressure variations within autoregulatory limits (mean arterial pressure of 50–140 mmHg) are not associated with significant changes in CBF or the BOLD signal [64], whereas large sudden changes in blood pressure have been shown to affect the BOLD response [26]. Our experience with relatively low dose remifentanil is that changes in arterial pressure are small, and thus are not likely to be a significant factor in interpreting neuronally related BOLD signal changes.

10.5 Opioid Effects on the Cerebral Vasculature

As endogenous opioids have a demonstrated role in the regulation of CBF [6, 13, 20, 43, 55], we investigated whether the synthetic opioid remifentanil has an effect upon cerebrovascular (characterized by BOLD signal) reactivity (one component of neurovascular coupling), independent of the hypercapnia associated with respiratory depression [39]. Although clinical studies in humans suggest there is little effect of remifentanil upon global cerebral CO_2 reactivity [28], regional differences in the brain had not been so extensively investigated and it is naturally specific brain regions that are of interest when trying to identify respiratory control networks.

Therefore as a precursor to attempting to image the neuronal effects of remifentanil, we felt it necessary to be certain that any direct vascular effects of remifentanil were assessed. Hypercapnic challenges are a useful technique with which to map vascular (BOLD FMRI signal) responsiveness [42, 50, 57, 60]. We ensured that comparisons between the drug and no-drug condition were made over the same CO_2 range by training participants to breath to a target $P_{ET}CO_2$ [42].

The reasoning behind this approach was that if remifentanil were to have a receptor mediated effect on vascular responsiveness then we would expect to see a pattern of changes in the BOLD response to hypercapnia that reflects the distribution of opioid receptors [5]. Greatest effects would be expected in areas of the brain that contain the highest opioid receptor densities, including pain-processing areas [62, 63].

As we found no change in BOLD responsiveness as a function of receptor density we concluded that the binding of remifentanil to its receptors in the brain does not have a great effect upon vascular reactivity. These findings are beneficial for interpreting pharmacological FMRI with opioids. It means that changes seen in the BOLD response to a neuronal stimulus can be interpreted with more confidence as they are unlikely to be confounded by a drug effect on the cerebral vasculature, and that we can safely assume that the changes in BOLD responsiveness during remifentanil administration are only due to hypercapnia. Studies of BOLD responsiveness are particularly important in situations where profound physiological effects are likely, not just for drug studies (particularly opioids, sedatives, and anesthetics), but also in hypoxia and in disease states (e.g. diabetes).

10.6 Opioids, Breathing, and FMRI

In comparison to much of cognitive neuroscience, FMRI of the respiratory control system is still in its infancy. Although FMRI studies of respiratory control are challenging, the potential benefits for the scientific and clinical communities are great. Applications include translation of animal models of respiratory control and the investigation of drug effects on respiratory control systems. Dyspnea secondary to chronic pulmonary disease represents a major burden of disease throughout the world [1], and the neural mechanisms are poorly understood. Likewise, in asthma neural factors may have an important role in its pathogenesis [47].

To determine brain mechanisms of opioid-induced respiratory depression we firstly determined that the drug we were investigating (remifentanil) was unlikely to have independent effects upon BOLD responsiveness [42], thus leaving us with only the CO_2 confounds to deal with. Secondly a CO_2 stimulation study identified respiratory targets in the lower brain and brainstem that we could further investigate with remifentanil [41]. We refined and validated noise reduction techniques to improve FMRI quality, especially in the brainstem [21].

Our final study of respiratory depression [40] with remifentanil focused upon its effect on conscious respiratory control (breath holding). By taking measurements of subjective "urge to breathe" we were able to determine the brain areas that mediate awareness of respiration. ASL was used to correct for CBF changes (described above) in the BOLD response to neuronal activity. The "urge to breathe" was correlated with BOLD activity in the anterior insula and frontal operculum (areas that mediate conscious awareness of unpleasant sensations), which was reduced with remifentanil. Remifentanil decreased the BOLD response to breath holding in brain areas that mediate task performance (prefrontal cortex, anterior cingulate, periaqueductal gray) but had no effect in areas mediating motor control (putamen, motor cortex) and sensory motor integration (supramarginal gyrus). Higher cortical centers provide "contextual awareness" of respiration that is depressed with remifentanil, compounding the known effects of opioids in the brainstem [39].

10.7 Application to the Study of Hypoxia

As hypoxia has profound effects upon CBF and on brain function, suitably designed control studies would be necessary to fully understand the neuronal consequences of hypoxia and to disentangle systemic effects on BOLD from those of altered localized neuronal activity. Some of the considerations outlined in this Chapter would be relevant. These are likely to need to be supplemented with modelling studies to identify the quantitative effects of hypoxia on the BOLD response to neuronal activity. It appears that hypoxia depresses BOLD responsiveness [22], and a preliminary study from our group suggests that this effect may be regionally dependent [54]. Areas of potential future research include understanding the effects of hypoxia upon the brainstem respiratory control network [54], and the brain mechanisms of acute mountain sickness, which have yet to be fully investigated.

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Chapter 11
Regional Cerebrovascular Responses to Hypercapnia and Hypoxia

Douglas R. Corfield and Leanne C. McKay

Abstract A limited number of studies using differing imaging approaches suggest that there are regional variation in the cerebrovascular response to hypercapnia and hypoxia. However there are limitations to these studies. In particular, it is not clear if existing studies of hypoxia have fully accounted for the confounding effects of the changes in arterial PCO_2 on cerebral perfusion that, if uncontrolled, will accompany the hypoxic stimulus. We determined quantitative maps of grey matter cerebral blood flow using a multi-slice pulsed arterial spin labelling MRI method at 3 T at rest, during conditions of isocapnic euoxia, hypercapnia, and mild isocapnic hypoxia. From these data, we determined grey matter cerebrovascular reactivity maps which show the spatial distribution of the responses to these interventions. Whilst, overall, cerebral perfusion increased with hypercapnia and hypoxia, hypoxia cerebrovascular reactivity maps showed very high variation both within and between individuals: most grey matter regions exhibiting a positive cerebrovascular reactivity, but some exhibiting a negative reactivity. The physiological explanation for this variation remains unclear and it is not known if these local differences will vary with state or with regional brain activity. The potential interaction between hypoxic or hypercapnic cerebrovascular changes and neurally related changes in brain perfusion is of particular interest for functional imaging studies of brain activation in which arterial blood gases are altered. We have determined the interaction between global hypoxia and hypercapnia-induced blood oxygen level-dependent (BOLD) MRI signal and local neurally related BOLD signal. Although statistically significant interactions were present, physiologically the effects were weak and, in practice, they did not change the statistical outcome related to the analysis of the neurally related signals. These data suggest that such respiratory-related confounds can be successfully accounted for in functional imaging studies.

Keywords Regional cerebral blood flow • Functional brain imaging

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11.1 Introduction

It is well known that the cerebral circulation is sensitive to changes in arterial blood gas tensions; hypercapnia and hypoxia both, independently, increase cerebral blood flow. There is therefore a close linkage between the regulation of breathing and the regulation of cerebral blood flow; changes in breathing will alter the concentrations of oxygen and carbon dioxide in arterial blood, and thus cerebral blood flow. The cerebral vascular response to hypercapnia has been used to assess the integrity of the cerebral circulation and has been shown to be altered in different physiological and pathophysiological states. For example, the cerebrovascular reactivity to hypercapnia is reduced in the morning compared to the evening [17] and is lower during slow wave sleep than during the waking state [16]. It is also modified in patients with sleep disordered breathing [23] and with heart failure [18]. These disease states, along with other cardiorespiratory conditions, are also associated with reduced levels of arterial oxygen (hypoxia). Hypoxia is of clinical significance as the brain is particularly vulnerable to the effects of low oxygen [5]. The physiological response of cerebral blood flow to hypoxia has been less extensively studied than that to hypercapnia. However, the consensus is that cerebral blood flow increases in response to moderate and severe hypoxia; the response to mild hypoxia is more equivocal (e.g. [17, 25]); like hypercapnia, the response to hypoxia is altered in certain physiological states, e.g. sleep [16]. Most investigations of the cerebral blood flow response to hypercapnia and hypoxia have been performed using transcranial Doppler ultrasound; this method has high temporal resolution but is limited to the study of flow velocity changes in individual arteries. Relatively little is known about the regional responses of the cerebral circulation to these stimuli. However, any differences in such responses may be significant as they may determine the relative degree of protection afforded to different brain areas in response to such insults.

11.2 Determination of Regional Cerebral Perfusion

A number of methodologies can be used to determine regional cerebral perfusion in humans. Single photon emission computed tomography (SPECT) determines the distribution of a gamma emitting radionucleotide across the brain. Usually for perfusion-related studies the ligand ^{99m}Tc-HMPAO (hexamethylpropylene amine oxime) is used (e.g. [21]); this will distribute around the brain in proportion to blood flow. The method is limited in application as it has relatively low spatial resolution and the relatively long half-life of ligands means that repeated measurements can be made relatively infrequently. The total number of measurements are limited by radiation dose. Regional cerebral blood flow can be determined using positron emission tomography (PET) using the $H_2{}^{15}O$ as the radioligand (e.g. [26]). As with SPECT the ligand distributes around the brain in proportion to brain blood flow. The positrons emitted during the decay of the ^{15}O are each annihilated in collision with an electron producing two co-incident gamma rays at 180° to each other. This

results in a greater spatial resolution than that of SPECT and the short half-life of ^{15}O allows measurements to be repeated every few minutes. Concomitant arterial sampling allows an accurate calibration of measurements and $H_2{}^{15}O$ PET is regarded as the "gold standard" for measurements of regional cerebral blood flow. However, like SPECT the scope of such studies are limited by increasingly stringent regulations limiting total radiation doses.

Considerable information on cerebral perfusion has been obtained using blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI), a method that is safe and repeatable and has high spatial and temporal resolutions. The methodology relies on using deoxyhaemoglobin, a paramagnetic compound, as an endogenous contrast agent. In particular, changes in the concentration of deoxyhaemoglobin alter the magnetic susceptibility of the blood and produce local inhomogeneities in the magnetic field that are evident in MRI T2* images. The change in the BOLD signal can be used as a marker of the change in regional brain perfusion that accompanies changes in brain activity [32]. The phenomenon is now widely exploited as the basis for functional neuroimaging. However, the relationship between the BOLD T2* signal and brain perfusion is not straightforward; local changes in the signal will reflect changes in both local haemoglobin saturation (i.e. the ratio of HbO_2 to Hb) and local blood volume (i.e. the concentration of Hb) [4]. The relationship to local blood flow will further depend on the rate of oxygen extraction in the local tissues.

Other MRI approaches offer more direct measurements of local cerebral perfusion. In particular pulsed arterial spin labelling (PASL) methods allow repeated and non-invasive determination of the cerebral blood flow or perfusion [7, 8]. PASL determines brain perfusion by monitoring the transit of magnetically labelled blood through a region of interest. It is well known that PASL methods for determination of cerebral blood flow suffer from low SNR compared to dynamic susceptibility contrast methods using paramagnetic contrast agents; the latter, however, are not feasible for applications requiring repeated determinations of cerebral blood flow as a full elimination of the contrast agent is necessary before repeating the scan. For this reason PASL methods are increasingly used to investigate and monitor brain perfusion under normal and pathological conditions [9, 11, 33] and where changes in cerebral blood flow related to physiological stimuli [13, 30, 31] or brain activation [14, 29, 31, 34] are of interest.

11.3 Regional Cerebrovascular Response to Hypercapnia

The first study of the regional cerebral blood flow responses to hypercapnia using $H_2{}^{15}O$ PET was performed by Pinard et al. [22], who determined responses in three anaesthetized baboons. The authors reported good reproducibility in the measurements of regional cerebral blood flow using hypercapnia and no notable differences in response were reported across 12 regions of interest. Subsequently, Ramsay et al. [26] determined differences in $H_2{}^{15}O$ PET regional cerebral blood flow in cortical grey and white matter regions of interest in humans using hypercapnic and hypocapnic stimuli. The study, however, did not explore differences between different cortical

sites. Ito et al. [10] specifically tested the heterogeneity of the response the hypercapnia and hypocapnia with $H_2{}^{15}O$ PET in humans and reported significant relative hyperperfusion during hypercapnia in the pons, cerebellum, thalamus, and putamen.

Kastrup et al. [12] investigated the regional variability of the BOLD response to breath-hold-induced hypercapnia in five predetermined regions of interest. They reported that BOLD signal changes in the cerebellum and visual cortex were significantly higher than in the frontal cortex and basal ganglia. BOLD signal changes in the cerebellum were also significantly higher than in the sensorimotor cortex.

Our own unpublished data show significant heterogeneity in the BOLD response to hypercapnia (produced by increasing inspired CO_2) across the cortex. However, MRI angiography suggests that the sites of the largest signal changes to BOLD include large veins and venous sinuses which are particularly evident at the boundaries of both the visual cortex and cerebellum (Fig. 11.1).

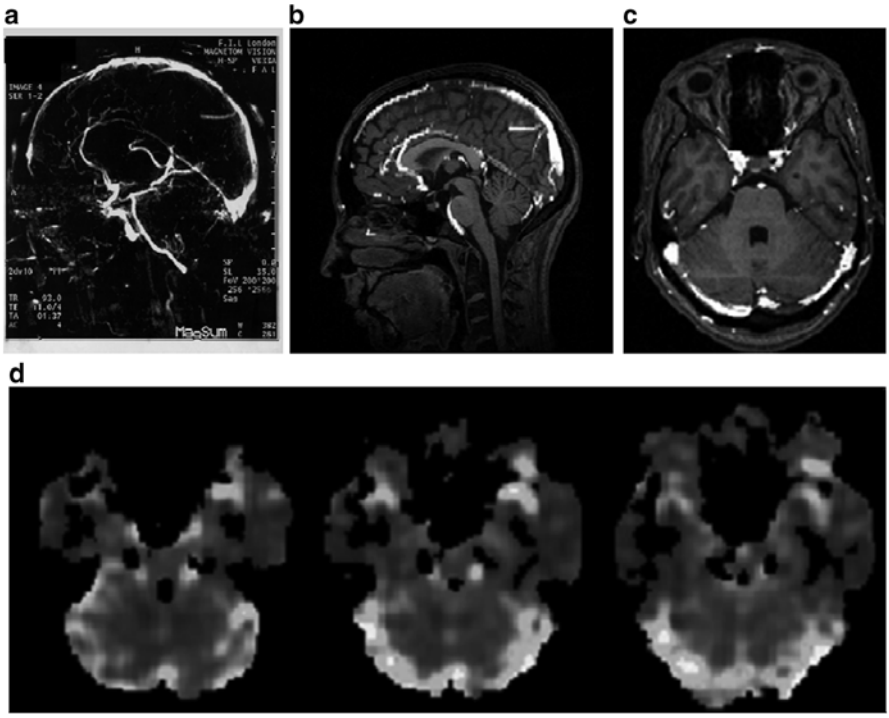

Fig. 11.1 Influence of large veins on inhomogeneities in the regional cerebrovascular response to hypercapnia. MRI angiography of venous vessels (**a**) has been superimposed upon anatomical (T1 weighted) MRI section of (**b**) the whole brain in sagittal section and (**c**) the cerebellum in transverse section. (**d**) Magnitude of BOLD signal change in response to hypercapnia in three contiguous sagittal sections of the cerebellum anatomically close to the section in (**c**). Increasing signal change is indicated in an arbitrary scale from grey to white. Note that the areas of greatest signal change in response to hypercapnia are anatomically similar to the location of large veins

A further note of caution must be made that all such mapping studies inducing hypercapnia by breath holding or by modulating inhaled gas concentrations, will necessarily include changes in neural activity associated with changes in the sensory motor task and with the "experience" associated with the changing blood gases. These neurally related signal changes must, at least in part, contribute to any observed differences in the regional cerebrovascular responses.

As described above, PASL is being increasingly used to determine regional cerebral blood flow. Typically, MRI scanners of different field strengths will be utilised for clinical investigations and the effects that this will have on quantification is uncertain. Therefore we determined cerebral blood flow at 1.5 and 3.0 T, under normo- and hypercapnia, using a pulsed arterial spin labelling technique [19]. To improve grey matter cerebral blood flow quantification white matter was excluded by using a high-resolution grey matter mask. The cerebral vascular reactivity to hypercapnia was derived from the quantitative grey matter cerebral blood flow maps. For both field strengths, the grey matter cerebral blood flow was significantly higher under hypercapnia compared to normocapnia. For both conditions, there was no significant difference of grey matter cerebral blood flow for 1.5 and 3.0 T; the same applied to the determination of cerebral vascular reactivity, which was 4.3 and 4.5 %/mmHg at 1.5 and 3.0 T, respectively. These data indicate that, with appropriate approaches, field strength will not affect quantitative determinations of cerebral perfusion.

11.4 Regional Cerebrovascular Response to Hypoxia

A limited number of studies have used perfusion-sensitive MRI to investigate the cerebral blood flow response to hypoxia. Some studies suggest that the response to hypoxia may be uniform across the brain, others indicate some inhomogeneity. However, it is not clear if these existing studies have fully accounted for the confounding effects of changes in arterial PCO_2 on cerebral blood flow that, if uncontrolled, will accompany the hypoxic stimulus [27, 28, 31].

Our laboratory determined quantitative cerebral blood flow maps of cortical grey matter with a pulsed arterial spin labelling technique at 3 T in a group of 19 subjects [20]. From these grey matter cerebral blood flow maps cerebrovascular reactivity maps to isocapnic hypoxia were calculated showing the regional distribution of the cerebrovascular reactivity (Fig. 11.2). Cerebrovascular reactivity maps of isocapnic hypoxia showed very high intra-subject variations, some grey matter regions exhibiting a positive others a negative cerebrovascular reactivity. Seventy percent of subjects showed an overall positive cerebrovascular reactivity and the remaining 30 % of subjects an overall negative cerebrovascular reactivity (Fig. 11.3); per 10 % decrease in arterial oxygen saturation, cerebrovascular reactivity to isocapnic hypoxia resulted in a 10.7 ± 2.5 % increase in cerebral blood flow in positive and in a 9.0 ± 2.6 % decrease in negative responders.

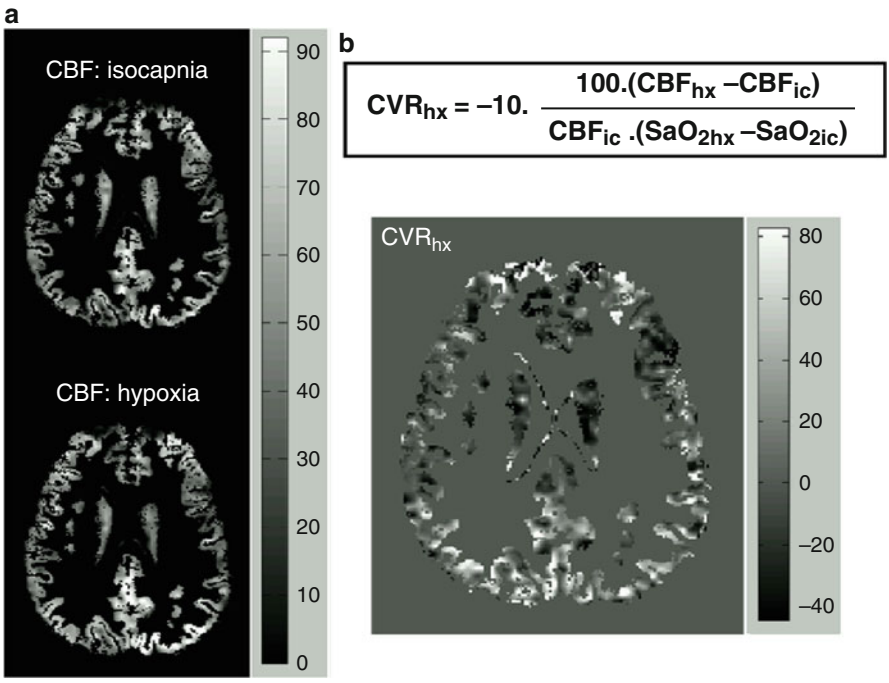

Fig. 11.2 (**a**) Quantitative grey matter cerebral blood flow maps from a single subject under isocapnia (ic) and isocapnic hypoxia (hx). Values are % change in cerebral blood flow per mmHg increase in PetCO2. (**b**) Quantitative map of cerebrovascular reactivity to hypoxia for the same subject as (**a**). Values are % change in cerebral blood flow per 10 % decrease in SaO2. Note both positive and negative values for cerebrovascular reactivity are present across the region. Modified from [20]

The high variability in hypoxic cerebrovascular reactivity between subjects could be due to conflicting physiological effects caused by hypoxia: vasodilatation and vasoconstriction. With low-level hypoxia, as applied in this study, both effects compete resulting either in an increase or a decrease of cerebral blood flow in single subjects depending on the overall dominant physiological effect in the subject studied. Also within individual subjects, there is a high variability in hypoxic cerebrovascular reactivity in grey matter; in some areas it is positive possibly indicating predominantly vasodilatation, in other regions it is negative indicating a vasoconstriction. At levels of hypoxia greater than those studied here, the vasodilator effect appears to dominate resulting in an increase in cerebral blood flow. We have also noted a vasoconstrictor effect of hypoxia in other studies from our laboratory that appear to be state dependent—this effect being prominent during slow wave sleep [16] and in the morning on awakening [17].

Recently Binks et al. [2] determined regional differences in the cerebrovascular response to moderate isocapnic hypoxia in cortical and subcortical grey matter using PET. Rather than applying an anatomical mask, white matter was excluded

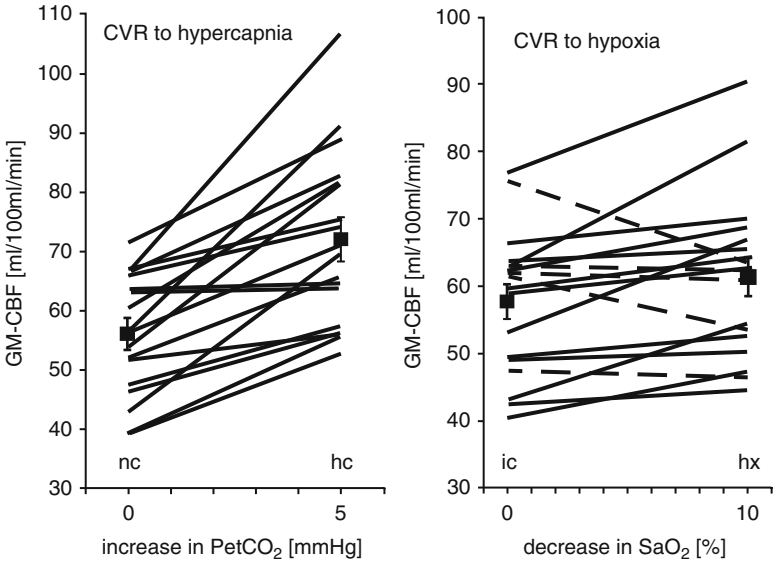

Fig. 11.3 Grey matter cerebral blood flow values for individual subjects under normocapnia and hypercapnia (*left*) and isocapnia and hypoxia (*right*). Positive and negative responders are shown with solid and dotted lines respectively. The group mean ± cerebral blood flow values for all subjects (including both positive and negative responders) are also indicated (Reproduced from [20])

by applying an absolute threshold to the cerebral blood flow measurements. They reported that generally regions of interest with higher baseline perfusion exhibited increased responses to hypoxia. Interestingly, cortical grey matter showed below average increases in response to hypoxia and phylogenetically older regions of the brain (including the putamen, brain stem, thalamus, caudate nucleus, nucleus accumbens, and pallidum) tended to show larger vascular responses.

These two complementary studies strongly indicate that there are marked differences in the cerebrovascular responses to hypoxia, within and between brain regions and across subjects, The underlying reasons for these differences remains unclear but may well determine the pathophysiological responses to hypoxia and the degree of detriment to brain tissue.

11.5 Implications for Functional Brain Imaging Studies

Neuronal activation studies using functional brain imaging are problematic when the task or stimulus is associated with any changes in the arterial blood gases: any change in arterial blood gases will change global brain blood flow such that the local neurally related change in cerebral blood flow are correlated with the global change (see Fig. 11.4). In such circumstances it is therefore not possible to uniquely

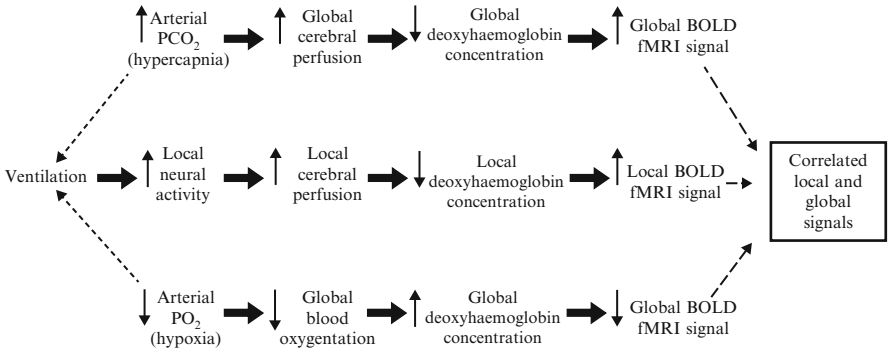

Fig. 11.4 Flow diagram summarising the effect of increased arterial PCO_2 and decreased arterial PO_2 on the local BOLD fMRI signal, which in this example is associated with an increase in ventilation

assign the change in local perfusion to being of neural origin. To address this we have developed paradigms for BOLD fMRI studies that dissociate the time course of the blood gas changes from the paradigm of interest and so dissociate the global perfusion signal from the local activation-related signals. This approach has had particular success in our study to determine the neural basis for voluntary breath holding [15]. Breath holding increases arterial PCO_2, which consequently leads to a vasodilation of the cerebral circulation and a concomitant increase in whole-brain BOLD signal that is highly correlated with local, neurally induced, BOLD signal changes of interest. To address this caveat, PCO_2 levels were manipulated throughout the experiment to dissociate the time course of the whole-brain BOLD signal from the time course of the local, neural-related BOLD signal; this allowed local activity associated with breath holding to be determined independent of whole-brain BOLD effects.

This approach is generally applicable to studies in which either CO_2 or O_2 blood gas levels change. However, one assumption for this is that there are no substantial interactions between the blood gas changes and the vascular response to activation. Changes in such neurovascular coupling could over or under estimate the degree of activation associated with a particular experimental activation. A number of studies have investigated this issue for hypercapnia and as yet the relationship between whole-brain and regional blood flow is incompletely characterised. Some studies propose that the two signals are independent and additive [6, 13, 26], while others report an interaction with an attenuation [1, 3] or augmentation [24] of the regional BOLD signal.

We have tested for an interaction between hypoxia and hypercapnia and a visual stimulus that would produce robust BOLD signal increases within the occipital lobe (visual cortex). Statistical analyses were performed in order to characterize the presence of any interactions between the effects of hypercapnia and hypoxia on whole-brain blood flow, with local changes in cerebral blood flow associated with the visual stimulus (Fig. 11.5). A first analysis determined the main effects of the visual

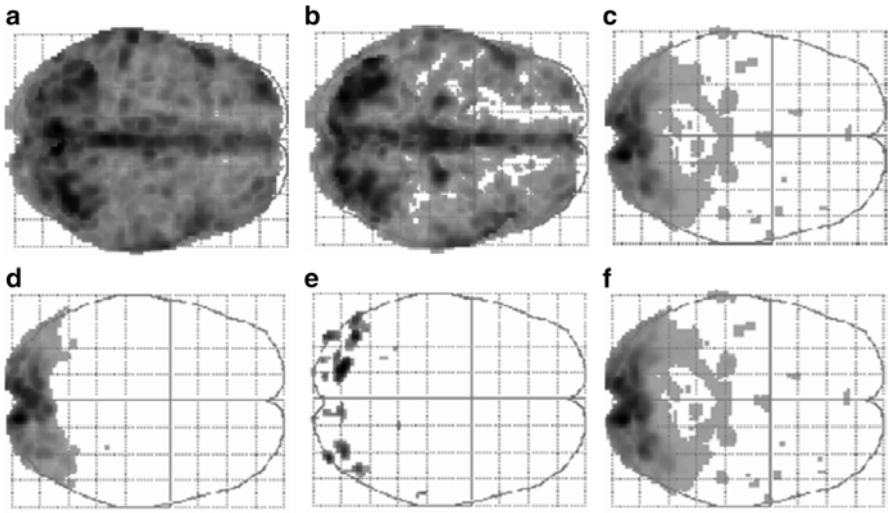

Fig. 11.5 "Glass brain" projections (viewed from above) of statistically significant changes in the BOLD signal (SPM{f} corrected for multiple comparisons $p<0.05$, $n=9$). (**a**) main effect of hypercapnia, (**b**) main effect of hypoxia, (**c**) main effect of visual stimulation accounting for the effects of hypercapnia and hypoxia, (**d**) interaction of hypercapnia and the visual stimulus (**e**) interaction of hypoxia with the visual stimulus and (**f**) main effect of visual stimulation without accounting for the effects of hypercapnia and hypoxia. Note the substantial similarity of (**c**) and (**e**) indicating that the interaction between global vascular effects and the neurally related effects has a negligible effect on the determination of the functionally related changes

stimulus and included components to model the presence of an interaction between whole-brain BOLD signal fluctuations with the visually induced changes in regional BOLD signal. A second analysis modelled only the main effects of the visual stimulus and omitted the interaction terms of the first model. These analyses identified significant interactions between whole-brain BOLD signal changes induced by hypercapnia and hypoxia and the local neurally related BOLD signal associated with the visual stimulus. Hypercapnia decreased the response to the visual stimulus and hypoxia increased the response. However, physiologically the effects were weak and, in practice, they did not change the statistical outcome related to the analysis of the neurally related signals.

11.6 Conclusions

The regional grey matter cerebrovascular responses to hypercapnia and hypoxia both show regional variation. However, this variation appears to be substantially greater for hypoxia. The reasons underlying these regional differences are unclear but may determine local neuroprotective responses to perfusion-related insults.

Correlation and interactions between global vascular changes due to hypoxia and hypercapnia and local neurally related vascular changes are a challenge to functional imaging studies. However, with appropriate study design, such respiratory-related confounds can be successfully accounted for.

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Chapter 12
Implications of Oxygen Homeostasis for Tumor Biology and Treatment

Boyan K. Garvalov and Till Acker

Abstract Tumors serve as a prototype system to study the role of the hypoxic microenvironment and gain insight in the regulation oxygen homeostasis. A series of biochemical and cell biological studies have significantly extended our knowledge of how tumor cells activate key regulatory mechanisms of oxygen homeostasis not only to adapt to the hostile tumor microenvironment but also to acquire a more aggressive tumor phenotype. Reduced oxygen levels and tumor-specific genetic alterations synergistically drive tumor progression by activating a key transcriptional system, the hypoxia inducible factors (HIFs). HIFs trigger a set of adaptive responses commonly associated with tumor malignancy including tumor angiogenesis, a shift in metabolism, proliferation, invasion, and metastasis. We and others could demonstrate that cancer stem cells are controlled by HIFs within a hypoxic niche, establishing an intriguing link between the well known function of hypoxia in tumor growth and stem cell biology. Additionally, HIF activation potentially conveys resistance to current tumor therapies including the evasive resistance phenotype observed after anti-angiogenic treatment. Together, these findings provide strong evidence that activation of the HIF system is a decisive step in cancer progression that critically shapes therapy response and clinical outcome. Recent insight into the precise mechanisms of oxygen sensing and signalling has offered new promising and potentially selective strategies to counteract this crucial pathway.

Keywords Anti-angiogenesis • Cancer stem cells (CSCs) • Epithelial mesenchymal transition (EMT) • Glioblastoma • Hypoxia • Hypoxia inducible factor (HIF) • Prolyl hydroxylase (PHD) • Tumor therapy

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12.1 Introduction

Oxygen is an indispensable substrate for aerobic metabolism and is therefore essential for the normal development and functioning of higher organisms. The efficient distribution of oxygen to tissues is a main function of the respiratory and cardiovascular systems. However, oxygen availability can vary greatly among individual cells and organs or during specific stages of development. While the arterial oxygen partial pressure is 90 mmHg (corresponding to ~12.5 %) under physiological conditions, differences in vascularization, tissue diffusion properties, and cell-specific oxygen consumption create a heterogeneous O_2 distribution, so that most tissues are exposed to lower oxygen concentrations. In particular, the brain experiences low O_2 levels down to 1 mmHg [24]. Reduced levels of oxygen (hypoxia) activate a set of adaptive responses which either enhance oxygen delivery or decrease oxygen consumption to promote survival under low oxygen conditions. Importantly, hypoxia arises not only in physiological situations, but is also a characteristic feature of various pathological conditions [1]. Particularly prominent among those is the process of neoplastic transformation and progression. Tumor growth and progression occurs as a result of the cumulative acquisition of genetic and epigenetic alterations in individual cells, followed by the selection of tumor cell clones with enhanced proliferation and survival potential. Once a tumor is formed, it creates a specialized microenvironment, which critically controls tumor progression. Highly proliferating tumors frequently outstrip their vascular supply leading to a tumor microenvironment characterized by low oxygen tension, low glucose levels, and an acidic pH. Tumor hypoxia is associated with an increased frequency of tumor invasion and metastasis and a poor therapy outcome. Notably, tumor cells not only adapt to survive under low oxygen, but also exploit hypoxia-induced mechanisms in order to promote their own growth and dissemination. Indeed, tumor hypoxia has become one of the main settings to study the mechanisms and functions of hypoxic signaling. Here, we will briefly summarize the mechanisms of cellular oxygen homeostasis and will focus on the function of hypoxic signaling in various aspects of cancer progression and resistance, as well as on possible strategies to target the hypoxic response as an anti-tumor therapy.

12.2 The Hypoxic Response and HIF

Since their discovery in 1995 [105], the hypoxia inducible transcription factors (HIFs) have emerged as the key transcriptional system initiating adaptive responses to hypoxia. HIFs act as heterodimers composed of a shared, stable HIFβ subunit and specific oxygen-regulated HIFα subunits. The stability of the α subunits is mainly controlled by prolyl hydroxylase domain proteins (PHDs), which use O_2 as a substrate to hydroxylate HIFα and target it for proteasomal degradation [15, 23, 62], following ubiquitination by the E3 ubiquitin ligase pVHL [48, 70]. An additional level of oxygen-dependent control is conferred by another hydroxylase, factor

inhibiting HIF-1α (FIH1), which modifies asparaginyl residues and inhibits the interaction between HIFα and its transcriptional coactivators p300/CBP [51]. More recently a number of additional mechanisms that modulate the HIF pathway have been identified, including the heat shock proteins HSP90 and HSP70, the histone deacetylases Sirt1 and Sirt6, TCA cycle-related metabolites, nitric oxide, the PHD E3 ubiquitin ligases Siah1/2, the microRNAs miR-17-92, and miR-107, as well as a number of oncogenes or tumor suppressor genes, e.g. PI3K/Akt, mTOR, Ras, p53 (reviewed in [69]). This high level of complexity of HIF regulation, which often involves an elaborate set of negative and positive feedback mechanisms [41], allows for precise, fine-tuned control of hypoxia-mediated responses and highlights the central importance of HIF signaling in cellular homeostasis [3]. In addition, hypoxia initiates adaptive responses independent of HIF through other redox sensitive systems, including activation of the NF-κB pathway, or global protein synthesis inhibition through the AMPK/mTOR or PERK/eIF2α pathways [11, 100], although the mechanisms mediating the O_2 dependence of these processes are less well understood.

There are two principal HIFα subunits, HIF-1α and HIF-2α, which mediate the hypoxic response through transcriptional regulation of an ever-growing number of genes. Although the functions of HIF-1α and HIF-2α partially overlap, it is now clear that the two isoforms are differently regulated by oxygen, are expressed in distinct normal and neoplastic cell types, possess different target specificities and generally appear to have complementary rather than redundant functions, as described in more detail in the following sections.

12.3 The Hypoxic Response and the Hallmarks of Cancer

Hypoxic signaling activates a large number of downstream biological responses, which together promote most of the defining properties of tumors (Fig. 12.1.) [4, 11]. One of the primary effects of hypoxia is the induction of a shift in cellular metabolism from oxidative phosphorylation to anaerobic glycolysis via HIF-mediated upregulation of glucose transporters and glycolytic enzymes [60]. This is accompanied by the upregulation of carbonic anhydrase IX (CA IX), transporters for lactate, H^+, HCO_3^- and other ions, as an adaptive mechanism of pH homeostasis, leading to a net acidification of the extracellular tumor environment. A decreased pH is a characteristic feature of many tumor types and has been shown to promote tumor growth and metastatic spread, at least in part through activation of extracellular matrix-degrading enzymes [18]. Another key response to tumor hypoxia is the stimulation of angiogenesis in order to improve the blood and oxygen supply of tumor cells. It is well established that tumors induce the formation of new vasculature as a key event in multistage carcinogenesis, a phenomenon termed “angiogenic switch” [8]. This is mainly accomplished through the upregulation of multiple angiogenic genes as direct HIF targets, including components of the VEGF, angiopoietin, and SDF-1 pathways [85].

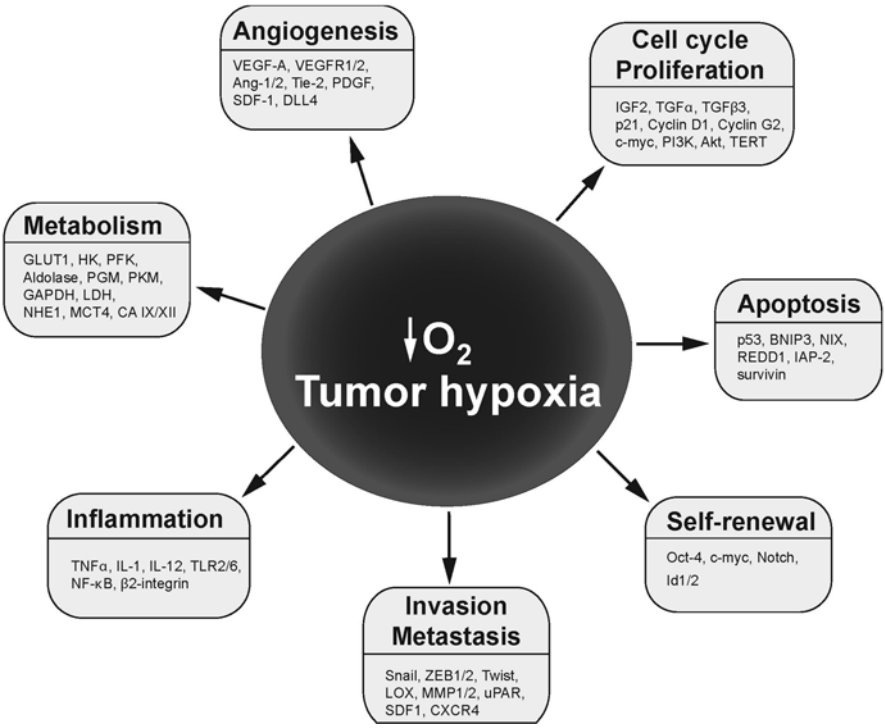

Fig. 12.1 Major aspects of tumor biology regulated by hypoxia. HIFs regulate multiple cellular processes relevant to tumor progression in response to hypoxia through trancscriptional upregulation of HIF-1α and/or HIF-2α target genes. In some cases, however, alternative mechanisms are involved, such as HIF-mediated protein stabilization (p53) or modulation of transcriptional activity (c-myc, Notch). *Ang-1/2* angiopoietin 1/2, *BNIP3* BCL2/adenovirus E1B 19 kDa interacting protein 3, *CA IX/XII* carbonic anhydrase IX/XII, *CXCR4* C-X-C chemokine receptor type 4, *DLL4* delta-like protein 4, *GAPDH* glyceraldehyde-3-phosphate-dehydrogenase, *GLUT1* glucose transporter 1, *HK* hexokinase, *IAP-2* inhibitor of apoptosis protein 2, *ID-1/2* inhibitor of DNA binding 1/2, *IGF2* insulin-like growth factor 2, *IL-1/12* interleukin 1/12, *LDH* lactate dehydrogenase, *LOX* lysyl oxidase, *MCT4* monocarboxylate transporter 4, *MMP1/2* matrix metalloproteinase 1/2, *NHE1* Na+/H+ exchanger 1, *NIX* NIP3-like protein X, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *PDGF* platelet-derived growth factor, *PFK* phosphofructokinase, *PGM* phosphoglycerate mutase, *PKM* pyruvate kinase M, *PI3K* phosphatidylinositol 3-kinase, *REDD1* protein regulated in development and DNA damage response 1, *TERT* telomerase reverse transcriptase, *TGF* transforming growth factor, *Tie-2* tunica interna endothelial cell kinase 2, *TLR2/6* Toll-like receptor 2/6, *TNF* tumor necrosis factor, *SDF-1* stromal cell-derived factor 1, *uPAR* urokinase plasminogen activator receptor, *VEGF-A* vascular endothelial growth factor A, *VEGFR1/2* vascular endothelial growth factor receptor 1/2, *ZEB1/2* zinc finger E-box-binding homeobox 1/2

Hypoxia also plays an important role in the regulation of tumor cell proliferation and cell cycle progression, via the control of growth factors (e.g. TGFs, IGFs), oncogenes (c-myc), the PI3K/Akt pathway, p21, cyclins, and telomerase [88, 92]. The regulation of tumor cell death is another major aspect of cancer cell biology modulated by the hypoxic response. HIFs can activate proapoptotic genes such as

BNIP3, NIX, and REDD1 [92] and can stabilize p53 [6]. However, tumor cells develop various mechanisms to evade hypoxia-induced cell death, e.g. by HIF-dependent upregulation of anti-apoptotic molecules such as IAP-2 and survivin or by exerting a selective pressure to acquire p53 mutations under hypoxia [34, 36, 78]. Moreover, we have shown that HIF-1/2α induce the expression of PHD2 and PHD3, which act in a negative feedback loop even under low O_2 concentrations to protect tumor cells against hypoxia-induced cell death by dampening the HIF response [42].

Solid tumors trigger an intrinsic smoldering inflammatory response modulated so as to create a protumorigenic environment, which plays a critical role at different stages of tumor progression [37]. Hypoxia/HIFs are central mediators of this process by modulating key aspects of immune cell function and inflammation to promote an immunosuppressive environment. This is mediated for example through the control of immune cell adhesion (via induction of β2 integrin), expression of toll-like receptors, production of NO, proinflammatory cytokines (e.g. TNF, IL-1, IL-12), and activation of NF-κB signaling [47, 76]. An additional central aspect of cancer progression that is under the control of hypoxia is tumor cell invasion and metastasis. Hypoxia can induce the process of epithelial-mesenchymal transition (EMT), which is thought to be an essential early determinant of metastatic dissemination [109]. Hypoxic signaling suppresses the expression of the epithelial cell adhesion molecule E-cadherin, an essential step of EMT [26], through HIF-1α or HIF-2α-dependent upregulation of EMT transcriptional repressors of the SNAI, ZEB, or TWIST families [27, 46, 59, 61, 110]. Furthermore, hypoxia can promote metastasis via upregulation of pro-invasive and metastatic HIF target genes including extracellular matrix-remodelling proteins such as lysyl oxidase (LOX) and urokinase plasminogen activator receptor (uPAR) [25, 58], matrix metalloproteinases [94] and the prometastatic chemokine receptor CXCR4 [79, 98, 111].

Additionally, hypoxia plays an essential role in the regulation of the self-renewal and differentiation of physiological stem cells in a variety of tissues, at least in part through the transcriptional activation of central stem cell regulators such as Oct4, Notch, and c-myc [53]. Importantly, work by us and others has demonstrated that hypoxia also has a critical function in the maintenance of cancer stem cells (CSCs), a population of tumor cells with properties of stem cells, that drives tumor initiation and progression [30]. Hypoxia promotes the self-renewal of CSCs, particularly in glioblastoma [40, 72, 91], while HIF knockdown blocks this effect and reduces CSC-mediated tumor growth [65, 73, 91, 95]. Current evidence indicates that HIF-2α is the main isoform to promote CSC maintenance as HIF-2α, but not HIF-1α, is highly expressed and strongly upregulated by hypoxia in glioma CSCs [65], enhances the CSC phenotype [91] and promotes tumor growth [40]. Moreover, HIF-2α regulates crucial signaling pathways that are involved in stem cell maintenance. HIF-2α interacts with and stabilizes the Notch ICD (intracellular domain), enhancing Notch signalling to control cellular differentiation [38]. Additionally, HIF-2α transcriptionally regulates Oct4 [21], a transcription factor that is important for the maintenance of the self renewal of embryonic stem cells and one of four factors necessary to induce pluripotency [99].

12.4 The Hypoxic Response and Tumor Progression

The brief overview provided above highlights the central role of hypoxic signaling in promoting the "hallmarks of cancer" [39, 88]. It is therefore not surprising that hypoxia and HIFs have been associated with tumor initiation and progression and a worse clinical outcome. It has been shown for a number of different cancer types that hypoxia correlates with a more aggressive tumor phenotype, including enhanced angiogenesis, metastasis, recurrence, therapy resistance, and decreased patient survival [49]. In comparison to adjacent tissue widespread HIF activation can be seen in various tumors types, correlating with tumor growth and progression. HIF overexpression in tumors is a result of hypoxia-dependent and hypoxia-independent mechanism such as oncogenic mutations or enhanced growth factor signalling. Various oncogenic mutations can directly lead to HIFα stabilization. For example, genetic alterations of pVHL are a characteristic feature of clear cell renal cell carcinomas and are linked to accumulation predominantly of HIF-2α [50]. In addition, activating mutations of PI3K, Akt, and Ras, as well as inactivating mutations of PTEN and TSC2 result in enhanced HIF-1α transcription, translation, or stabilization [14, 31]. An elevated expression of HIFs has been associated with multiple cancers, based on immunohistochemical analysis. HIF-1α has been found to be upregulated compared to the nonmalignant tissue in a broad variety of tumor types, including oligodendroglioma, breast, cervical, colon, ovarian, endometrial, lung, prostate, bladder, pancreatic, and oropharyngeal cancer [49, 92]. In most of these cancers higher HIF-1α levels have been associated with poor patient survival [11]. Elevated HIF-2α, on the other hand has been linked to worse prognosis in a distinct set of tumor entities, including clear cell renal carcinoma, non-small-cell lung carcinoma, head and neck squamous cell carcinoma, neuroblastoma, and glioma [65, 82].

In support of a distinct, nonredundant function of the two main HIFα isoforms, HIF-1α overexpression correlated with decreased patient mortality in head and neck cancer and non-small-cell lung cancer, in both of which HIF-2α showed the opposite association [92]. Furthermore, silencing of HIF-2α, but not HIF-1α, in a number of cancer cell lines reduced cell proliferation and tumor growth [29]. Such functional differences between HIF-1α and HIF-2α could be due to differential expression in the different tumor entities, as well as to the distinct sets of target genes controlled by the two isoforms [41]. In addition, the two HIFα subunits can modulate the activity of key oncogenes or tumor suppressors in opposing fashion. For example, HIF-1α antagonizes myc transcriptional activity, while HIF-2α promotes it [32]. Renal cell carcinomas that express HIF-2α but not HIF-1α upregulate myc target genes, have increased proliferation and enhanced resistance to replication stress [33]. HIF-1α and HIF-2α also have contrasting effects of the function of p53: while HIF-1α binds to p53 and stabilizes it [6, 75], HIF-2α indirectly suppresses p53, promoting radio- and chemoresistance [10, 86]. Compared to HIF-1α, HIF-2α accumulates at higher oxygen concentrations [43, 108], which more closely resemble the in vivo conditions under which tumors arise and grow. At the same time,

while HIF-1α gets only transiently upregulated under chronic hypoxia, HIF-2α levels remain elevated in these conditions [44]. In addition, HIF-2α appears to be the primary isoform regulating the self-renewal capacity of the CSC pool [65, 91], which may have different contributions to the progression of distinct tumor entities [54, 83].

Although HIFs are typically perceived as protumorigenic molecules, in some settings they can also act as tumor suppressors. For example, HIF-1α-deficient teratomas grow faster due to the refractoriness of the mutant tumor cells to stress-induced apoptosis [16]. Similarly, HIF-1α and HIF-2α were stabilized in Vhl−/− ES cells, but the resulting teratomas were smaller than in controls [68]. Furthermore, HIF-2α overexpression in glioma cells enhanced apoptosis and decreased tumor growth, whereas HIF-2α inhibition or genetic deletion had the reverse effect [2]. The complexity of HIF function in tumorigenesis was further highlighted by studies using a Kras mutant lung tumor model. Expression of nondegradable HIF-2α in this system increased tumor burden, angiogenesis, EMT, and decreased survival [55]. Paradoxically, deletion of HIF-2α in the same model also promoted tumorigenesis, whereas HIF-1α deletion had no apparent effect [71]. Such contrasting results indicate that the effect of HIFs on tumor progression is likely to depend on the cellular context as well as the precise extent of functional inhibition or activation of specific isoforms and the balance of competing signaling pathways that can be activated by their stimulation or suppression [69].

In glioma, hypoxia plays a prominent role in several aspects. First, the characteristic necrotic regions which represent one of the key criteria for the histological diagnosis of glioblastoma (GBM) are associated with hypoxia [49]. Hypoxia and the activation of HIF also contribute to the second characteristic feature of GBM, the high degree of vascularization [2]; the GBM microcirculation, however, is leaky and functionally inefficient, failing to restore normal oxygenation [103]. Glioma cells overexpress HIF-1α and HIF-2α both in culture and in situ, especially in the perinecrotic pseudopalisading areas [49, 91]. Interestingly, the cells found in those regions have been implicated in hypoxia-induced migration away from the necrotic areas [12, 84]. Furthermore, the expression of classical HIF target genes like CA IX and glucose transporter 1 (Glut-1) correlates with higher brain tumor grade and poor response to treatment [28, 45, 52, 67, 89, 96, 102, 112]. Finally, as discussed above, hypoxia and HIF-2α play a particularly prominent role in the maintenance of glioma CSCs within a hypoxic niche, which is thought to be responsible for determining key aspects of GBM malignancy [30].

12.5 Hypoxia and Therapy Resistance

The capacity of hypoxia to protect tumor cells from radiation damage was first noted in the 1950s and has been extensively corroborated since then (reviewed in [11]). In GBM, for example, elevated hypoxia before radiotherapy is strongly

associated with decreased time to progression and patient survival [97]. In addition, hypoxic cells have an increased resistance to a variety of standard chemotherapeutic agents [101]. Hypoxic signaling converges on multiple pathways that contribute to therapy resistance. For example, hypoxia selects for cancer cell clones with mutant p53, a key mediator of therapy-induced apoptosis [34]. Additionally, in GBM cells hypoxia induces the activation of the antiapoptotic protein Bad and subsequent inhibition of programmed cell death [74]. Moreover, the multidrug resistance gene MDR1 is a direct HIF-1α target, which can mediate the efflux of chemotherapeutic drugs [20, 106]. The ability of hypoxia to increase the CSC pool, as discussed above, may provide an additional explanation for the decreased sensitivity of hypoxic tumors to treatment. Indeed, a series of studies have demonstrated that CSC have enhanced resistance to chemo- and radiotherapy [30]. This is due to a combination of properties characteristic of CSCs, including the high expression of ABC drug pumps, relative quiescence, resistance to oxidative DNA damage and enhanced DNA repair capacity [7, 90]. The increased resistance of CSCs, combined with the ability of only a very small number of CSCs to reinitiate tumor growth is thought to be a major reason for cancer persistence and relapse after treatment.

Anti-angiogenic therapies have become an established tool in the treatment of several cancers, including colorectal, lung, breast cancer, and GBM [66]. However, following the initial wave of enthusiasm, it has become clear that the inhibition of angiogenesis has complex consequences and smaller than expected benefits for cancer patients. Typically tumor shrinkage is initially observed, but this is followed by adaptation and renewed growth of the tumor, often resulting only in extension of progression-free survival, but not overall survival. The major problem underlying the relative inefficacy of angiogenic therapies is that tumors quickly adapt and manage to circumvent them. The mechanisms of this “evasive resistance” are poorly understood, but preclinical studies have started to suggest several possible explanations. By definition, anti-angiogenic agents are designed to curtail the blood supply of the tumor, thus inducing tumor hypoxia. This may provide one key explanation of the limited therapeutic efficiency of current anti-angiogenic drugs, since hypoxia activates a number of mechanisms that contribute to the evasive resistance following anti-angiogenic therapy. As discussed above, several alternative proangiogenic signals are HIF target genes. In addition, hypoxia enhances the recruitment of bone marrow derived cells to the tumor, which can promote the formation of blood vessels either through secretion of cytokines and growth factors or through direct differentiation into blood vessel cells, such as endothelial cells or pericytes [8]. Interestingly, inhibition of angiogenesis also elicits enhanced local invasion and distant metastasis in different tumor types, including breast tumors, pancreatic neuroendocrine tumors, melanoma, and glioma [22, 77]. The elevated hypoxia observed under these conditions [77] suggests several possible mechanisms for anti-angiogenesis driven metastasis, as outlined in the previous sections, however, the precise pathways involved in the evasive resistance phenotype remain to be elucidated.

12.6 Strategies for Therapeutic Targeting of Tumor Hypoxia

Based on the realization that hypoxia plays a key role at various steps of tumor progression and resistance, substantial effort has been invested in targeting or exploiting tumor hypoxia as an anti-cancer therapeutic strategy (Fig. 12.2). Early attempts were aimed at preventing hypoxia by increasing tumor oxygenation during irradiation, but the clinical efficacy of such interventions was unsatisfactory [11]. Given the relevance of the hypoxic tumor fraction in shaping the tumor phenotype, a different strategy is to take advantage of the hypoxic state of tumor cells in order to selectively eliminate them. Several chemical classes of agents have been proposed which can be specifically converted from a nontoxic to a toxic form by reduction under low oxygen conditions [13]. An example of such a hypoxia activated prodrug is tirapazamine. In hypoxic cells, it gives rise to free radical species which block the function of topoisomerase II and lead to double-stranded DNA breaks. Phase III clinical trials of tirapazamine in combination with chemotherapy have demonstrated benefits in lung cancer patients [104]. Another bioreductive prodrug, the potent DNA intercalator and topoisomerase poison AQ4N, has shown selective activation in hypoxic regions in phase I trials [5]. Other classes of drugs (e.g. CB 1954 or SN 23862) are designed to release more stable cytotoxins upon reduction, which can diffuse away from hypoxic cells and kill additional cells in the tumor, in a "bystander effect" [13].

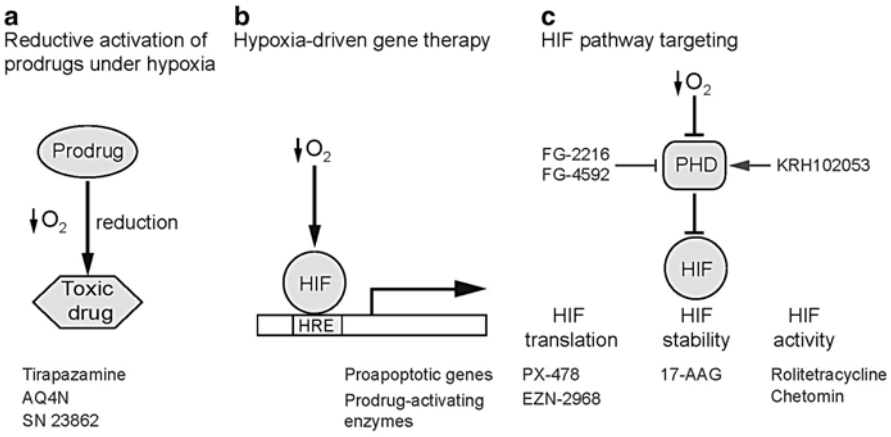

Fig. 12.2 Strategies for therapeutic targeting of tumor hypoxia (**a**) The hypoxic state of tumor cells can be exploited for the chemical conversion of prodrugs into a toxic form under the reducing conditions created by the shortage of O_2. (**b**) The stabilization of HIFs under hypoxia can also be used for driving the expression of cytotoxic gene products in hypoxic cells. (**c**) A third strategy is to interfere with the tumor promoting functions of hypoxic signalling, e.g. by specific targeting of components of the HIF pathway. Shown below the schemes are specific compounds or types of genes that are being explored in preclinical studies or clinical trials. FG-2216 and FG-4592 are PHD inhibitors currently in clinical trials for renal anemia [9, 17, 69]

An alternative set of approaches in preclinical development aims to exploit tumor hypoxia for the selective activation of gene expression. Such hypoxia-targeted gene therapy approaches involve constructs containing HIF-binding sequences (hypoxia response elements (HREs)), which are virally transduced into tumor cells and drive transcription of therapeutic genes in cells that experience hypoxia. Examples of such strategies include the hypoxia-dependent expression of proapoptotic genes [87] or of prodrug-activating enzymes [93]. Others have designed conditionally replicative oncolytic viruses that are specifically activated in hypoxic cells, causing their lysis [81]. A somewhat different approach consists in the coupling of diphtheria toxin to the oxygen-dependent degradation domain (ODD) of HIF-1α [57]. Under normoxic conditions such a fusion protein would be targeted for proteasomal degradation following ubiquitination of the ODD, however, under hypoxia it would be stabilized allowing the toxin to kill the hypoxic cell.

The largest group of hypoxia-targeting agents in current development are centered around HIF and the molecules that regulate its stability and function. Being a transcription factor, HIF represents a challenging target, but several different approaches have provided interesting hits. Interestingly, a surprisingly broad array of established drugs has been shown to suppress HIFα stability or activity. Examples include the HSP90 antagonists geldanamycin and 17-AAG, the histone deacetylase inhibitors trichostatin A and FK228, the DNA intercalating agents doxorubicin, daunorubicin, and acriflavine, the topoisomerase inhibitor topotecan, cardiac glycosides, as well as inhibitors of central signal transduction pathways like Ras/MAPK, PI3K/Akt, and mTOR [63, 64, 80]. While there is evidence that some of the anticancer effects of these drugs may be mediated by HIF inhibition [63, 64], the diversity of "nonselective" compounds that block HIF may rather be seen as an indication of the central role of this protein in the control of cellular homeostasis, than as an optimal strategy for the design of HIF-targeted therapies. More specific strategies to suppress HIF activity include the blockade of HIF-1α/HIF-1β dimerization or HIF binding to p300/CBP, which has been achieved with small molecule inhibitors such as rolitetracycline, chetomin, or YC-1 [80]. A specific inhibitor of HIF-1α translation, PX-478, exhibited antitumor activity against human xenografts and is currently in phase I clinical trials [56, 107]. The RNA antagonist of HIF-1α, EZN-2968, inhibits tumor cell growth and is also being tested in phase I clinical trials [35, 69]. A further possibility for suppressing HIF function is to promote activation of PHDs. For instance, a potent small molecule activator of PHD2, KRH102053, has been shown to decrease HIF-1α levels in tumor cells [19]. In principle, a variety of RNAi or gene therapy approaches, e.g. aimed at the silencing of HIFs, expression of dominant negative HIF mutants or overexpression of PHDs are potentially powerful alternative treatment strategies, provided that safe and efficient methods for clinical delivery become available.

It has to be noted that nearly all therapeutic agents so far have been targeted against the more ubiquitous family member HIF-1α. However, as discussed in the previous sections, HIF-2α plays a dominant role in some tumor types or subpopulations of tumor cells, such as CSCs. Small molecule inhibitors have been identified, which suppress HIF-2α translation in renal cell carcinoma cells through a mecha-

nism dependent on an iron response element in the 5′ UTR of the HIF-2α mRNA; however, the same compounds also decreased the levels of HIF-1α, albeit via unrelated mechanisms [113]. Therefore concentrating greater efforts on specifically targeting the HIF-2α isoform remains an important objective for future drug discovery screens. In addition, given that under some circumstances HIFs can also elicit tumor suppressive functions (see above), exploring HIF activating strategies, e.g. by using PHD inhibitors, may in some cases also prove valuable. Our own work, for example, has shown that PHD inhibition in GBM cells facilitates cell death induction by staurosporine or TRAIL [42].

Our growing understanding of the mechanisms mediating the hypoxic response and the signaling pathways involved in its regulation have allowed us to more fully comprehend central aspects of tumor cell biology and malignant progression. Our deepened knowledge of O_2 homeostasis in tumors has formed the basis for the design of novel therapeutic strategies targeted at hypoxic signaling, which carry the potential to become a powerful weapon in our battle against cancer.

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Chapter 13
Hyperoxia and Functional MRI

Daniel Bulte

Abstract Oxygen plays a fundamental role in functional magnetic resonance imaging (FMRI). Blood oxygenation level-dependent (BOLD) imaging is the foundation stone of all FMRI and is still the essential workhorse of the vast majority of FMRI procedures. Hemoglobin may provide the magnetic properties that allow the technique to work, but it is oxygen that allows the contrast to effectively be switched on or off, and it is oxygen that we are interested in tracking in order to observe the oxygen metabolism changes. In general the changes in venous oxygen saturation are observed in order to infer changes in the correlated mechanisms, which can include changes in cerebral blood flow, metabolism, and the fraction of inspired oxygen. By independently manipulating the fraction of inspired oxygen it is possible to alter the amount of dissolved oxygen in the plasma, the venous saturation, or even the blood flow. The effects that these changes have on the observed MRI signal can be either a help or a hindrance depending on how well the changes induced are understood. The administration of supplemental inspired oxygen is in a unique position to provide a flexible, noninvasive, inexpensive, patient-friendly addition to the MRI toolkit to enable investigations to look beyond statistics and regions of interest, and actually produce calibrated, targeted measurements of blood flow, metabolism or pathology.

Keywords MRI • Oxygen • Calibration • $CMRO_2$

13.1 Introduction

The inspiration of supplemental oxygen has been used in conjunction with magnetic resonance imaging (MRI) for over a decade [27, 48]. However, due to the complexity of its interaction with both a subject's physiology and with the inherent MR signal it has not been generally adopted by either the research or clinical communities. The interaction of the components of the BOLD signal itself is still not entirely elucidated and so the addition of a further potentially confounding factor that has

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the potential to alter both the physiology and the behavior of the MR parameters is sufficient to make most users wary. It is, however, this very behavior that makes supplemental oxygen such a valuable mechanism for investigating vascular and metabolic physiology and pathology. By considering the effects that hyperoxia will have on each element of the imaging process; from physiological effects to the changes to the resonance properties of the molecules in the system; we can form a basis from which to explore the potential of the techniques.

Hyperoxia is an increase in the inspired level of oxygen above the normal concentrations leading to an increase of the partial pressure of oxygen in the tissues and organs. As the normal oxygen tension at sea level is approximately 20.9 %, hyperoxia is a term that can mean any increase in the oxygen tension brought about by the inhalation or administration of oxygen that will increase levels beyond this. As such hyperoxia is rather a catchall term that can refer to a range of circumstances. From a research or clinical perspective the most common and convenient method of inducing hyperoxia is by the administration of supplemental oxygen to the inspired air at normobaric pressure. This can be via an open mask or nasal cannula, which will result in mild levels of hyperoxia depending on the flow rate, or via a closed non-re-breathing system such as a Laerdal bag which can increase the fraction of inspired oxygen (Fi_{O2}) up to 1.0.

13.2 Basic Physiology of Hyperoxia

An increase in the fraction of inspired oxygen leads to an increase in the partial pressure of oxygen dissolved in the arterial plasma (Pa_{O2}), and thus also in the saturation level of arterial hemoglobin (Sa_{O2}) [3, 26]. This results in a multitude of physiologic and biochemical effects [23, 53], which alter the acidity of the blood, the binding of carbon dioxide and oxygen with hemoglobin, the partial pressures of oxygen and carbon dioxide in the tissue, plasma, and expired gases, as well as changes in ventilation, metabolism, and cerebral blood flow (CBF) [1, 2, 4, 7, 11, 25, 37]. A characteristic response to hyperoxia is a tendency to hyperventilate; this can in turn result in a reduction in end-tidal P_{CO2} [2, 15, 53]. A decrease in PET_{CO2} is accompanied by vasoconstriction in the arterioles that in turn reduces CBF [13, 57]; however, the increase in Pa_{O2} also has a direct vasoconstrictive effect independently of the PET_{CO2} response [7, 15, 28]. The temporal responses of these two parallel effects are different, as are the magnitudes. Both responses are dose-dependent, but have absolute boundaries beyond which they no longer cause a change in CBF. It has also been shown that CBF will tend to restore back towards a normal level during hyperventilation-induced reductions in PET_{CO2}, except when hyperoxia is also present in which case the CBF will remain at a constant reduced level [37].

Oxygen is carried in the blood in two forms: dissolved in the plasma and bound to hemoglobin. Dissolved oxygen obeys Henry's law—the amount of oxygen dissolved

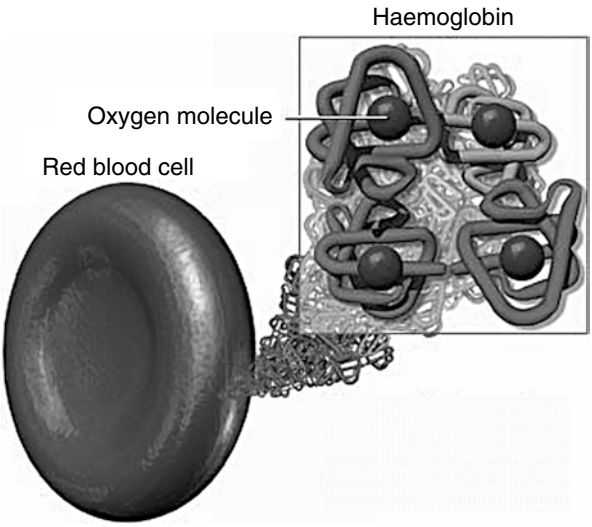

Fig. 13.1 Oxygen binding sites on the hemoglobin molecule

is proportional to the partial pressure. For each mmHg of P_{O2} there is 0.0031 ml O_2/dl (100 ml of blood). Hemoglobin is the main carrier of oxygen; each gram can carry 1.34 ml of oxygen. This means that with a hemoglobin concentration of 15 g/dl, the O_2 content is approximately 20 ml/100 ml. With a normal cardiac output of 5 l/min, the delivery of oxygen to the tissues at rest is approximately 1000 ml/min.

Hemoglobin has 4 binding sites for oxygen (Fig. 13.1), and if all of these sites in each hemoglobin molecule were to be occupied, then the oxygen capacity would be filled or saturated. This is rarely the case: under normal conditions, the hemoglobin is 97–98 % saturated. The amount of oxygen in the blood is thus related to the oxygen saturation of hemoglobin. The arterial oxygen content (Ca_{O2}) in ml O_2/dl of blood may be calculated, with contributions from O_2 bound to Hb, and O_2 dissolved in the arterial plasma, as:

$$Ca_{O_2} = \underbrace{\left(\varphi \times [Hb] \times Sa_{O_2}\right)}_{O_2 \text{boundtoHb}} + \underbrace{\left(Pa_{O_2} \times \varepsilon\right)}_{\text{dissolved}O_2} \tag{13.1}$$

where φ represents the species-dependent O_2-carrying capacity of hemoglobin (1.34 ml O_2/g_{Hb} for humans), and ε is the solubility coefficient of oxygen in blood (0.0031 $ml_{O2}/(dl_{blood}$ *mm Hg)). We assume here a normal value for the concentration of hemoglobin ([Hb] =15 g Hb/dl blood). Thus at normal atmospheric pressure the absolute theoretical maximum (but practically impossible) oxygen carrying capacity of human blood is approximately 22.5 ml/dl. As the concentration of oxygen in air is normally about 20 %, then during the inspiration of 100 % oxygen it is about five times higher, thus we can assume that the arterial partial pressure will increase proportionally. Therefore if normoxic levels produce Pa_{O2} values of approximately 100 mmHg, then $Fi_{O2} = 1.0$ will produce values of approximately 500 mmHg [48].

Normally about 97 % of the oxygen is carried by hemoglobin whereas only 3 % is carried dissolved in the blood [19]. As the Pa_{O2} increases, the amount of oxygen that is bound to hemoglobin, or the percent saturation of hemoglobin, also increases; normally at a Pa_{O2} of about 95 mmHg the saturation of hemoglobin is about 97 %. Above a P_{O2} of approximately 100 mmHg the oxygen-hemoglobin dissociation curve becomes almost flat, and so large changes in the partial pressure of oxygen do not result in substantial changes in arterial saturation. As a result, the changes in arterial content for hyperoxia occur considerably more as a result of changes in the partial pressure than due to changes in saturation, whereas the opposite is true at lower levels of partial-pressure/saturation.

Greater amounts of oxygen can be delivered to the tissues if administered at greater than normal pressures, usually referred to as hyperbaric oxygen therapy (HBOT) [49]. This is sometimes administered to speed wound healing, or for pressure-related pathology such as the bends. However, exposure to hyperbaric oxygen is not without risks as there is a danger of developing oxygen toxicity, a condition resulting from the harmful effects of breathing oxygen at elevated partial pressures [29]. Severe oxygen toxicity can result in cell damage and death. Effects are most often observed as damage to the central nervous system, lungs, and eye. Depending on the type of exposure the body is impacted in different ways. Central nervous system toxicity is caused by short exposure to high concentrations of oxygen at greater than atmospheric pressure. Pulmonary and ocular toxicity result from longer exposure to elevated oxygen levels at normal pressure. Symptoms include disorientation, breathing difficulty, and vision changes. Serious incidents can cause oxidation damage to cell membranes, collapse of the alveoli in the lungs, myopia, retinal detachment, and seizures.

As hyperoxia can induce changes in both respiration rate and tidal volume, these can in turn change the partial pressures of carbon dioxide, a powerful vasoactive agent. In order to avoid these complications the subject can either be trained to regulate their own breathing, or else be cued to breathe via some metronomic system to prevent hyperventilation. More sophisticated methods include end-tidal forcing which controls the delivered fraction of all the respiratory gases [54], and mechanical ventilation which directly controls the breathing rate and depth of the subject [47]. These are generally termed controlled breathing methods as opposed to uncontrolled methods where the subject is able to breathe spontaneously. Controlled methods sacrifice ease and practicality to obtain higher levels of accuracy and repeatability. There are also feed-forward methods that monitor a specific subject's response to changes in respiratory gases, and then produce a protocol of sequential gas delivery mixtures to target a particular response from that subject [38, 39, 45]. These are quite reliable; however, they do require substantial preparation time to obtain the specific data for each subject. Such methods are neither directly controlled nor spontaneous.

13.3 FMRI and Hyperoxia

MRI contrast relies on the temporal characteristics of the MRI signal being different in different tissues, and so by applying certain conditions of magnetic field within the imaging region, and then waiting a specific amount of time before obtaining an image,

contrast in the form of differing signal strength develops between the tissues types. MRI contrast is generally derived from one of two means. The first kind of contrast is dependent on the absolute relaxation of the MRI signal back to zero, this occurs with a particular time constant for each tissue type. The time constant is called T1. The second kind of contrast occurs due to the signals within a region going out of phase with each other; meaning the sum of the signals from the protons tend to cancel each other out rather than adding together to produce a large signal. This effect also has a distinct time constant called T2. If an imaging technique relies on the first effect it is said to use T1 contrast or be T1 weighted, and similarly for T2 methods [20].

The basis of most functional MRI is blood oxygenation level-dependent (BOLD) imaging [36]. BOLD derives contrast between a baseline and an active state from localized changes in cerebral blood volume (CBV) and blood oxygen saturation. This is due to the fact that oxyhemoglobin is diamagnetic (the same as most tissue) whereas deoxyhemoglobin is paramagnetic, meaning it is much more susceptible to magnetic fields. This property of hemoglobin means that the resonant behavior of the hydrogen protons in water, which are the source of the MRI signal, changes in relation to the number of molecules of deoxyhemoglobin within a given volume [24]. This is effectively a T2-based (T2*) contrast mechanism and the signal is found almost exclusively in venous regions where the concentration of deoxyhemoglobin is at its highest.

Inducing a state of hyperoxia introduces a number of changes in both physiology and resonance behavior of tissues and fluids, these in turn manifest as changes in image contrast. The venous oxygen content (Cv_{O2}) of blood is normally on the order of 15.2 ml/dl. Thus, ~5.2 ml/dl is extracted by the tissue, leaving ~15.1 ml/dl bound to hemoglobin and ~0.11 ml/dl in the plasma [42]. Assuming a constant demand for oxygen by the tissue, this implies that under normobaric hyperoxic conditions with $Fi_{O2} = 1.0$, the Cv_{O2} would be on the order of 17.2 ml/dl, with a corresponding increase in venous saturation (Sv_{O2}) over normoxic values of up to 10 %, whereas the increase in Sa_O_2 will be only 1 or 2 %. As a consequence, the T2*-based MRI contrast produced by hyperoxia is heavily weighted to the venous side of the vasculature [27].

Molecular oxygen is itself weakly paramagnetic, with a magnetic moment of 2.8 Bohr magnetrons [17, 56]. During inhalation of 100 % oxygen the concentration of dissolved oxygen in arterial blood plasma increases by approximately five times without significant increase in the amount of oxygen combined with hemoglobin because of the characteristic sigmoid-shaped oxygen-hemoglobin dissociation curve. The observed T1 change in the arterial blood during hyperoxia is therefore considered to directly reflect the dissolved oxygen concentration [12, 48]. However, dissolved plasma oxygen does not become an important contrast mechanism until the arterial oxygen tension exceeds ~350 mmHg [3]. The vasoconstriction associated with hyperoxia also contributes to the signal differences between the normoxic and hyperoxic states, as the OEF (although much decreased due to the high Sa_O2) will be slightly greater than would occur with no change in CBF.

Supplemental inspired oxygen inducing differing levels of hyperoxia has been used to induce changes in either image contrast or in physiology while investigating the human brain, sometimes in parallel with the use of hypoxia and/or hypercapnia which both produce their own characteristic changes in MRI signal, blood flow, or

metabolism. These investigations have primarily considered oxygen-induced changes observed in BOLD imaging [30, 39, 41, 44], studied the effects on physiology such as blood flow [1, 7, 13, 57] or simply investigated the impact of hyperoxia on relaxation times (T1 and T2) [12, 35, 48, 51].

Hyperoxia in combination with FMRI has the potential, however, to be used passively to obtain physiological measurements. For example the CBV may be calculated from the BOLD data during hyperoxia epochs using the hyperoxia contrast method described by Bulte et al. [8] which uses the BOLD signal change with the oxygen contrast in a pure blood voxel (in this case within the sagittal sinus vein) as a calibration voxel to calculate a baseline CBV map. The blood volume in each voxel is then given by

$$\mathrm{CBV} = \frac{h}{n\rho}\sum_{j=1}^{n}\left(\frac{-\ln\left(\frac{S_r(j)}{S_{r,0}}\right)}{-\ln\left(\frac{S_v(j)}{S_{v,0}}\right)}\right) \tag{13.2}$$

where ρ (the density of brain tissue) is 1.04 g/ml; $h=(1-\mathrm{Hct})/(1-r * \mathrm{Hct})$ corrects for the fact that the hematocrit (Hct) is greater in large vessels than in brain microvasculature [50], where r was assigned a value of 0.85 based on PET data; n is the number of ratios measured during the hyperoxic epochs once the signal has stabilized; $S_r(j)$ and $Sv(j)$ are the jth signal measurements during the plateau portion in the tissue voxels and venous-blood-filled voxels, respectively; and $S_{r,0}$ and $S_{v,0}$ are the average signals in tissue and in a vein-filled voxel, respectively, during the baseline period prior to arrival of the hyperoxic contrast.

Hyperoxia has also been used as a contrast mechanism in tissues and organs other than brain. Oxygen has been used as an intravascular contrast agent in muscle to show differences in vascular density or vessel recruitment [6, 33, 34], or simply to monitor the oxygen usage [46]. Under these conditions hyperoxia can be utilized to investigate the effects of exercise or use, or the effect of vasoactive or metabolically active pharmaceuticals. As the oxygen contrast travels within the blood stream it can be used in almost any organ or tissue for a multitude of purposes [35, 48].

13.4 The Hyperoxia-Calibration Model

Hyperoxia and FMRI have been shown to be able to produce measurements of changes in blood flow, blood volume, and oxygen metabolism during functional activation. There has recently been proposed a hyperoxia-based model for calibrated FMRI measurement [10], which is analogous to the hypercapnia-calibrated model [22]. Hyperoxia calibration makes use of the expression for BOLD signal change,

$$\frac{\Delta \mathrm{BOLD}}{\mathrm{BOLD}_0} = -\mathrm{TE}\Delta \mathrm{R}_2^*\big|_{d\mathrm{Hb}} \tag{13.3}$$

where TE is the echo time and R_2^* is the relaxation rate. The expression for $R_2^*|_{d\mathrm{Hb}}$ derived by Boxerman et al. [5] is:

$$\mathrm{R}_2^*|_{d\mathrm{Hb}} = \mathrm{A}\left(\mathrm{CBV}\left[d\mathrm{Hb}\right]_{\mathrm{v}}^{\beta}\right) \tag{13.4}$$

where $[d\mathrm{Hb}]_v$ represents the concentration of deoxygenated Hb in the venous vasculature, A is a proportionality constant related to the magnetic field strength and the volume susceptibility difference between blood and tissue, and β is a factor which adjusts for the relative contribution to the signal from small versus large vessels. Combining Eqs. (13.1) and (13.2) to describe changes in $R_2^*|_{d\mathrm{Hb}}$, and factoring out the resting condition,

$$\frac{\Delta \mathrm{BOLD}}{\mathrm{BOLD}_0} = \mathrm{TE} \times \mathrm{A}\left(\mathrm{CBV}\left[d\mathrm{Hb}\right]_{\mathrm{v}}^{\beta} - \mathrm{CBV}_0\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}^{\beta}\right) \tag{13.5}$$

$$\frac{\Delta \mathrm{BOLD}}{\mathrm{BOLD}_0} = \underbrace{\mathrm{TE} \times A \times \mathrm{CBV}_0\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}^{\beta}}_{M}\left(1 - \left(\frac{\mathrm{CBV}}{\mathrm{CBV}_0}\right)\left(\frac{\left[d\mathrm{Hb}\right]_{\mathrm{v}}}{\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}}\right)^{\beta}\right) \tag{13.6}$$

Equation (13.6) is the general BOLD equation and is valid regardless of the origin of the signal changes. During the special case of hyperoxic stimuli, however, the CBV is only changed via the mild reduction in CBF as high levels of hyperoxia yield a small relative decrease in grey matter CBF, on the order of ~7 % at 100 % inspired O_2 [7]. It is desirable to account for this CBF decrease, which will influence the BOLD signal through a direct effect on [dHb] concentration, and through a secondary effect on CBV. Thus for a hyperoxic stimulus Eq. (13.6) becomes:

$$\frac{\Delta \mathrm{BOLD}}{\mathrm{BOLD}_0} = M\left(1 - \left(\frac{\mathrm{CBV}}{\mathrm{CBV}_0}\right)\left(\frac{\left[d\mathrm{Hb}\right]_{\mathrm{v}}}{\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}} + \frac{\Delta\left[d\mathrm{Hb}\right]_{\mathrm{v}}}{\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}}\right)^{\beta}\right) \tag{13.7}$$

The CBV correction term in Eq. (13.7) can be replaced with CBF by assuming the Grubb relationship:

$$\frac{\mathrm{CBV}}{\mathrm{CBV}_0} = \left(\frac{\mathrm{CBF}}{\mathrm{CBF}_0}\right)^{\alpha} \tag{13.8}$$

where α is a constant which has been experimentally determined to have a value of 0.38 in humans [18]. Furthermore, the $\Delta[d\mathrm{Hb}]_v/[d\mathrm{Hb}]_{v0}$ contribution can be replaced with a term related to CBF.

$$\frac{\Delta\left[d\mathrm{Hb}\right]_{\mathrm{v}}}{\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}} = \frac{\left[d\mathrm{Hb}\right]_{\mathrm{v}}}{\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}} - 1 \tag{13.9}$$

$$\frac{\left[d\mathrm{Hb}\right]_{\mathrm{v}}}{\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}} = \frac{\mathrm{CBF}_0}{\mathrm{CBF}} \tag{13.10}$$

$$\frac{\Delta\mathrm{BOLD}}{\mathrm{BOLD}_0} = M\left(1-\left(\frac{\mathrm{CBF}}{\mathrm{CBF}_0}\right)^{\alpha}\left(\frac{\left[d\mathrm{Hb}\right]_{\mathrm{v}}}{\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}} + \frac{\mathrm{CBF}_0}{\mathrm{CBF}} - 1\right)^{\beta}\right) \tag{13.11}$$

Equation (13.10) is derived from Fick's principle, and is used similarly by Hoge et al. under the assumption that $\mathrm{CMR_{O2}}$ does not change during the calibration procedure [22]. The hyperoxia calibration model is given by Eq. (13.11), where $M = \mathrm{TE}\times A\times \mathrm{CBV}_0\times\left[d\mathrm{Hb}\right]_{\mathrm{v}_0}^{\beta}$ is the maximum theoretical BOLD signal change, and β is assumed to be 1.3 at 3 T [9]. The CBF terms are the corrections applied to account for those changes known to be induced by hyperoxia and are of the order of a 5–10 % reduction in flow from baseline [7].

Using experimentally acquired BOLD, CBF, and $\mathrm{Pa_{O2}}$ data, Eq. (13.11) can be used to obtain the calibration value *M* [10]. CBF data may be obtained using arterial spin labeling (ASL) methods [55], and $\mathrm{Pa_{O2}}$ can be estimated from end-tidal measurements.

13.4.1 Estimation of Venous Oxygen Saturation

Changes in $[d\mathrm{Hb}]_{\mathrm{v}}$ can be estimated by reformulating standard physiological relationships of oxygen transport in blood, and by assuming a value for the baseline oxygen extraction fraction (OEF) [14, 32, 52]. OEF has been found to be remarkably consistent throughout the brain, in contrast to $\mathrm{CMR_{O2}}$ and CBF [21, 40]. There are a number of MRI methods for measuring either OEF [16, 21] or $\mathrm{SvO_2}$ [31] in patients where the assumption of uniform OEF is not valid.

Arterial oxygen tension ($\mathrm{Pa_{O2}}$) can be inferred via the sampling of end-tidal $\mathrm{O_2}$ partial pressure ($\mathrm{P_{ET O2}}$). The fractional oxygen saturation of arterial Hb ($\mathrm{Sa_{O2}}$) may be calculated from $\mathrm{Pa_{O2}}$, although this value is ≈1.0 at fractions of inspired oxygen ($\mathrm{Fi_{O2}}$) at or above atmospheric concentration ($\mathrm{Fi_{O2}} \geq 0.21$). In the general case the Severinghaus equation [42, 43] may be used to relate measured $\mathrm{Pa_{O2}}$ to $\mathrm{Sa_{O2}}$, as follows:

$$\mathrm{Sa_{O_2}} = \frac{1}{\left(\dfrac{23400}{\left(\mathrm{Pa_{O_2}}\right)^3 + 150\left(\mathrm{Pa_{O_2}}\right)} + 1\right)} \tag{13.12}$$

Once Pa_{O2} and Sa_{O2} are determined, the arterial oxygen content (Ca_{O2}) may be calculated, with contributions from O_2 bound to Hb, and O_2 dissolved in the arterial plasma using Eq. (13.1).

$$Ca_{O_2} = \underbrace{\left(\varphi \cdot [Hb] \times Sa_{O_2}\right)}_{O_2\,bound\,to\,Hb} + \underbrace{\left(Pa_{O_2} \times \varepsilon\right)}_{dissolved\,O_2}$$

Alternatively, this value may be measured by sampling blood from individual subjects. The resting OEF is used to calculate the amount of O_2 removed from arterial blood, yielding the venous oxygen content (Cv_{O2}).

$$Cv_{O_2} = Ca_{O_2} - \left(Ca_{O_2}\big|_0 \times OEF\right) \tag{13.13}$$

Equation (13.13) indicates that the total amount of extracted O_2 is assumed to remain constant during hyperoxia. Given a value of Cv_{O2}, the venous oxygen saturation of Hb (Sv_{O2}) may be calculated as follows:

$$Cv_{O_2} = \left(\varphi \times [Hb] \times Sv_{O_2}\right) + \left(Pv_{O_2} \times \varepsilon\right) \tag{13.14}$$

$$Sv_{O_2} = \frac{Cv_{O_2} - \left(Pv_{O_2} \times \varepsilon\right)}{\varphi \times [Hb]} \tag{13.15}$$

Pv_{O2} represents an estimated measure of oxygen dissolved in venous plasma, and is expected to be a minor fraction (<1 %) of the total O_2 content in venous blood, due to the high affinity of Hb for O_2 and the significant deoxygenation of venous blood, even at high Fi_{O2}. The calculation of the deoxygenated fraction of Hb from Sv_{O2} is straightforward ($F_{dHb} = 1 - Sv_{O2}$). The change in the deoxygenated fraction represents the experimental *x*-axis variable in the hyperoxia-calibrated model ($[dHb]_v/[dHb]_{v0}$).

The relative change in the cerebral metabolic rate of oxygen during any given task may then be calculated using the measured BOLD and CBF changes and the value for *M* calculated using Eq. (13.11), along with the model presented by [22]:

$$\frac{CMR_{O_2}}{\left(CMR_{O_2}\right)_0} = \left(1 - \frac{\left(\frac{\Delta BOLD}{BOLD_0}\right)}{M}\right)^{1/\beta} \left(\frac{CBF}{CBF_0}\right)^{1-\alpha/\beta} \tag{13.16}$$

Using this information and assuming the Grubb relationship, it is possible to produce a set of data for a given functional task that incorporates the induced changes in BOLD signal, CBF, CBV, and $CMRO_2$ from a single scanning session.

13.5 Clinical Translation

One of the most promising aspects of using hyperoxia in conjunction with FMRI is the potential to translate many of the methods into clinical environments. Many of the techniques developed in laboratory settings are either impractical for use in the clinic or are too demanding or time consuming to be suitable for use with patients. Oxygen is readily available in most clinical MRI suites, and is not contraindicated for the majority of patient populations. It is inexpensive in comparison to pharmaceuticals, and the methods require little or no patient compliance in regards to breathing patterns. The analysis of the functional images is also relatively simple, even when the results are producing measurements of changes in CBF, CBV, or CMR_{O2} rather than simple maps of activated regions. The added benefit of the calibration methods is that the results are meaningful to clinicians as they are changes in actual physiological parameters rather than just a percentage change in BOLD signal, which can be extremely difficult to interpret in terms of pathology.

13.6 Conclusion

Although still at an early stage of development functional MRI incorporating hyperoxia (O2FMRI) has shown a great deal of potential. It is a very flexible technique that is capable of producing images and data from a variety of organs and physiological systems. As a safe, inexpensive intravascular contrast agent it has many uses, but its unique position as a physiologically active compound that is an essential part of metabolism lends it to have significant advantages of many other methods. It can be used to investigate baseline physiological parameters, measure changes due to activation or stimulation, specify pathology or monitor treatment and recovery. The fact that oxygen can do all of this and yet be safe, inexpensive, and piped in to almost every room in every hospital makes it all the more remarkable. As more sites start to investigate the possibilities of O2FMRI there will no doubt be a multitude of potential uses discovered.

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Chapter 14
Astrocytes and Brain Hypoxia

Nephtali Marina, Vitaliy Kasymov, Gareth L. Ackland, Sergey Kasparov, and Alexander V. Gourine

Abstract Astrocytes provide the structural and functional interface between the cerebral circulation and neuronal networks. They enwrap all intracerebral arterioles and capillaries, control the flux of nutrients as well as the ionic and metabolic environment of the neuropil. Astrocytes have the ability to adjust cerebral blood flow to maintain constant PO_2 and PCO_2 of the brain parenchyma. Release of ATP in the brainstem, presumably by local astrocytes, helps to maintain breathing and counteract hypoxia-induced depression of the respiratory network. Astrocytes also appear to be involved in mediating hypoxia-evoked changes in blood–brain barrier permeability, brain inflammation, and neuroprotection against ischaemic injury. Thus, astrocytes appear to play a fundamental role in supporting neuronal function not only in normal conditions but also in pathophysiological states when supply of oxygen to the brain is compromised.

Keywords Adenosine • Astrocytes • ATP • Breathing • Glutamate • Hypoxia • Prostaglandins • Vasodilation

14.1 Introduction

The central nervous system is highly vulnerable to changes in energy supply. Permanent damage to the human brain occurs when oxygen delivery is interrupted for more than several minutes. However, brain cells differ significantly in terms of their susceptibility to the effects of energy depletion. For example, in vitro studies have shown that glucose deprivation affects ionic gradients associated with decreases in cellular levels of ATP, ADP, phosphocreatinine and

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creatinine in both major types of brain cells—neurons and astrocytes. However, these changes are more pronounced and occur faster in neurons than in astrocytes [30]. Similarly, in conditions of hypoxia, neurons show a substantial decrease in both ATP and phosphocreatinine, whilst astrocytes remain largely unaffected if glucose is available [1].

Cerebral microcirculation in specific brain regions is regulated by mechanisms that match local blood flow to the levels of neuronal activity and metabolism. When neuronal activity increases local microvasculature dilates in order to divert more oxygenated blood to the active area. This neurovascular coupling mechanism is termed functional hyperaemia. When the brain is exposed to hypoxic conditions, adaptive molecular mechanisms are rapidly activated in order to limit the damage and to protect the viability and function of the brain cells. In this brief report, we focus on the possible role(s) played by astroglial cells in maintaining brain function under conditions when oxygen supply is compromised.

14.2 Astrocytes and Neurovascular Coupling

Astrocytes are the most abundant type of brain glial cells. Their functions are versatile and have attracted considerable interest over the last few years. The traditional view of the functional role played by astroglial cells was limited to providing neurons with structural and nutritional support. Indeed, they enwrap all penetrating and intracerebral arterioles and capillaries, control the traffic of nutrients and other chemicals from and into the blood stream, and regulate the ionic and metabolic environment of the neuropil. Astrocytic processes surround neuronal bodies and make contacts with thousands of individual synapses and thus can "sense" the level of neuronal activity by responding to neurotransmitter spillover from the synaptic clefts [4, 14]. On the other hand, pial arteries rest on the underlying glia limitans, while all the penetrating arterioles are surrounded by astrocytic end-feet. Therefore, astrocytes can affect the activity of vascular smooth muscle cells [23].

There is significant evidence supporting the idea that brain astrocytes provide a neurovascular coupling interface. Several in vitro studies have demonstrated that astrocytic activation by Ca^{2+} uncaging changes the diameter of associated arterioles [2, 10, 21]. Multiple mechanisms underlying astrocyte-mediated neurovascular coupling have been proposed. One of these putative mechanisms is believed to be via release of potassium ions from astrocytic endfeet onto the arterioles in response to neuronal activity [8, 24]. Increases in extracellular K^+ concentration could hyperpolarize smooth muscle cells resulting in dilation of cerebral vasculature [18]. Another mechanism whereby astrocytes control the local blood flow in brain areas involves the products of arachidonic acid (AA) metabolism. Increased synaptic activity is detected by astrocytes when glutamate is released by presynaptic nerve terminals. Glutamate activates astrocytic metabotropic glutamate receptors, leading to increases in $[Ca^{2+}]i$ and activation of phospholipase A_2, which generates AA from membrane phospholipids. AA is then converted to prostaglandins

(PGEs) and epoxyeicosatrienoic acids (EETs). Astroglial release of both, PGEs and EETs has vasodilator effects [10, 21, 36] whilst another AA product 20-hydroxy-eicosatetraenoic acid (20-HETE) produced in vascular smooth muscle cells promotes vasoconstriction [21, 22]. The main prostaglandin involved in neurovascular coupling is believed to be PGE_2 whose vasodilator effect involves decreased phosphorylation of the myosin light chain and the activation of the K^+ channels in arteriole vascular smooth muscle cells [29, 32].

In vitro slice studies have shown that activation of astrocytes can produce either dilation or constriction of brain arterioles. The precise contribution of glial control over the cerebral vasculature appears to depend on the pre-existing tone of the vessel [2] and most importantly, on tissue O_2 concentration [10]. Activation of perivascular astrocytes through stimulation of metabotropic glutamate receptors or via Ca^{2+} uncaging produces arteriolar constriction when the preparation is exposed to hyperoxic conditions. However, activation of astrocytes under lower O_2 conditions leads to vasodilation [10]. This suggests that tissue PO_2 level is one of the main factors that determines the cerebrovascular responses to astroglial activation. These differential effects appear to be produced by changes in the synthesis of astrocytic messengers involved in neurovascular coupling. When PO_2 is high, low levels of extracellular lactate facilitate clearance of PGE_2 (in exchange for intracellular lactate) by prostaglandin transporters. In conditions of low extracellular PGE_2 levels, vasoconstrictor effects of 20-HETE produced by vascular smooth muscle cells dominate [22]. In contrast, low PO_2 promotes astrocytic glycolysis resulting in increased lactate release. Increased level of extracellular lactate reduces the activity of prostaglandin transporters leading to accumulation of extracellular PGE_2, which dilates cerebral arterioles [10]. In addition, cellular hypoxia triggers release of adenosine, which binds to adenosine A_{2A} receptors on vascular smooth muscle cells to promote vasodilation [11].

14.3 Astrocytes as Hypoxia Sensors

Recent studies from our laboratory have shown that astrocytes show robust increases in $[Ca^{2+}]i$ when PO_2 is reduced below ~17 mmHg. Our previous studies in anesthetized, artificially ventilated and peripherally chemodenervated rats demonstrated that systemic hypoxia (10 % O_2 in the inspired air; 5 min) elicits release of ATP (a key gliotransmitter) in the brainstem areas responsible for generation and patterning of the respiratory rhythm. Blockade of ATP receptors in the same brainstem areas facilitates hypoxia-evoked respiratory depression [13]. Further experiments in slice preparations confirmed that ATP release in response to hypoxia occurs within the anatomical regions corresponding to the location of the medullary respiratory networks and immediately ventral to it [13]. These data suggest that ATP is released within (and in close proximity to) the respiratory network and plays an important role in maintaining breathing in conditions when hypoxia-induced slowing of respiration occurs. Astrocytes are likely to be the source of ATP released in the brainstem

during hypoxia and the mechanism(s) of ATP release include vesicular exocytosis. Interestingly, in our earlier study we showed that ventral brainstem astrocytes also respond to increases in the inspired levels of CO_2 in vivo as well acidification of the extracellular medium in vitro with elevations in intracellular Ca^{2+} leading to exocytotic release of ATP [12]. ATP propagates astrocytic Ca^{2+} excitation within the astroglial network and activates local chemoreceptor neurons resulting in adaptive increases in breathing. These data obtained in various brainstem preparations demonstrate that astrocytes are capable of sensing both decreases in PO_2 and increases in PCO_2 and respond to these chemosensory challenges with increases in intracellular [Ca^{2+}]. These results suggest that ATP released as a result of chemosensory activation contributes to the development of the ventilatory response to CO_2 and helps to maintain breathing in face of the hypoxia-evoked depression of the respiratory network.

14.4 Astrocytes and Inflammation

Glial cells are capable of releasing chemokines while hypoxia is associated with upregulation of a number of inflammatory mediators, including interleukin (IL)-1β (IL-1β), IL-8, monocyte chemoattractant protein, intercellular adhesion molecule-1 (ICAM-1) and others [31, 34]. In human fetal astrocytes, hypoxia has been shown to evoke a marked upregulation of NF-kappaB—a crucial transcription factor necessary for synthesis of many inflammatory mediators [31]. There is evidence that NF-kappaB upregulates the expression of astrocytic IL-1β and IL-8 in response to hypoxia followed by amplification through autocrine IL-1β-induced NF-kappaB activation during reoxygenation [31]. It is not yet clear whether glial-derived cytokines have a protective or harmful effect on neurons exposed to hypoxic conditions, although the inflammatory process may contribute to brain repair following a hypoxic event. Removal of the damaged cells may be facilitated by macrophage infiltration, whilst neurogenesis may be promoted in response to increased cytokine production [7, 27, 35].

14.5 Astrocytes and Blood–Brain Barrier During Hypoxia

The blood–brain barrier (BBB) separates the circulating blood and the brain parenchyma. The BBB is composed of endothelial cells and astrocytic end-feet. Tight junctions between adjacent cells and sparse pinocytic vesicular transport are the landmarks of cerebral endothelium which under normal physiological conditions limit the permeability of microorganisms, T lymphocytes and hydrophilic molecules from the arterial blood into the brain. Efficacy of this barrier also relies on astrocytic end-feet which cumulatively circumscribe all cerebral blood vessels.

During hypoxia the BBB may become disrupted, leading to vasogenic edema [15, 19]. Astrocytes may increase BBB permeability through the release of a range of cytokines and chemical mediators [31, 34]. Glial-derived chemokines can upregulate the synthesis of the other factors such as IL-8, ICAM-1, E-selectin, IL-1β, TNF-α, and MCP-1 by the cerebrovascular endothelial cells [33], facilitating infiltration of leukocytes across the BBB. This triggers further signal transduction cascades leading to increased phosphotyrosine production, loss of tight junction proteins (occludin and zonula occludens-1), and redistribution of vinculin [3]. These events result in junctional disorganization and increased BBB permeability.

There is also evidence that astrocytes can protect the integrity of the BBB during hypoxia. It was demonstrated using an in vitro model that hypoxia-induced paracellular hyperpermeability is significantly decreased if astrocytes or astrocytic-conditioned medium are present [9]. These effects are associated with inhibition of hypoxia-induced vascular endothelial growth factor synthesis which enhances transcytosis in endothelial cells [6] and induces endothelium fenestrations [26].

14.6 Astrocytes and Neuroprotection During Hypoxia

In the last decade it has also become clear that astrocytes are capable of protecting neurons under conditions of hypoxia. One of the possible mechanisms is related to the phenomenon called “hypoxic preconditioning” where an initial brief period of mild hypoxia confers neuroprotection to subsequent, normally lethal to neurons, ischemic events. Mild hypoxia stimulates the production of various protective astrocytic factors that enhance neuronal viability [16]. Hypoxia upregulates the expression of certain proteins that may mediate egress of ATP/adenosine into the extracellular compartment like connexin 43 [17]. In response to hypoxia [20] astrocytes have been found to produce another factor with potent neuroprotective effect—erythropoietin [25]. Erythropoietin expression in astrocytes is upregulated following activation of hypoxia inducible factors—HIF-1α and HIF-2α [5, 28].

14.7 Summary

Astrocytes appear to play an important role in maintaining neurophysiological function under conditions when oxygen supply to the brain is compromised. At the system level, release of ATP by the brainstem astrocytes helps to maintain breathing and counteract hypoxia-induced depression of the respiratory network. At the tissue level, astrocytes appear to be involved in mediating hypoxia-evoked changes in BBB permeability, brain inflammation and neuroprotection against ischaemic injury. Under normal physiological conditions astrocytes mediate neurovascular coupling and have the ability to “sense” increased neuronal activity to trigger dilation of local microvasculature and divert oxygenated blood to brain areas with increased activity.

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Chapter 15
Bidirectional Control of Blood Flow by Astrocytes: A Role for Tissue Oxygen and Other Metabolic Factors

Grant R.J. Gordon, Clare Howarth, and Brian A. MacVicar

Abstract Altering cerebral blood flow through the control of cerebral vessel diameter is critical so that the delivery of molecules important for proper brain functioning is matched to the activity level of neurons. Although the close relationship of brain glia known as astrocytes with cerebral blood vessels has long been recognized, it is only recently that these cells have been demonstrated to translate information on the activity level and energy demands of neurons to the vasculature. In particular, astrocytes respond to elevations in extracellular glutamate as a consequence of synaptic transmission through the activation of group 1 metabotropic glutamate receptors. These Gq-protein coupled receptors elevate intracellular calcium via IP3 signaling. A close examination of astrocyte endfeet calcium signals has been shown to cause either vasoconstriction or vasodilation. Common to both vasomotor responses is the generation of arachidonic acid in astrocytes by calcium sensitive phospholipase A_2. Vasoconstriction ensues from the conversion of arachidonic acid to 20-hydroxyeicosatetraenoic acid, while vasodilation ensues from the production of epoxyeicosatrienoic acids or prostaglandins. Factors that determine whether constrictor or dilatory pathways predominate include brain oxygen, lactate, adenosine as well as nitric oxide. Changing the oxygen level itself leads to many downstream changes that facilitate the switch from vasoconstriction at high oxygen to vasodilation at low oxygen. These findings highlight the importance of astrocytes as sensors of neural activity and metabolism to coordinate the delivery of essential nutrients via the blood to the working cells.

Keywords Cerebral blood flow • Brain • Nitric oxide • Glutamate

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15.1 Functional Hyperemia

More than 100 years ago Roy and Sherrington discovered that the brain is intrinsically capable of controlling cerebral blood flow (CBF) [57]. This is termed functional hyperemia, whereby the brain communicates to the vasculature to enlarge vessel diameter in order to augment CBF so that energy demands are met. Enhanced CBF augments the delivery of oxygen (O_2) rich hemoglobin, glucose, and other nutrients to the working cells, while simultaneously clearing by products of metabolism like carbon dioxide (CO_2). Understanding the cellular mechanisms of how the cerebrovasculature changes diameter may be critical for developing effective treatments for an array of neurological afflictions such as hemorrhage, focal ischemia, and migraine. In addition to these pathologies, understanding CBF control within the context of brain signaling and energy metabolism is important for the proper interpretation of functional magnetic resonance imaging data, which uses the paramagnetic signal of deoxyhemoglobin as an indirect measure of brain activity. Contributing towards our understanding of CBF regulation in recent years, brain glial cells known as astrocytes are now understood to be important participants in coupling changes in neuronal activity to alterations in the diameter of brain vessels. In this review there is a focus on the role of astrocytes, and in particular that of astrocyte endfeet calcium (Ca^{2+}) signals and ambient oxygen concentration, in the control of brain blood vessels. The participating signaling molecules, cellular pathways and the link to O_2 and metabolism will be highlighted as well as the different experimental models used to test the involvement of astrocytes in CBF control. Other studies examining astrocytes in vivo will not be covered here as this has been covered in a recent review [24].

15.2 Astrocytes are Ideal Regulators of CBF

Astrocytes form a physical bridge between synapses and the microvessels of the brain, a feature identified as early as 1913 by the great Ramon y Cajal. On the vascular side, this occurs through modified astrocytic process termed perivascular "endfeet" [58]. Endfeet are enlarged astrocytic compartments that are specialized for the direct interaction with vessels, which includes the smooth muscle cells (SMCs) and endothelial cells. Of the CNS that all cerebral vessels in the brain are circumscribed by endfeet, suggesting a prominent role in vasculature physiology. The diameter of arterioles, and thereby blood flow, is regulated by contraction and relaxation of SMCs. A current hypothesis for functional hyperemia receiving much interest is that astrocytes can directly sense changes in synaptic activity, which is tightly linked to metabolism and oxygen consumption, and relay this information to the cerebrovasculature to initiate appropriate changes in CBF.

Early work with calcium indicator dyes demonstrated that exogenous glutamate caused oscillating increases in internal free Ca^{2+} resulting via metabotropic glutamate receptors (mGluRs) activation in cultured astrocytes [9]. These Ca^{2+}

oscillations propagated as a wave through connected astrocytes, indicating that mGluR could elicit long-range signaling cascades incorporating many astrocytes. Further studies demonstrated the same phenomena using more physiological techniques and preparations such as afferent stimulation in acute brain slices [10, 49, 53, 54]. These data inspired decades of research into how astrocyte Ca^{2+} signals propagate, and what intracellular pathways might this Ca^{2+} signal target and why. One molecule of interest is soluble phospholipase A_2 (PLA_2), which is highly expressed in astrocytes including their endfeet processes [16]. Once activated, PLA_2 generates freely diffusible arachidonic acid (AA) from the plasma membrane, which can be converted into an array of vasoactive lipids, some of which act to induce vasodilation and others to induce vasoconstriction. Dilating products include prostaglandin E_2 (PGE_2) generated from the action of cyclooxygenase (COX) enzymes [46, 60, 64] and several epoxyeicosatrienoic acids (EETs) including 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET [13, 22] generated from the activity of a type of CYP450 epoxygenase enzyme known as CYP2C11. Constricting molecules consist of PGF_2 [14] and thromboxane A_2 [3, 4, 18, 32] from COX activity, endothelin peptide [15, 38], as well as 20-Hydroxyeicosatetraenoic acid (20-HETE) [34, 43] from the conversion of AA by a different CYP450 epoxygenase enzyme than that mentioned for EETs known as CYP4A. In cultured astrocytes, stimulating soluble PLA2 causes the release of AA [59]. However, AA can also be converted while still within the cells. Cultured astrocytes can produce PGE_2 or EETs in response to glutamate agonists [1, 65]. These in vitro data show that astrocytes are capable of releasing several vasoactive lipid products.

15.3 Astrocytes Control Cerebrovascular Diameter

The first evidence showing that glutamate-induced elevations in intracellular Ca^{2+} were a major component of astrocyte-mediated neurovascular coupling was acquired by the Carmignoto laboratory [64]. Activating astrocytes indirectly with patch electrodes (which disrupts their membrane and increases intracellular Ca^{2+}) or by applying mGluR agonists triggered the release of diffusible factors that subsequently acted on SMCs to cause vasodilation. Further, an elevation of extracellular glutamate from evoked synaptic activity evoked Ca2+ increases in astrocyte endfeet via mGluRs and caused vasodilation, mimicking the other effects. The dilation was reduced by attenuating COX activity with aspirin or indomethacin, suggesting products from COX activity, such as PGE_2 and/or prostacyclin, were involved in the effect. These experiments followed a previous report from the same group showing that mGluR-mediated Ca^{2+} oscillations in astrocytes resulted in the release of prostaglandin [65]. The results from the vessel experiments, which were obtained from acute brain slices, were also replicated in vivo. CBF responses were triggered by forepaw stimulation and measured with laser Doppler flowmetry. Supporting their hypothesis, the functional hyperemia responses were dramatically reduced by mGluR antagonists. Iadecola's group performed experiments that broadly affect the

COX enzymes to test their role in cerebrovascular control. Pharmacological inhibition or knockout of COX-2 dramatically reduces functional hyperemia responses to whisker stimulation [45]. In contrast, pharmacological inhibition or knockout of COX-1 failed to affect CBF responses to whisker stimulation, but these treatments did reduce a form of acetylcholine-mediated vasodilation [46]. Collectively, these results suggest that products derived from COX activity play a crucial role in functional hyperemia and that astrocytes might be important participants in this process.

A direct and unequivocal demonstration of the role of astrocytes in the regulation of arteriole diameter came from Mulligan and MacVicar [43] who used two-photon fluorescence microscopy and uncaging techniques to trigger Ca^{2+} transients within the volume of individual astrocytes. This allowed them to causally test the impact of astrocyte Ca^{2+} transients on arteriole diameter. In their experiments, the liberation of intracellular Ca^{2+} caused profound vasoconstrictions in neighboring arterioles immediately following rapid astrocyte endfeet Ca^{2+} transients. The authors found a strong, positive relationship between the extent of the Ca^{2+} signal within the astrocyte endfeet and the extent of the vasoconstriction. Pharmacology experiments showed that PLA2 and CYP4A enzymes were important. These experiments suggested that the underlying mechanism for vasoconstriction was AA formation in astrocytes and the generation of the vasoconstrictor molecule 20-HETE, which inhibits smooth muscle K^+ channels leading to depolarization and contraction of these cells [34].

Similar to the work by the Carmignoto lab, using acute slices the Nelson laboratory demonstrated that afferent stimulation causes an increase in free intracellular Ca^{2+} in astrocyte somas and endfeet, which can be blocked by mGluR antagonists [18], but the vessel movements in response to the stimulation were not, suggesting other receptors and signaling molecules were participating, though they were not explored in this work. However, in basal conditions Nelson's group observed spontaneous and repetitive vasomotion that were well timed with Ca^{2+} oscillations in SMCs. Rather than showing an increase in vessel diameter when stimulating afferents, they showed a reduction in the contractile portion of the rhythmic vessel movements and in the spontaneous Ca^{2+} signals. This indicates distinct differences between the brain slice preparations used in different laboratories.

Studying retinal explants, Newman's lab showed that uncaging Ca^{2+} in retinal glial cells, known as Muller cells, using UV light caused constriction or dilation of nearby arterioles [40]. The observation of vasoconstriction was shown to be caused by the generation of astrocyte AA and conversion to 20-HETE, similar to the results of the MacVicar Laboratory. Interestingly, the vasodilations were also caused by AA generation in astrocytes, but the AA was instead converted to EET by a CYP2C11 enzyme. They pondered what factors determined the direction of the vessel's response. They reasoned that the level of NO would be important because the epoxygenase enzyme involved is sensitive to NO [19, 56]; thus, by regulating the enzymatic conversion of AA to either EET or 20-HETE, the vessels response might change. Consistent with this idea, in the presence of the NO donor

SNAP, vasodilations were transformed into vasoconstrictions. The fact that NO could change the vessel response to activity generated more questions as to whether other variables might do the same.

15.4 Astrocytes in Brain Energetics and the Link to Blood Flow

It is now well appreciated that changes in synaptic activity and neuronal action potentials correlate with changes in blood oxygen [42], with regional alterations in CBF [12, 63] and glucose consumption [27, 29]. Astrocyte metabolism may in fact be an important variable linking changes in the energy demand of the tissue to changes in blood flow. Subsequent to the initiation of neural activity there is an immediate and transient reduction in oxygen content within the vessel, which precedes the increase in CBF, suggesting that brain cells utilize oxygen very rapidly when excited [2]. Imaging the intrinsic fluorescence of the metabolic electron carrier NADH using two-photon microscopy as a measure of brain metabolic redox state, also supports this idea. At the onset of activity changes to the NADH signal suggests that dendritic oxidative metabolism is enhanced [33], which is consistent with a rapid consumption of O_2. Notably, the NADH signal also indicates that this is followed by a sustained increase in astrocyte glycolytic metabolism [5, 61]. There are a few additional lines of evidence in support of the idea that neurons and astrocytes preferentially utilize a different type of metabolism, aerobic vs glycolytic respectively. First, CBF changes occur in close proportion to brain glucose utilization, yet there is not a similar proportional increase in the consumption of O_2 [20, 21]. This suggests significant ATP production from an anaerobic source such as glycolysis. In fact, the CBF increase actually overshoots the needed O_2, resulting in an oversupply of oxygenated blood, which is the basis of the BOLD fMRI signal [48]. A dramatically enhanced glycolytic pathway is also supported by an increase in extracellular lactate, an end product of anaerobic metabolism, measured with lactate electrodes [17, 28]. In accord with astrocyte derived lactate, astrocytes are the primary source of brain glycogen [31]. Glycogen, a large polymer of glucose molecules, is thought to be important for a fast, on-demand source of ATP via glycogen breakdown (glycogenolysis) and subsequent glycolysis [6]. Lactate is also thought to arise from astrocytes for the purposes of the astrocyte–neuron lactate shuttle. In this model, glutamate released from synapses causes Na^+ loading in astrocytes as a consequence of glutamate transporter (EAAT1 and EAAT2) activity. Elevated intracellular Na^+ instigates energy dependent Na^+-K^+ ATPases to restore ionic gradients, driving astrocytes to generate more ATP by glycolytic means. The generated lactate is released through monocarboxylate transporters that operate by facilitated diffusion. The lactate can then be taken up and consumed as fuel in oxidative metabolism in neurons [50, 51]. The astrocyte–neuron lactate shuttle hypothesis is interesting in light of an in vivo study conducted in the olfactory bulb, which used intrinsic optical imaging (IOS) as an indirect measure of alterations in CBF.

Gurden et al. [25] found that the IOS evoked by physiological odor presentation in glomeruli did not rely on the activation of ionotropic or metabotropic glutamate receptors. Instead, glutamate uptake through astrocyte transporters was found to be the major contributor [25]. This was consistent with the idea that glutamate transport was an important trigger for vasodilation, which has now been repeated by another group [52], while also suggesting that the generation and release of lactate may be important for both feeding neurons and CBF control.

That the metabolic state of the brain is an important factor controlling CBF is supported by experiments in both animals and humans. First, by manipulating the external lactate/pyruvate ratio, which controls the intracellular NADH/NAD^+ ratio, CBF responses can be changed in vivo [30, 41, 62]. If lactate is increased, the CBF response to physiological stimulation is augmented, whereas if pyruvate is increased, the CBF response is reduced. Consistent with this, an NMR spectroscopy study demonstrates a strong correlation between the lactate signal and CBF increases [35]. Interestingly, other variables such as ATP production and the cerebral metabolic rate of oxygen consumption did not correlate nearly as well as lactate to CBF, despite the fact that they were expected to have a stronger relationship. Other studies have shown lactate can affect vessel tone and CBF. In the retina, lactate has direct effects on SMCs via activation of NOS and opening of K^+-ATP channels causing vessel relaxation [26]. In the hippocampus and cortex, lactate increases extracellular PGE_2 to cause vasodilation [23] (more details below).

15.5 The Switch from Vasoconstriction to Vasodilation: Oxygen, Lactate, and Adenosine

MacVicar's group [23] was also interested in how metabolism might be critical for determining vascular responses to brain activity. They reasoned that metabolic factors known to change dynamically with brain activation would be a good candidate to dictate whether astrocytes induced vasoconstriction or vasodilation of arterioles. In the parenchyma for instance, rapid changes in neural activity result in an immediate drop in the partial pressure of O_2 (pO_2) [47] and increase the extracellular lactate concentration [28]. By treating pO_2 as a variable and clamping it at either a high or a low level, Gordon et al. [23] found that they could change the "polarity" of the vessel's response when Ca^{2+} was uncaged in astrocytes. In high O_2 astrocytes triggered vasoconstriction, whereas in low O_2 astrocytes triggered vasodilation. High O_2 content is standard for acute brain slice experiments where the saline solution perfused over the preparation is bubbled with 95 % O_2 and 5 % CO_2. Historically this was used to prevent an anoxic region developing within the core of the brain slice. Low O_2 was achieved by bubbling their input solution with 20 % O_2 (5 % CO_2 and balanced N_2), which resulted in a pO_2 of 18.94 mmHg at a depth of 50 μm below the surface of the slice where the vast majority of the experiments are recorded from. This value mimicked the lower end of O_2 levels recorded in vivo in an

anesthetized healthy Sprague Dawley rat with a range of 12.7–64.4 mm Hg [47]. Further experiments showed that oxygen was not the only variable. In fact, the pO_2 was altering the metabolic state of the tissue leading to several downstream changes that were ultimately responsible for the observations. These changes were higher lactate and adenosine levels in the low O_2 condition and each had a separate role in switching the vessel's response. High extracellular lactate promoted vasodilation by hindering prostaglandin uptake by affecting the prostaglandin transporter [8]. This allows PGE_2 to accumulate in the extracellular space until a high enough concentration is reached to cause vasodilation. Adenosine acted in the reverse way. Instead of facilitating vasodilation, adenosine prevents the vessel's ability to constrict by activating A2A receptors directly on smooth muscle cells which results in a reduced Ca^{2+} permeability of these cells [44]. Both the elevation in external lactate and adenosine were required simultaneously to switch the vessel's response from vasoconstriction to vasodilation in high O_2 and low O_2 respectively. More experiments are needed to know where the oxygen concentration cross over point occurs and whether or not this falls within the physiological range. Though the physiological basal range of pO_2 in vivo is notably large, the decrease in pO_2 caused by brain activity is quite small (up to 10% reduction from strong afferent activity) [47]. However, this points to the idea that different brain regions have their own unique profiles, depending on the number, type and density of cells and their activity level. If the pO_2 in a given brain region is around the cross over point, neural activity would be able to decrease the local pO_2 enough to ensure the switch from constriction to dilation by astrocytes. Alternatively, in vivo, the oxygen mechanism described by Gordon et al. [23] may better apply to basal blood flow control. In this scenario, changes in arteriole diameter occur across a continuum, whereby subtle changes in the extracellular concentration of lactate and adenosine cause progressive adjustments in vessel tone. Furthermore, by simply breathing high or low O_2 to change tissue pO_2 causes changes in basal CBF in the directions predicted by MacVicar's group according to their mechanism described in brain slices [37, 39, 55]. However, changing tissue pO_2 by a similar method fails to change the increases in CBF triggered by sensory stimulation [36]. This interesting observation suggests that either the O_2 mechanism described above applies only to basal flow, not functional hyperemia, or that O_2 is not the correct variable to test this mechanism in vivo because it is in fact lactate and adenosine that are end effectors, not oxygen. For this idea, it would be important to know whether lactate and adenosine still increase in vivo when tissue pO_2 is completely saturated when breathing high O_2. Finally, it is important to know that changes in the metabolic state of the brain do not always couple logically to CBF changes in vivo. For example, unilateral forepaw stimulation causes an increase in CBF and glucose metabolism in the contralateral hemisphere, as expected, yet the ipsilateral hemisphere displays a decrease in CBF while glucose metabolism still increases [11]. This effect highlights a complex interaction of metabolic and neurogenic factors that ultimately determine how CBF and energy metabolism are controlled. Clearly we still have much to learn about the totality of brain metabolism and blood flow regulation.

15.6 Conclusions

The experiments described here show that astrocytes can elicit either vasoconstriction or vasodilation of brain arterioles. Synaptic glutamate release is an important signal initiating these changes by activating group I mGluRs to increase intraglial Ca^{2+}. The subsequent activation of Ca^{2+} sensitive PLA_2 in astrocytes leads to the production of an array of lipids derived from AA metabolism that affect arterioles. The vaosconstriction mechanism occurs when AA is converted to 20-HETE in smooth muscle by the CYP4A enzyme. The vasodilation mechanisms result from the conversion of AA to PGE_2 or EETs from COX or CYP2C11 enzymes, respectively. CYP2C11 is sensitive to NO and is thought to help switch the polarity of vessel responses as NO levels change. Metabolic factors are also critical determinants of how astrocytes control the diameter of arterioles. In high O_2, astrocytes recruit the vasoconstriction mechanism, yet in low O_2 they promote the vasodilatory PGE_2 pathway. Elevations in external lactate and adenosine are important for switching from constriction to dilation, as well as the intracellular $NADH/NAD^+$ ratio. A role for astrocytes in controlling basal blood flow remains a distinct possibility. Astrocytes appear to be important regulators of CBF, but a comprehensive understanding is far from complete. More experiments and new technologies will help shed light on this intriguing subject.

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Chapter 16
Hypoxic Adaptation in the Nervous System: Promise for Novel Therapeutics for Acute and Chronic Neurodegeneration

Rachel Speer and Rajiv R. Ratan

Abstract Homeostasis is the process by which cells adapt to stress and prevent or repair injury. Unique programs have evolved to sense and activate these homeostatic mechanisms and as such, homeostatic sensors may be potent therapeutic targets. The hypoxic response mediated by hypoxia inducible factor (HIF) downstream of oxygen sensing by HIF prolyl 4-hydroxylases (PHDs) has been well-studied, revealing cell-type specific regulation of HIF stability, activity, and transcriptional targets. HIF's paradoxical roles in nervous system development, physiology, and pathology arise from its complex roles in hypoxic adaptation and normoxic biology. Understanding how to engage the hypoxic response so as to recapitulate the protective mechanism of ischemic preconditioning is a high priority. Indeed, small molecules that activate the hypoxic response provide broad neuroprotection in several clinically relevant injury models. Screens for PHD inhibitors have identified novel therapeutics for neuroprotection that are ready to proceed to clinical trials for ischemic stroke. Better understanding the mechanisms of how to engage hypoxic adaption without altering development or physiology may identify additional novel therapeutic targets for diverse acute and chronic neuropathologies.

Keywords HIF • Hypoxia sensing • Prolyl hydroxylase • Stroke

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16.1 Molecular Mechanisms of Oxygen Homeostasis

16.1.1 Homeostatic Programs as Targets of Injury and Targets for Therapy

The term homeostasis was first coined by Walter Cannon in 1930. It comes from the Greek words for "steady" or "same," and refers to the process by which living things respond to physiological perturbations in order to maintain fairly stable conditions necessary for survival. Changes in the environment activate evolutionarily conserved adaptive programs, which alter gene transcription, protein activities, and cellular and organismal behavior to help restore favorable internal conditions, prevent damage, and promote repair. The effectors of these adaptive programs typically have very broad beneficial effects under the range of conditions to which organisms have been subjected over the course of evolution, but they may be impotent or even deleterious under novel conditions in which modern humans find themselves, such as diet-induced obesity [89].

Homeostatic adaptive mechanisms are engaged by injuries such as cerebral ischemia, in which reduced blood flow subjects neurons to reduced supplies of oxygen and glucose. The hypoxic stress that results from oxygen supply not meeting demand activates homeostatic programs. If these programs are successful, they prevent or sufficiently attenuate any energy deficit and enhance blood flow to restore oxygenation, protecting neurons from potential injury. This likely happens frequently, but is not observed clinically because the homeostatic programs have adapted to the stress and prevented any injury. If, however, the homeostatic programs fail, then cells are exposed to persistent hypoxia and suffer damage that can lead to necrotic or apoptotic cell death.

Under conditions of hypoxia, homeostatic programs engage many compensatory mechanisms at systemic, local, and cellular levels. Rapid responses involve post-translational modification of existing proteins, but sustained responses involve changes in gene transcription. For example, hypoxia stimulates erythropoiesis, the production of red blood cells to increase the oxygen-carrying capacity of the blood, by upregulating expression of the *Epo* gene encoding erythropoietin. At a local level, in ischemic myocardium, *Vegf* gene induction promotes local revascularization. Within hypoxic cells, ramping up anaerobic respiration to compensate for a deficit in aerobic respiration requires the transcription of genes encoding glycolytic enzymes. The changes in gene expression induced by hypoxia mediate a comprehensive program to help organisms adapt to hypoxia, both in terms of the number and range of genes that are upregulated, and in terms of the multiple functions that these genes play. Many hypoxia-induced gene products, including EPO, VEGF, and neuroglobin not only enhance oxygen delivery or ATP production, but are directly neuroprotective.

The coordination of the hypoxic response is particularly striking in its alteration of cellular metabolism. Hypoxia stimulates a shift away from oxidative phosphorylation and toward glycolysis by coordinately inducing many genes including those

encoding glycolytic enzymes such as lactate dehydrogenase to replace NADH for glycolysis to continue, and pyruvate dehydrogenase kinase to shunt pyruvate away from conversion to Acetyl Co A and subsequent entry into the TCA cycle [27, 51]. This ensemble of changes together allows cells to maintain their ATP levels despite lowered oxygen availability.

The homeostatic program by which cells adapt to hypoxia may be disrupted or aberrantly activated in the many pathologies in which hypoxia is a component. The homeostatic program itself may be targeted during the development of chronic diseases such as neurodegenerative syndromes. Thus, reengaging or augmenting the endogenous repair mechanisms may slow or relieve disease progression, and identifying the mediators and modulators of the hypoxic response could reveal promising therapeutic targets.

16.1.2 HIF Mediates the Adaptive Response to Hypoxia

Hypoxia-induced alterations in gene expression and many cellular processes have been studied for decades, but only in the 1990s was a hypoxia-responsive transcription factor identified that coordinates the hypoxic adaptive program. Semenza and colleagues hypothesized that a single protein was responsible for the hypoxic induction of the many genes known to be hypoxia responsive. Using a strategy of in vivo promoter bashing, they made transgenic mice carrying mutations in untranslated regions in and around the EPO gene, and then evaluated whether the mutations blocked the induction of EPO by hypoxia. They identified an 8 bp sequence necessary for hypoxia-dependent Epo expression, which was later found in the promoter regions of many hypoxia-induced genes. Using a DNA binding column and lysates from hypoxia-treated cells, they were then able to isolate and clone the protein that bound to that sequence. They called the protein "Hypoxia Inducible Factor 1," what we now know well as HIF [90]. They further showed that it mediated canonical hypoxia-induced VEGF and glycolytic enzymes as well [28, 91], bolstering the case that HIF is a central effector of hypoxia-induced transcriptional changes.

HIF is a member of the basic helix-loop-helix/PAS family of proteins. The HIF heterodimer consists of the hypoxia-responsive alpha subunit and the constitutively nuclear beta subunit. Multiple isoforms have been identified but HIF-1 is best studied. The sequence to which HIF-1α binds in the promoter region of the EPO gene is also present in the promoters of other hypoxia-induced genes including VEGF, glycolytic enzymes, and heme oxygenase, suggesting that HIF plays a general role in mediating compensatory responses to hypoxia. Although a given HIF target gene may only be expressed in a few tissues—e.g., EPO is only expressed in kidney, liver, and brain—HIF is expressed throughout the body, so it can mediate hypoxia responses in many different cell types and body systems.

After a decade of work on HIF, we now know that HIF coordinates the expression of many functionally related target genes, so HIF is able to comprehensively regulate cellular processes including not only glucose metabolism and oxygen

transport and delivery but also cell growth and fate. Dozens, if not hundreds, of studies have been published in the last 15 years demonstrating that HIF upregulation is necessary for previously identified consequences of hypoxia, including both the adaptive responses that help cells survive stresses and the delayed cell death observed after hypoxia in many cell types and injury models in vivo and in vitro.

16.1.3 Prolyl Hydroxylases Are Oxygen- and Cellular Stress-Sensors That Regulate HIF

Over the past 20 years, elegant studies have revealed that the stability of HIF alpha subunits is regulated by a family of iron- and oxygen-dependent enzymes called HIF prolyl 4-hydroxylases, or HIF-PHDs [14, 46]. Hydroxylation at proline 402 or proline 564 allows HIF to be recognized by von Hippel–Lindau protein, ubiquitinated by the E3 ligase complex, and targeted for proteasomal degradation. Under hypoxic conditions, PHD activity is inhibited, which allows HIF-a to accumulate, translocate to the nucleus, and induce the transcription of target genes. Under normoxia, stimuli long referred to as "hypoxia mimics," including cobalt chloride and iron chelators, can promote HIF-1α stabilization and translocation to the nucleus. HIF can be sumoylated, but there are conflicting reports as to whether this increases or decreases stability [46]. Once HIF-alpha is stabilized, its activity is subject to further regulation, e.g., its interaction with p300/CBP is necessary for transactivation of target genes [6] and is regulated by an asparaginase called factor inhibiting HIF or FIH [64].

It is noteworthy that, under normoxic conditions, cells are constantly transcribing HIF mRNA, translating HIF protein, hydroxylating it, and breaking it down. This is an energy-intensive process, suggesting that it is important for cells to have HIF protein available to become stabilized quickly by hypoxia or by other forms of cellular stress. During hypoxia, HIF stabilization leads to the rapid induction of over a hundred hypoxia-responsive genes with complementary functions. This HIF-mediated hypoxic response is remarkably well-conserved across species. From these observations, we can infer that HIF is a master regulator of the hypoxic response.

The dogma that HIF is the major mediator of the hypoxic response is being challenged and broadened as HIF-independent effects of hypoxic adaptation are being elucidated. As oxygen sensors, PHDs are the first line of response to hypoxia. Accumulating evidence points to other substrates for PHDs including IRP2 and RNA polymerase [68, 106], building a case that HIF is only one of many downstream effectors of the broad response to hypoxia. Moreover, PHDs likely play important roles aside from oxygen-sensing. PHDs have been identified in species that lack HIF, and even in photosynthetic species such as *Chlamydomonas reinhardtii*. Because PHD's prolyl hydroxylase activity is coupled to the decarboxylation of 2-oxoglutarate into succinate, it has been proposed that PHDs regulate cell metabolism, for instance in sensing and mediating a cellular response to amino acid deprivation [12].

Indeed, many intracellular and intercellular signaling pathways have been found to impinge upon PHD-dependent regulation of HIF. PHD enzyme activity requires oxygen, iron, 2-oxoglutarate, and ascorbate, and thus can be inhibited by lack of these cofactors or by competitive inhibition by analogs. High levels of the PHD enzymatic end product succinate can inhibit PHDs, and some reports suggest that fumarate and other metabolic intermediates inhibit PHDs as well [41, 75]. Mitochondria play several roles in regulating HIF expression and activity (reviewed by Ref. [17]), including redistributing intracellular oxygen [35]. Under mild to moderate hypoxia, complex III in the electron transport chain acts as an oxygen sensor and produces reactive oxygen species (ROS), which inhibit PHD when O_2 concentration is not low enough to limit PHD enzymatic activity (reviewed by Ref. [34]). PHD enzyme activity is modulated by nitric oxide and carbon monoxide and by autoregulatory feedback loops from HIF target gene products, all of which participate in other signaling pathways. For example, under normoxia NO inhibits PHDs leading to HIF-1α stabilization, but under hypoxia NO suppresses HIF binding to DNA and target gene transcription without altering HIF protein levels [60, 67]. One explanation for this paradoxical action is that under both normoxia and hypoxia, NO treatment produces a biphasic response in which initially it inhibits PHD activity directly, but a later consequence of this inhibition is HIF-dependent upregulation of PHDs, with PHD2 being primarily responsible for regulated HIF-1α degradation under NO treatment [10]. NO and TNF-alpha, two mediators of inflammatory signaling, have been shown to regulate HIF, which in turn regulates gene expression of many other cytokines [88]. Cytokines and growth factors engage HIF during many physiological and pathological processes [117].

These observations suggest that HIF-PHDs are sensors of a variety of cellular stress states and endocrine signaling pathways. As such, their ability to integrate these signals into transcriptional changes via HIF or other as yet unknown mechanisms of action via other substrates may give them uniquely potent roles in physiological and pathological processes, with important tissue-specific mechanisms and consequences.

PHD isoforms have been shown to have cell-type specific levels of mRNA, which do not uniformly correlate with cell-type specific protein levels, e.g., PHD1 protein levels were lower than predicted by PHD1 mRNA levels in most cell lines. Hypoxic induction of mRNA of PHD isoforms is also cell-type specific: in most cell types, PHD1 is unchanged or downregulated by hypoxia and PHD3 is the isoform most strongly induced by hypoxia, but for instance in OVCAR-3 cells hypoxia induces PHD2 much more strongly than PHD3 in most cell types [5]. One example of the biological significance of PHD isoform expression levels is the demonstration that PHD3, as the isoform whose expression is most significantly upregulated by hypoxia, uniquely limits the accumulation of HIF-2a under hypoxia and is uniquely required for rapid decay of HIF-2α upon restoration of normoxia after hypoxia, indicating it may play a uniquely important role in preventing ischemia–reperfusion injury [5]. Given this cell-type specificity of basal and inducible PHD expression patterns with important biological consequences, in order to predict neuroprotective benefits of PHD manipulations, careful experiments must be conducted in primary neurons and intact neural systems in vivo.

Global or isoform-specific PHD inhibition may have distinct consequences in post-mitotic neurons relative to the immortalized cancer cell lines in which PHD has been most extensively studied to date. PHDs are tumor suppressor genes that negatively regulate HIF, which can act as a proliferative factor (reviewed by Ref. [97]). The aberrant attempt to reenter into the cell cycle has been proposed as one mechanism leading to dysfunction in post-mitotic neurons (reviewed by Ref. [38]). Experiments using structurally diverse PHDs inhibitors show that they are neuroprotective across several injury models; e.g., they delay sympathetic neuron death after growth factor deprivation [62], protect HT22 immortalized mouse hippocampal neurons and rat primary neurons from oxidative stress, and reduce infarct volume in rats subjected to the middle cerebral artery occlusion (MCAO) model of cerebral ischemia [95]. These studies all lend support to the safety and therapeutic benefits of PHD inhibition in neurons. Still, more work needs to be done to clarify the consequences of PHD inhibition in neurons under distinct types of cellular stress.

16.1.4 HIF Can Promote Survival or Death in Neurons

In addition to upregulating genes that help cells survive hypoxia, HIF also upregulates genes encoding proteins that are known to be involved in executing programmed cell death. Activating HIF with the iron chelator deferoxamine (DFO), or overexpressing HIF under the control of a VP16 promoter within HT22 cells increases protein levels of pro-survival genes such as *Vegf* and *Enolase*, but also increased levels of proteins that can promote apoptosis under certain circumstances, including BNIP3, Nix, and PUMA. This reminds us that apoptosis at the single cell level can be adaptive for the body at a system level. It is noteworthy that these increases in potentially pro-death factors occurred in cells that remained healthy, raising the question, what determines whether HIF will promote cell survival or cell death?

We conceptualize hypoxia as bringing the cell to the edge of a cliff, from which point it can either recover from the stress and move back to familiar ground, or, overwhelmed by stress, it can mobilize programmed cell death, which protects surrounding cells from toxicity associated with necrosis [83]. HIF activation leads to the transcription of both prosurvival and prodeath proteins very early after hypoxia, and the fate of the cell is determined by the context over the following hours, days, or even weeks. These pro-death proteins are only activated if the cell faces persistent stress such as acidosis or oxidation, but it makes sense that the cell would transcribe them as soon as it senses a stress rather than waiting until energetic crisis renders it incapable of transcription.

The prodeath effects of HIF activation in HT22 cells can be prevented by siRNA against BNIP3 and PUMA, two established HIF-dependent prodeath genes. As BNIP3 can be activated by secondary stressors, we also found that the antioxidants *N*-acetyl-cysteine and butylated hydroxyanisole abrogate the prodeath effects of

HIF-1. Finally, and most surprisingly, PHD inhibition can also abrogate the pro-death effects of HIF [4]. These results support the notion that HIF expression per se does not commit the cell towards an apoptotic cell death pathway. Rather, post-translational modification of HIF dependent genes, including BNIP3 and possibly PUMA, triggers a cell to jump off the cliff. By contrast, anything that modifies cell context towards survival, such as antioxidants or global PHD inhibitors, push HIF away from the edge of the cliff by biasing the cell against executing the death pathway, perhaps through HIF-independent as well as HIF-dependent mechanisms.

Finally, despite the potential for HIF activation to induce apoptosis under certain cellular states, or to promote tumorigenesis under other circumstances, HIF activation clearly regulates many survival-promoting processes. To harness HIF activation for therapeutic benefit in acute and chronic neuronal injury, it is critical to understand its roles in normal CNS development and physiology as well as its participation in the adaptive response to, or progression of, neuropathologies.

16.2 The Hypoxic Response in CNS Development, Physiology, and Pathology

Relatively little is known about the hypoxic response in neurons compared to other cell types, despite the importance of oxygen supply to neurons given their uniquely high energy demands, and despite the intriguing paradoxical observations of pro-survival and pro-death consequences of activating the hypoxic response in neurons.

Within the body, the partial pressure of oxygen varies widely between tissues; even within tissues, cells lying closer to vasculature are subject to higher oxygen gradients than those a bit further away. Thus, defining normoxia and hypoxia for the purposes of in vitro modeling requires some care. In vivo pO_2 has been estimated at 2–9 % for neurons, but primary neuronal cultures or cell lines are typically maintained at 20 % hypoxia. Cortical neurons cultured in physiological O_2 levels of 1 % continuously from plating have been reported to survive better than those cultured at 20 % O_2, suggesting that standard cortical preparations and plating procedures introduce cortical cells to nonphysiologically high oxygen levels [57]. Indeed, HT22 cells cultured at different O_2 tensions appear able to reset their normoxic set points by up or down regulating PHD levels so that they can still rapidly respond to changes in oxygen tension with normal O_2-sensitive biological responses including HIF activation [50].

16.2.1 Hypoxic Signaling in Development

Within the developing mammalian embryo, low oxygen availability signals proliferation and differentiation of neural precursor cells and HIF is necessary for proper brain development. Neural cell-specific HIF-1α-deficient mice exhibit hydrocephalus accompanied by a reduction in neural cells and an impairment of spatial

memory. The effects of HIF here, as in many cases, have been linked to its regulation of VEGF, as apoptosis of neural cells coincided with vascular regression in the telencephalon of mutant embryos [102]. Normal proliferation of mouse neural precursors has also been shown to require EPO, another canonical HIF target gene [21]. Roles of HIF-regulated EPO and VEGF expression in neurogenesis have implications for regeneration in neurodegenerative disorders as well as excessive proliferation leading to neuroplasias (reviewed by Ref. [1]).

In neuronal stem cells in culture, hypoxic conditions (~5–10 % O_2) promote proliferation in a HIF-dependent manner and skew cellular differentiation toward specific fates, particularly dopaminergic fates presumed to result from HIF-dependent upregulation of tyrosine hydroxylase (TH), discussed further below [70, 99, 111, 116]. In contrast, with human neuroblastoma cell lines hypoxia induces a more immature phenotype, and the ability of hypoxic signaling to lead to dedifferentiation may contribute to the heterogeneity of cells lying within solid tumors that display hypoxic gradients [7, 45]. These paradoxical effects may be mediated by Notch signaling; in primary neural stem cells, hypoxia increased stabilization of the transcriptionally active Notch intracellular domain, stimulation of Notch target genes, and blocked differentiation in a Notch-dependent manner [33]. However, in certain neural stem cells Notch activation actually promotes terminal differentiation [71].

In bone-marrow-derived mesenchymal stem cells (MSCs) which do not normally give rise to neurons in vivo, $CoCl_2$ promotes neuronal differentiation via HIF activity, an effect that is potentiated by ROCK inhibition [74]. The ability of another HIF activator, the 2-oxoglutarate analog dimethyloxalyl glycine (DMOG), to promote mesenchymal stem cell survival by blocking cytochrome c release and to activating the Akt pathway in mesenchymal stem cells gives promise to the strategy of using MSC transplants to replace lost neurons [59].

Oxygen tension regulates the development not only of neuronal precursors but also of astrocytes and oligodendrocytes [78]. Despite the requirement for physiological HIF activity, excessive HIF activation seen in gestational hypoxia leads to a hyper-induction of glutamate transporters and other genes important for neuronal function, indicating a role for aberrant HIF activation during fetal development in alterations in the nervous system observed post-natally [86].

Tyrosine hydroxylase (TH), the rate-limiting enzyme for synthesis of the neurotransmitter dopamine, is a HIF target gene, and thus the hypoxic response plays a role in the development of dopaminergic neurons and their later secretion of dopamine. In rat mesencephalic cells, from which neuronal populations are derived, hypoxia increases TH and dopamine levels as well as HIF reporter activity [56]. Interestingly, treating neural progenitors with either a mixture of cytokines or low oxygen both had the effect of activating HIF and driving differentiation of TH-positive cells, but the gene expression patterns of neural markers including THP, GAD67, GluT1, and beta-tubulin III, were different between treatments [52]. HIF-mediated regulation of TH occurs not only during development; HIF-dependent upregulation of TH has been observed in glomus cells and in PC12 cells in vitro as well [69]. In the adult rat brain in vivo, HIF prolyl hydroxylase inhibition augments dopamine release, suggesting a potential therapeutic role for PHD inhibition in

Parkinson's disease, restless legs syndrome, and other disorders arising from selective loss of dopaminergic neurons [110].

In addition to specifying neuronal fate and negotiating cellular-level survival, hypoxic signaling effects the development of neuronal circuits that control systemic physiological and behavioral responses to changes in oxygen availability. In C. elegans, oxygen levels affect axon guidance and neuronal migration via HIF-mediated upregulation of the Eph receptor VAB-1 [79].

Normal perinatal development of sympathoadrenal innervation in vivo requires the intact hypoxic response program. PHD3−/− mice show hypofunctional blood pressure regulation arising from an aberrant reduction in apoptosis among neurons innervating the adrenal medulla, which are normally culled by gradients in NGF [11]. PHD3 is known to preferentially stabilize HIF-2a [5], but these findings do not exclude HIF-2a-independent effects of PHD3. A series of studies by Freeman and colleagues have demonstrated that hypoxia or pharmacological PHD inhibitors block apoptosis in primary sympathetic neurons isolated from the superior cervical ganglion following NGF-deprivation, which serves as a model of both the pruning of neurons in response to gradients of secreted growth factors that is necessary for normal development and the loss of trophic factors required for neuronal survival such as is observed in progressive neuropathologies. They first showed that HIF activation and BIMEL suppression play distinct roles in the pathway by which hypoxia blocks NGF-deprivation-induced apoptosis [112]. They later showed that PHD inhibition promotes glucose uptake and is protective when glucose is available in the extracellular medium but is toxic under low-glucose conditions, and that HIF-2α stabilization is sufficient to induce apoptosis after NGF-deprivation [61].

16.2.2 HIF in Neuronal Control of Systemic Physiological and Behavioral Responses to Changes in Oxygen Levels

Systemic responses to hypoxemia include ventilatory acclimatization to hypoxia (VAH) which are regulated by the glomus cells of the carotid body. Glomus cells are derived from the neural crest and provide afferent input to the CNS regarding oxygen and carbon dioxide levels in the blood. HIF plays a role in allowing the carotid body to sense hypoxemia (reviewed by Ref. [30]). As in other cell types, HIF-1α is expressed at low levels in glomus cells under normoxia, but its binding to HIF-1β and translocation to the nucleus are induced by ciclopirox (an iron chelator) and hypoxia [8]. In chronically hypoxic rats, carotid body glomus cells show an upregulation of HIF-1α and VEGF-1 that plateaus after 4 weeks of inspiring 10 % oxygen [101]. VAH requires the activities of HIF-1α and its downstream target genes including VEGF, ET-1, and Ca^{2+} channels in the glomus cells of the carotid body [80]. Acute hypoxia increases afferent neuronal activity without induction of HIF, while prolonged hypoxia upregulates HIF, and both of these effects are dependent upon oxygen sensing by mitochondrial cytochrome c oxidase [54]. Iron chelators, which mimic hypoxia by inhibiting PHDs, instantaneously block the

ion-channels in excitable glomus cells of the rat, exciting the chemosensory discharge, and also induce a gradual accumulation of HIF-1α [85]. In chronic intermittent hypoxia, a component of increasingly common obesity-related and other sleep apneas, HIF upregulation in glomus cells is necessary for sensory long-term facilitation of the chemoreceptor activity, and hypoxic induction of HIF can be blocked by a superoxide scavenger [76]. Differential morphological and neurochemical responses of the carotid body to chronic versus intermittent hypoxia result from distinct regulation of HIF subunits [55].

Oxygen-sensitive behaviors in nonmammalian organisms are sensitive to modification by hypoxic exposure and HIF activity in neurons. In mature nematodes, HIF-1 activation can reorganize the neuronal circuit for oxygen-dependent behavior from a distributed, regulated neuronal network into a smaller, fixed network that is constitutively active, with the functional effect of shifting behavioral oxygen preferences to lower concentrations and eliminating a regulatory input form food [15]. In *Lymnaea stagnalis* freshwater snails, peripheral chemosensors detect changes in ambient oxygen and control a central pattern generator for respiration, which requires HIF [9]. Exposure to hypoxia delays responses to light stimuli, and suppresses locomotor activity and righting responses; on an acute time-scale, hypoxia increased the frequency of spontaneous postsynaptic excitatory potentials (sEPSPs), but reduced the firing frequency, the amplitude, and the duration of spontaneous action potentials; hypoxia also induces stress-response proteins such as HSP-70 and alters phosphoproteomics, the consequences of which are still being investigated [25, 96].

16.2.3 HIF Activation in Protective Ischemic Preconditioning and in Ischemic Damage

Hypoxia is a major component of ischemic stroke, which is often modeled in vitro by oxygen and glucose deprivation (OGD) or in vivo by transient or permanent occlusion of the middle cerebral artery (MCAO). In rat cortical neurons exposed to hypoxia, HIF-1 binding activity is enhanced, but hypoxic preconditioning blunts this effect, suggesting that the sensitivity of the HIF response to hypoxia can be altered based on prior experience and that the neuroprotection seen after preconditioning depends in part on limiting the activation of HIF [87]. However, the ability of DFO to protect primary immature hippocampal neurons from hypoxia-induced death is partially dependent upon HIF-1α [37]. Hypoxia causes delayed cell death in neurons, which is dependent upon HIF and p53 [36]. In one study of neural progenitor cells exposed to OGD, a reduction in ERK phosphorylation was associated with inhibited cell growth [47]. Hypoxia without glucose deprivation, however, enhanced proliferation and differentiation in primary neural stem cells, a finding that has been interpreted as evidence that mild hypoxia could make the ischemic penumbra a niche for resident neural stem cells to replace damaged neurons [39].

In cocultures, preconditioned glia can protect neurons from hypoxia-ischemia. EPO released from astrocytes in a mixed primary culture, or recombinant human EPO given up to 6 h after reoxygenation following 6 h exposure to 2 % O_2, protected primary neurons from hypoxia-induced death [58]. EPO stimulates hippocampal neurogenesis, which is thought to be a key component of and target for anatomical and functional repair of this particularly vulnerable brain structure after ischemia [81].

HIF is likely not the only path to protection by preconditioning. Although HIF has been implicated in the mechanism by which ischemic preconditioning affords protection, some studies have shown that HIF is not necessary and have suggested that activation of adenosine receptors, ATP-dependent potassium channels, heat-shock proteins or activation of NMDA receptors are key mechanisms mediating ischemic tolerance (reviewed by Ref. [20]). Preconditioning with 3-nitropropionic acid (3-NP) inhibits succinate dehydrogenase and was considered an inducer of "chemical hypoxia" before the succinate-sensitive PHDs were discovered [84]. 3-NP protects neurons from damage and death upon subsequent oxygen and glucose deprivation (OGD) without inducing HIF translocation to the nucleus, suggesting HIF-independent consequences of PHD sensing, perhaps via other transcription factors such as CREB or AP-1 [108].

The cellular context appears to be critical in determining the outcome of HIF activation in hypoxia-ischemia. In human NT2-N neurons exposed to hypoxia and reoxygenation under neutral or acidotic conditions, distinct pH-dependent patterns of activation of cytokine receptors and other members of inflammatory pathways were observed [29]. In rat primary cortical neurons exposed to hypoxia, HIF-1 alpha protein levels were inversely proportional to ROS levels, and a reducing environment, e.g., as created by treatment with the glutathione precursor *N*-acetyl cysteine, allowed HIF stabilization in response to hypoxia, and proposed that the stabilization of HIF observed in OGD but not under hypoxia alone is a consequence of glucose regulation of redox status [32]. In primary hippocampal cultures exposed to hypoxia (with normal glucose), NAC (50 μM) improved cell survival, reduced ROS generation, and inhibited DNA strand breaks induced by hypoxia [44].

In in vivo models of ischemia, HIF activation has been reported to have both beneficial and detrimental effects. Although HIF-1 is necessary for hypoxic preconditioning, paradoxically, in the absence of preconditioning, HIF has been reported to promote ischemia-induced neuronal death in perinatal ischemia [16]. In a transient focal ischemia–reperfusion rat model of neonatal stroke, HIF-1α and VEGF were induced peaking at 8 h post-occlusion and declined by 24-h post-occlusion [72]. Focal ischemia upregulates HIF and EPO rapidly, then VEGF and BNIP3 later, and BNIP3 is associated with delayed cell death in striatal and cortical neurons [2]. After mild transient global brain ischemia, the HIF-regulated proapoptotic Bcl-2 family member BNIP3 is induced and precedes apoptosis [92]. HIF-1 mediates the hypoxic induction of the receptor for advanced glycation end products (RAGE), which participates in multiple signaling pathways that, like HIF itself, can be either injurious or pro-survival [77]. In one study, siRNA inhibition of HIF was shown to reduce ischemic–reperfused brain injury in rats [19]. The antioxidant vitamin E, which protects rats from ischemic brain tissue loss, induced HIF-dependent gene transcription

[115]; however, transglutaminase 2, which protects neurons from delayed cell death after OGD in vitro, binds HIF-1β and inhibits HIF-dependent gene transcription [26]. HIF activates iNOS after permanent MCAO [65]. Neuroprotection in a chronic hypoxia model with phoshodiesterase-5 inhibition indicates that the NO/cGMP signaling pathway prevents apoptosis independent of HIF signaling. Neuronal targeting of AAV vectors that overexpress VEGF under control of the hypoxia response element reduced infarct volume after transient MCAO in mice, while limiting adverse effects of VEGF expression on adjacent normal parenchyma [93].

Glial cells are more resistant to hypoxic injury than neurons, and coculture experiments indicate that the activation of the hypoxic response in glia can alter neuronal viability in the face of later challenges. Pyruvate kinase expression is regulated by HIF and is induced in glia but not neurons in response to hypoxia; overexpression of PK in neurons attenuates hypoxic cell death, suggesting that PK induction is part of the mechanism by which glia are more resistant to hypoxic injury than neurons [94]. Conditioned medium from cortical astrocytes exposed to nonlethal OGD protects neurons from later lethal OGD, and this protection requires HIF-2 regulated EPO expression [18]. Other reports hold that selective loss of HIF-1 function in astrocytes protects neurons from hypoxic-induced death, likely because HIF activity in astrocytes induces iNOS, causing astrocytes to secrete NO at levels harmful to neurons [104]. In microglia, hypoxia induces iNOS expression under the regulation of both HIF-1α and the PI3-kinase/Akt/mTOR signaling pathway, and microglial iNOS modulates inflammation after hypoxia, a critical component of delayed phases of ischemic damage [63].

In the retina, ischemic preconditioning by hypoxia or $CoCl_2$ requires HIF-mediated upregulation of Hsp27 [109] and EPO [31]. HIF-1 is induced by transient optic nerve ischemia that causes blindness, but it is unclear whether HIF here is limiting injury, contributing to injury, or epiphenomenal [24]. Both periventricular white matter damage and retinal damage after 3–14 days of hypoxia is associated with increases in mRNA and protein expression of HIF-1α NMDAR1, GluR2, GluR3, VEGF, eNOS, nNOS, and iNOS, suggesting that NO and excitotoxicity play roles in retinal damage [49].

Numerous drugs that protect spinal neurons from death after ischemia–reperfusion or contusion injuries appear to act at least in part through HIF. L-arginine reduced spinal neuron loss and paralysis and induced HIF after spinal ischemia–reperfusion [103]. HIF-1α is induced after spinal cord injury, and a lecithinized superoxide dismutase (PC-SOD) further increased HIF-1α as well as reducing ROS levels and reduced motor dysfunction even if given 24 h after injury [100]. Our lab's recent work with a newly identified potent HIF activator, tilorone, protected rats from spinal cord injury as well [82].

16.2.4 Hypoxic Signaling in Other Neuropathologies

Inducing the hypoxic adaptive response provides broad neuroprotection in several injury models distinct from hypoxia. A diverse array of HIF activators, including $CoCl_2$, DFO, and mimosine protect neurons from oxidative stress-induced death in

a glutathione depletion model [114]. Overexpressing HIF-1α or using siRNA to knockdown HIF-1α in mouse hippocampal HT22 cells showed that HIF promotes survival in response to DNA damage or ER stress but increases sensitivity to oxidative stress induced by glutathione depletion [3]. This finding suggests HIF-independent mechanisms by which PHD inhibitors acting upstream of HIF might protect against oxidative stress while genetic manipulation of HIF alone is deleterious, and demonstrates injury-type-specific effects of HIF. HIF activation and subsequent upregulation of glucose flux was observed in neuronal cell lines and primary neurons that are resistant to A-beta pathology, indicating that the hypoxic response may protect cells from Alzheimer's disease [98]. Cortical spreading depressions (CSDs), a component of cerebral ischemic damage and other neuropathologies, induce HIF, iNOS, and LDH-A, which are proposed to mediate the protective effect of preconditioning CSDs to produce tolerance to subsequent ischemia [105]. The cerebral cortex of rats exposed to acute anemia showed increased HIF and expression of target genes VEGF, erythropoietin, CXCR4, iNOS, and nNOS, consistent with the dependence of HIF-PHDs upon iron, but it is unclear whether HIF activation is protective or deleterious in this paradigm [66].

As with neurons, the hypoxic response plays roles in determining the consequences of non-hypoxic injury to glia. HIF-1 induction secondary to preconditioning with cobalt chloride or iron chelators may mediate the protective effects against metabolic insult induced by the mitochondrial inhibitor 3-NP in C6 astroglial cells [113].

HIF activation can cause disease through aberrant activation of cell survival and proliferation pathways. In neuroblastoma cells derived from sympathetic nervous system cells, hypoxia or hypoglycemia caused cells to lose their neuronal/neuroendocrine features and revert to an immature, neural crest-like phenotype, which may result from prolonged HIF-2 stabilization and may lead to aberrant proliferation and tumorigenesis [73]. As in many poorly vascularized and thus hypoxic solid tumors, HIF-2α is upregulated in human paragangliomas, and familial paragangliomas and pheochromocytomas have been associated with mutations in the genes encoding HIF PHDs, VHL, and SDH, all of which are necessary for hypoxic signaling ([53], Dahia et al. [23]).

In summary, HIF plays important roles in the normal development of the CNS, in neuronally regulated physiological and behavioral responses to changes in environmental oxygen, and in CNS pathologies. HIF and PHD isoforms show cell-specific expression and activity patterns even within neuronal cells of different types and at different points in development. There is complex crosstalk between the hypoxia response and other signaling pathways and markers of metabolic stress that leads to often paradoxical observations of the consequences of HIF activation. These observations reveal a need for further investigations in neuronal systems in vivo and a variety of models of neuropathology in order to clarify under what conditions the hypoxic response is beneficial and under what conditions its activation contributes to pathology, and how we can bias the activation of this response toward promoting cellular survival and tissue repair after hypoxia or other types of injury or disease.

16.3 Harnessing the Hypoxic Response FOR Neuroprotection and Repair

Despite its complex roles in physiology, the breadth, depth, and coordination of the hypoxic response makes HIF an attractive therapeutic target. Cerebral ischemia is a heterogeneous disorder that affects many physiological pathways. Historically, therapeutic trials for stroke have been disappointing, and one possible reason for the failure of these many drugs is that their effects were too narrow, but targeting HIF might engage a more comprehensive program for protection and repair. Further, because HIF activation is part of what the body naturally tries to do to adapt to hypoxia, the breadth of this response should not show the proportional increase in toxicity that would be expected by combining many narrowly targeted drugs to evoke a similarly broad response. In light of the potential pro-death or tumorigenic consequences of HIF activation, however, we wanted to identify ways to bias the cell toward survival. To engage broad homeostatic processes, our strategy was to intervene at the point where the cell senses stress, that is, the oxygen-sensing PHDs. HIF-PHDs are iron-dependent, and promising clinical trials showing that iron chelators are neuroprotective, so we hypothesized that PHD inhibition would provide the benefits seen from iron chelators without the potential toxicity of disrupting iron uptake and intracellular storage, and use by other enzymes.

16.3.1 Neuroprotection by Iron Chelators and PHD Inhibitors

Small molecule iron chelators have long been known to provide therapeutic benefits both in human disease and in a variety of animal models of neuronal injury and degeneration. Iron chelators enhance metabolic recovery in an animal model of stroke [40], attenuate severity and duration of experimental allergic encephalomyelitis in rats [13], and attenuate functional deficits caused by MPTP in animal models of Parkinson's disease [48]. In a small human clinical trial, iron chelators slow progression of Alzheimer's disease [22].

Iron chelators have long been known to mimic the effects of hypoxia, that is, to stabilize HIF and activate HIF-mediated gene expression. DFO was shown to prevent the association of HIF-alpha with the von Hippel–Lindau protein, which is necessary for HIF degradation under normoxia. In 2001, two groups reported that they had identified prolyl hydroxylation as the modification that targets HIF-1α for degradation, and showed that DFO inhibits the HIF prolyl hydroxylases [42, 43]. Since HIF-PHDs require iron along with other cofactors, it is logical to think that an iron chelator would competitively inhibit PHDs with respect to iron binding. In fact, this mechanism is controversial, but in any case iron chelators certainly activate HIF and protect neurons from a variety of types of stress.

There are clinically approved iron-chelating PHD-inhibitors ready for human trials, including Desferal. Desferal is not ideal for a neuroprotectant because it pen-

etrates the blood brain barrier poorly. Further, because iron is necessary for many physiological functions, chronic treatment with iron chelators has the potential to cause toxicity. These limitations provided rationale to identify drugs that have the PHD-inhibiting properties of DFO, but do not chelate iron.

We first investigated whether the salutary effects of iron chelators are related to HIF prolyl hydroxylase inhibition. Three structurally diverse HIF-PHD inhibitors (DFO, DHB, and Compound A), as well as a 19-amino acid-long peptide consisting of the conserved oxygen dependent domain of the HIF protein, stabilize HIF-1, upregulate the mRNA and protein levels of HIF-target genes including erythropoietin in the CNS in vivo, and reduce infarct volume when administered prior to permanent occlusion of the middle cerebral artery, a rat model of severe stroke [95].

16.3.2 Identifying Novel Neuroprotective Agents and Mechanisms

Given the therapeutic potential of targeting HIF PHDs or HIF directly, we wondered if we could identify novel HIF activators that do not chelate iron and that are nontoxic. We used a cell-based assay for HIF activity to screen a library of FDA-approved drugs so that we would be able to move our findings directly into the clinic [82]. A reporter construct containing the firefly luciferase gene downstream of the hypoxia response element (HRE-luc) was stably transfected into HT22 cells, allowing HIF-activated transcription to be measured by quantifying luminescence upon addition of the substrate, luciferin. This assay was used to screen a library of over a thousand FDA-approved drugs for their ability to activate HIF binding to the HRE and subsequent transactivation of gene expression.

The most potent inducer of HRE-luciferase activity identified in the initial screen was tilorone. Tilorone was identified in 1970 as an antiviral agent; it is an interferon-inducing agent, and is thought to induce cytokine expression by acting as a DNA intercalator. While DFO induced the HRE-luc reporter activation almost twofold compared to background signal with our DMSO control, tilorone induced reporter activation almost tenfold, i.e., with fivefold greater efficacy than DFO.

To validate the screen, we used several commercially available tilorone analogs to begin to investigate whether a structure–activity relationship exists. Tilorone and three tested analogs were all much more potent activators of our HRE-luc reporter than three positive controls: reduced oxygen, cobalt chloride, and DFO. Tilorone and its analogs induced HIF-protein levels in HT22 cells, shown here, and also in primary embryonic rat neurons, and they induced mRNA of HIF target genes in both of those cell types as well.

To determine whether tilorone crosses the blood brain barrier, that is, whether i.p. or oral administration would induce HRE-regulated gene transcription in rat brain in vivo, we used bioluminescent imaging (IVIS). This technique allows us to measure the near-infrared light that passes through body tissues, and thus to measure luciferase activity in real time within a live, intact animal. An i.p. injection of

tilorone produced robust induction of the HRE-luc reporter in the rat brain in vivo, indicating that tilorone crosses the BBB in healthy animals. Notably, we did not see this effect with i.p. administration of DFO. To validate these findings with the reporter construct, we showed that i.p. tilorone stabilizes HIF protein levels in rat brain in a dose-dependent manner. Further, tilorone and its analogs induced mRNA of HIF target genes in both HT22 cells and in primary neurons, again, more potently than our positive controls, for VEGF, LDH, and Epo.

To demonstrate whether tilorone is neuroprotective in an in vivo model of hypoxia-ischemia, we administered tilorone 24 h before permanent middle cerebral artery occlusion, and observed significant reductions in infarct volume. To determine whether tilorone could protect in other CNS injury paradigms, we gave i.p. tilorone 40 min before a spinal cord contusion induced by weight-drop and observed reduced lesion volume 24 h later. Taken together, these findings indicate that tilorone is a promising drug candidate that we identified in our screen as an inducer of the hypoxia response, and validated in vivo as a neuroprotective agent in at least two injury paradigms. We are in the process of validating other hits in this manner.

Although we see robust activation of HIF and neuroprotection by tilorone, we do not yet know tilorone's mechanism of action. It is clearly different from iron chelators such as DFO because tilorone does not bind iron, nor does it inhibition PHDs. This is exciting for two reasons; first, our screens of FDA-approved compounds have identified novel activators of the HIF pathway that are poised for testing in animal models that could be transitioned rapidly to human studies. But not only that, our screen has also identified novel chemical entities by which we can probe activation of the hypoxia response in neurons; tilorone is now a tool we can use to uncover mechanisms that regulate HIF activity other than PHD inhibition.

Other groups have used high-throughput screening of small molecule libraries to identify novel mediators of neuroprotection in various assays. For example, a brain slice-based model for ischemic stroke used as the primary assay for a compound screening platform in a study that identified cardiac glycosides as novel neuroprotectants whose putative target is the Na^+/K^+-ATPase [107]. Findings of this nature underscore the important role that hypothesis-neutral, high-content, tissue-based screens can play in the identification of new candidate drugs and drug targets for the treatment of diseases for which validated therapeutic pathways are not currently available.

16.4 Conclusion

Investigations into one of the most primordial of cell stresses, hypoxia, have elucidated a comprehensive homeostatic program by which the HIF transcription factors regulate a well characterized set of genes involved in hypoxic adaptation. Subsequent studies defined the HIF prolyl 4-hydroxylases as the family of oxygen dependent enzymes that convert a deficiency in oxygen supply into increases in oxygen delivery and decreases in demand. While an elegant program is coalescing around

hypoxic adaptation, each new discovery has fostered the identification of new proteins and genes that participate in cross-talk with the hypoxic response. These studies are forging a more complete understanding of how the brain utilizes oxygen and adapts to its deficiency, how the hypoxic response participates in normal development and physiology and may be harnessed or hijacked during pathology, and how we may manipulate these endogenous programs for therapeutic advantage. Together this exciting new body of knowledge has set the table for treatments of some of the most devastating and unexpected ailments that confront humans, including cardiac and cerebral ischemia. Based on these findings, we are sanguine that better treatments for myriad diseases are within our reach.

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Part IV
Molecular Oxygen Sensing

Chapter 17
Optical Analysis of Hypoxia Inducible Factor (HIF)-1 Complex Assembly: Imaging of Cellular Oxygen Sensing

Jun Hu, André Bernardini, and Joachim Fandrey

Abstract Hypoxia is a common phenomenon that occurs in a variety of diseases such as cardiovascular ischemia, anemia, and cancer. Cellular oxygen sensors measure changes in tissue oxygenation and induce responses aimed at restoring sufficient supply with oxygen. Genetic adaptation to hypoxia is under control of hypoxia-inducible factors (HIFs), of which two highly homologous subunits HIF-1α and HIF-2α are regulated by oxygen tension. Together with HIF-1β (=ARNT; aryl hydrocarbon receptor nuclear translocator) they form transcriptionally active complexes under hypoxia which drive the expression of hypoxia inducible genes. The meaning of different HIF complexes, i.e., HIF-1α/ARNT versus HIF-2α/ARNT with respect to target gene or tissue specificity has not been fully resolved. We applied modern microscopic methods like fluorescence resonance energy transfer (FRET) to elucidate protein–protein interactions and fluorescence recovery after photo-bleaching (FRAP) to study mobility of HIF proteins inside the nuclei of living cells. We found differences both in nuclear mobility and the assembly of HIF-1 versus HIF-2 which might help to better understand the assembly of HIF complexes.

Keywords HIF-1 assembly • Dimerization • Mobility

17.1 Introduction

All mammalian cells require oxygen for proper function and survival. To ensure an adequate supply with oxygen, tissues and cells have developed strategies to adapt to shortage of oxygen, called hypoxia. In general, these mechanisms are aimed at keeping the balance between oxygen supply and consumption, thus ensuring a stable oxygen tension in the tissue [1]. While an increase in oxygen consumption is physiologically balanced by improved supply in healthy tissue, an imbalance with

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reduced tissue oxygenation is more likely to develop in pathophysiology. It is well known that tumors, for example, develop central regions of hypoxia where the increasing oxygen demand by fast growing tumor tissue outpaces the growth of new capillaries which is intended to provide more oxygen [32]. The pathophysiology of cardiovascular disease is almost exclusively caused by impaired oxygen supply to tissue which remains high in its oxygen demand. While these causes of hypoxia develop due to inappropriate perfusion with respect to the consumption of oxygen in the tissue, other reasons for tissue hypoxia are obviously reduced oxygen capacity of the blood, such as in anemia, or a decrease in the inspiratory oxygen tension [12].

17.2 Hypoxia Induced Erythropoietin Production

Both anemia and hypoxemia have classically been linked to the erythropoietic hormone erythropoietin. It was known from expeditions in the late nineteenth century that high altitude exposure increased the number of red blood cells [10]. It was soon discovered that the reduced inspiratory oxygen tension was the cause of polycythemia as a compensatory increase in oxygen capacity of the blood. Studies in the early twentieth century clearly defined an erythropoietic substance in the serum of anemic animals that was able to stimulate red blood cell production [6]. Common to hypobaric hypoxia and anemic hypoxia was the increase in erythropoiesis to compensate for reduced tissue pO_2 by increasing or restoring oxygen capacity of the blood.

Nevertheless, it still took decades to identify erythropoietin as the main regulator of erythropoiesis. After isolation and cloning of the gene [23] research on the physiology rapidly focused on hypoxia-induced gene expression. The fact that cells and tissue—despite under reduced oxygen tension—start to increase production of the proteohormone erythropoietin is remarkable: In general, protein production is reduced to spare as much oxygen as possible. Erythropoietin, in contrast, was found to be several hundredfold increased upon severe hypoxic stimuli [11]. Further studies revealed that de novo gene expression of the erythropoietin gene mainly in the kidneys of adults but to a lesser degree also in the liver, the brain, the spleen and, potentially, the bone marrow was responsible for an exponential increase of erythropoietin protein in the serum after anemic or hypobaric hypoxia [10]. Using transgenic mice with different parts of the erythropoietin gene, both including regulatory and coding sequences, Gregg Semenza and colleagues defined critical regulatory elements of the erythropoietin gene [28, 29]. Within these regulatory DNA elements a short highly conserved hypoxia response element (HRE) was isolated to which the transcription factor complex hypoxia-inducible factor-1 (HIF-1) was found to bind. Subsequent work revealed that many genes in the human genome contain hypoxia response elements and that the transcription factor HIF-1 is part of a widespread cellular oxygen sensing mechanism [21, 30].

17.3 Hypoxia Inducible Factor 1

HIF-1 turned out to be a heterodimer of an oxygen-sensitive α-subunit and a constitutively nuclear localized β-subunit. While HIF-1β was identical to a previously known transcription factor subunit ARNT (= Aryl hydrocarbon Receptor Nuclear Translocator) HIF-1α turned out to be a new member of a transcription factor family that is characterized by a DNA binding basic helix loop helix (bHLH) domain as well as of two domains important for dimerization of the transcription factor subunits [33]. These domains are called PAS A and B—for Period, Aryl hydrocarbon receptor, Single-minded—three other previously known members of this transcription factor family [13].

Soon after the initial description of HIF-1α other members of the HIF family that also formed complexes with ARNT were described. HIF-2α, regulated in a very similar fashion as HIF-1α, was found to be preferentially expressed in endothelial cells but showed a more tissue-specific expression while HIF-1α was essentially found in all cells of the human body [24]. A further isoform, HIF-3α and splice variants thereof, can like HIF-1α or -2α form complexes with ARNT. Its role as a transcription factor for hypoxia-inducible gene expression, however, is less clear. In fact, one isoform of HIF-3α was found to inhibit the activity of HIF-complexes [20]. Therefore, for the rest of this chapter, regulation of HIF-1α and -2α as oxygen-sensitive subunits of the HIF complex and dimerization partners of HIF-1β are discussed.

17.4 Regulation of the HIF-1 Complex

Formation of the HIF complex after a hypoxic stimulus is instantaneous [17]. Within a few minutes the HIF complex accumulates and binds to the respective DNA element to start transcription. For the erythropoietin gene it was found that already 20–30 min after the onset of hypoxia, the HIF target gene erythropoietin is found to be increasingly expressed on the mRNA level [11]. Research on the rapid activation of HIF complexes revealed an interesting mode of regulation: Instead of synthesizing new HIF-1α/HIF-2α oxygen-sensitive subunits to form the HIF complex, it was found that hypoxia causes a block of degradation of continuously synthesized HIF-α subunits [25]. Upon reoxygenation and the return of oxygen, degradation of HIF-α subunits is rapidly taken up again causing a half-life of less than 5 min of HIF-α proteins under well-oxygenated conditions. HIF-α degradation critically depends on recognition by the protein product of the von Hippel–Lindau tumor suppressor gene (pVHL). Binding of pVHL to HIF-α subunits causes the recruitment of an E3 ubiquitin ligase, subsequent poly-ubiquitination and degradation through proteasomes [22]. Therefore the recognition of the HIF-α subunits by pVHL is critical for degradation. Binding of pVHL to HIFα is only possible under oxygenated conditions when posttranslational modification of HIF-α subunits by proline hydroxylation is enabled. Seminal work at the beginning of the twenty-first

century lead to the identification of these bona fide cellular oxygen sensor enzymes, namely prolyl hydroxylases, of which the activity is regulated by the oxygen tension within the cells [5, 9, 16]. Posttranslational hydroxylation of two proline residues in HIF-1α and HIF-2α marks the α-subunits for recognition by pVHL and subsequent degradation. Under hypoxic conditions, hydroxylation ceases and HIF-α subunits can evade degradation. In this scenario prolyl hydroxylases of which three iso-enzymes have been described so far, called PHD1, PHD2, and PHD3, fulfill the criteria of cellular oxygen sensors: Their enzymatic activity depends on cellular pO_2 leading to high activity under normoxic conditions and subsequent degradation of HIF-α subunits while under hypoxia the activity rapidly decreases and allows stabilization of the α-subunits. Interestingly, the steep part of the curve that describes enzyme activity as a function of oxygen tension lies in the physiological range observed in different tissues [3].

An additional hydroxylase modifies an asparagine in the C-terminal end of HIF-α subunits [18]. This part of the protein is responsible for recruitment of transcriptional adapter proteins such as CBP/p300. Only when these adapter proteins bind to the HIF complex, tissue-specific expression of hypoxia-inducible, HIF-dependent genes is possible. This has been shown for the erythropoietin gene where binding through the C-terminal end of the HIF complex allows recruitment of further transcription factors such as hepatic nuclear factor 4α [10]. This asparagine hydroxylase was originally termed factor inhibiting HIF-1 (FIH-1) because the initial description of this protein showed that the activity (= hydroxylation) under well-oxygenated condition impedes the binding of transcriptional co-activators and thus inhibits the activity of HIF [19]. Under hypoxia, oxygen-sensitive enzymatic activity of FIH-1 decreases, thus allowing co-activators to bind under hypoxic conditions.

Collectively, HIF complexes are regulated in a dual way: by protein abundance through normoxic destruction of the α-subunits and also by inhibition of the activity of the HIF complex by asparagine hydroxylation under normoxia. Under hypoxia, when enzymatic activity of PHDs and FIH-1 decreases HIF target genes like erythropoietin are expressed.

17.5 Analysis of HIF Complex Assembly

To get further insights into the process of HIF complex assembly for hypoxia-induced gene expression we analyzed nuclear mobility and the interactions of HIF-1α and HIF-2α with ARNT by fluorescence recovery after photo bleaching (FRAP) and fluorescence resonance energy transfer (FRET) microscopy respectively [34, 35]. As described above, under hypoxia HIF-α subunits accumulate and translocate into the nucleus via a nuclear localization sequence (NLS) and binding to importins [7]. Within the nucleus, they dimerize with ARNT. It was of considerable interest to determine whether HIF dimers are immediately formed after entry of HIF-α subunits or whether DNA binding at HRE is a prerequisite for HIF complex formation. We decided to approach this question by determining nuclear

mobility of the HIF subunits. To this aim we applied the Gateway™ cloning system to generate fusion proteins of HIF subunits with fluorescent derivatives of green fluorescent protein (GFP), namely enhanced cyano fluorescent protein (ECFP) and enhanced yellow fluorescent protein (EYFP). Resulting fusion proteins were ECFP-HIF-1α, EYFP-HIF-1α, ECFP-HIF-2α, EYFP-HIF-2α and ECFP-ARNT and EYFP-ARNT. The DNA-plasmids encoding the fusion proteins were pair wise (ARNT with HIF-1α or with HIF-2α, respectively) co-transfected into human osteosarcoma cells (U2OS) at equal concentrations. As shown in Fig. 17.1, fluorophores in a square-like region within the nucleus were irreversibly destroyed by photobleaching with a high-intensity laser beam. The co-transfected ECFP or EYFP fused proteins were bleached with laser of different wave length (according to their absorption maxima) and fluorescence signals in their respective emission channel. Intact molecules from the area outside the bleached square then diffuse into the bleached area and recover fluorescence intensity within this square (Fig. 17.1). The process of fluorescence recovery is recorded over time (here over 300 s in intervals of 15 s) which provides information about the mobility of the fusion proteins. Finally, maximum recovery is achieved and allows calculating the half recovery time t_{50} to quantify the mobility of the investigated proteins. Interestingly, both HIF-α subunits redistributed differently compared to each other and to their partner ARNT (Fig. 17.2), indicating independent mobility between HIF-αs and ARNT. Table 17.1 gives estimates of the t_{50} values which were substantially different. This finding provides evidence that the dimerization of HIF-αs and ARNT does not occur immediately after the translocation of the α-subunits because in this case HIFαs and ARNT should have the same mobility due to the formation of a single

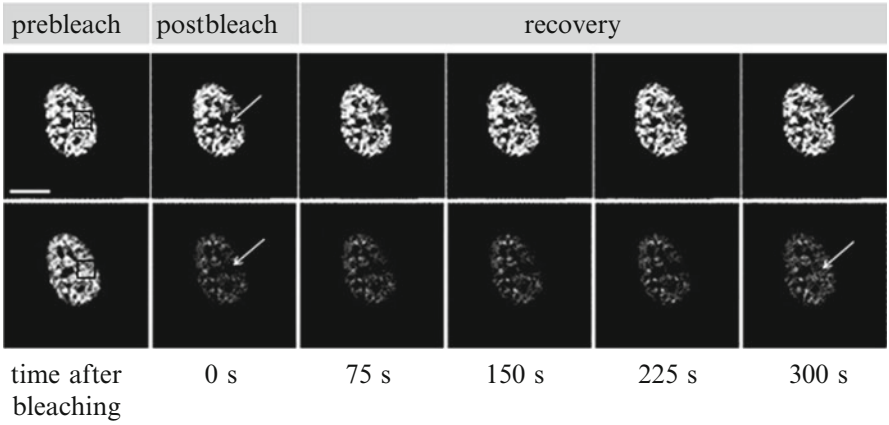

Fig. 17.1 Fluorescence recovery after photo-bleaching (FRAP). U2OS cells were co-transfected with EYFP-HIF-1α (recorded in the *upper row*) and ECFP-ARNT (recorded in the *lower row*). An area of 3 µm×3 µm (*black square*) was bleached and redistribution of both fluorescent fusion proteins was measured over 300 s at time intervals of 15 s. *Arrows* indicate the fluorescence intensity directly after photo-bleaching and after maximum recovery. The *white calibration bar* on the left represents 7.5 µm

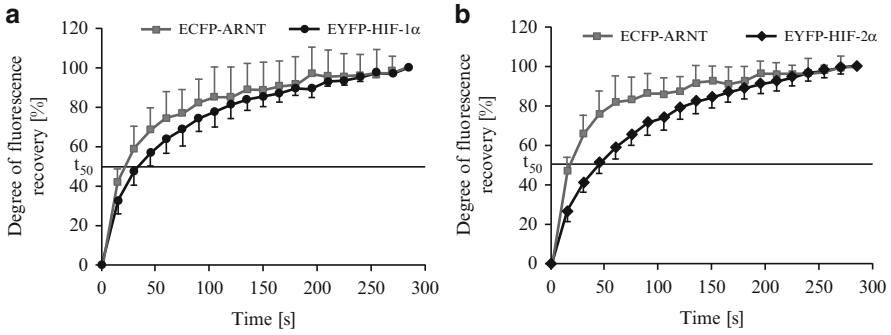

Fig. 17.2 Analysis of mobility HIF-1α, HIF-2α, and ARNT. U2OS cells were co-transfected with EYFP-HIF-1α in (**a**) or EYFP-HIF-2α in (**b**) and ECFP-ARNT. Cells were bleached as described in Fig. 17.1 and recovery of fluorescence intensity was recorded during redistribution. After 300 s, the maximum recovery of approximately 100 % of the pre-bleach value was achieved. Values are presented in % of the initial value. Each data point represents the mean value ± SD from ten cells. Finally, half recovery time t_{50} (*black line*) to quantitate the mobility of the HIF subunits was calculated

Table 17.1 Half recovery time of HIFs after co-expression

Half recovery time [s]	HIF-1α	HIF-2α	ARNT
co-expression	34		22
		53	18

complex. Additionally, HIF-1α mobility with a t_{50} of 34 s differed considerably from that of HIF-2α with a t_{50} of 53 s: HIF-2α diffused much slower than HIF-1α. Interestingly, ARNT also exhibited different mobility depending on whether it was cotransfected with HIF-1α or HIF-2α. So far, the reasons for the different mobility have not been resolved. Additional studies using deletion constructs in which the C-terminal end of the α-subunits have been removed to prevent binding of coactivators such as CBP/p300 did not show any change of mobility (data not shown) suggesting that binding of coactivators does not affect the mobility or that coactivators are first recruited when the complex is bound to HREs. One other observation is mentionable: Careful evaluation of the microscopic images before bleaching and after fluorescence recovery revealed that fluorescence recovers to exactly the same spots within the nucleus displaying the typical heterogeneous distribution of HIF subunits within the nucleus [2, 35]. It thus appears that HIF subunits are "directed" to these spots and do not randomly distribute within the nucleus (Fig. 17.1).

Collectively, the fact that HIFαs and ARNT move separately within the nucleus is very well in line with reports that besides canonical HIF signaling through binding to DNA regulatory elements HIF-1α has been found to be involved in noncanonical signaling by binding to other nuclear proteins. HIF-1α was found to bind the intracellular domain (ICD) of Notch and contribute to maintenance of stem cell state [14]. It has been reasoned that depending on the number of binding partners for HIF-1α—either ARNT or Notch ICD—competition for canonical versus noncanonical signaling may occur. In view of our findings that ARNT and HIFαs appear

to move independently competition for binding to Notch ICD or other proteins will most likely not be influenced by the affinity for ARNT.

HIF-1, formed from HIF-1α and ARNT, and HIF-2, made of HIF-2α and ARNT bind to the same consensus DNA sequence of HREs. Still, it is well known that certain hypoxia inducible genes are regulated by HIF-1 such as carbonic anhydrase 9 while others are under control of HIF-2 such as erythropoietin. It has been reasoned that different coactivators recruited to the C-terminus of either HIF-1 or HIF-2 may be responsible for the target gene selectivity [8]. This could also imply that the HIF complexes may differ in their assembly to allow binding of different coactivators. We therefore decided to determine the assembly of the two HIF complexes by determining protein–protein interaction between the α-subunits and ARNT. To this aim we applied fluorescence resonance energy transfer (FRET) as recently described [35].

According to the theory of FRET, the donor ECFP fused to HIF-α subunits may transfer energy nonradiatively (by dipole–dipole coupling) to the acceptor EYFP fused to ARNT. Importantly, the emission spectrum of ECFP needs to sufficiently overlap with the excitation spectrum of EYFP. If the two fluorophores and thus the subunits fused to them are in close proximity to each other (typical distance below 10 nm), energy from ECFP is transferred to EYFP instead of emitting fluorescent light [27]. The transferred energy excites EYFP which will emit fluorescent light although the fluorophore was not directly excited (Fig. 17.3). The efficiency of this energy transfer increases when intermolecular distance decreases. Due to this dependence of FRET efficiency on the distance between donor and acceptor FRET can be used as a molecular ruler. The distance *d* between two fluorophores can be

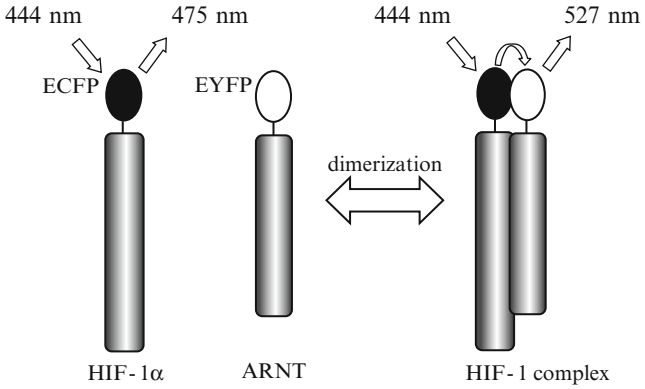

Fig. 17.3 Principle of fluorescence resonance energy transfer (FRET). Fusion proteins of enhanced cyano fluorescent protein (ECFP) and HIF-αs, called donors, are excited by a 444 nm laser to emit fluorescence light at 475 nm. If acceptor molecules, here enhanced yellow fluorescent protein (ECFP) fused with ARNT, are closer than 10 nm, energy may be transferred through nonradiative dipole–dipole coupling to the acceptor which then emits light at 527 nm although it was not directly excited. The efficiency of the energy transfer (FRET-efficiency) depends on the distance between the two chromophores which is the higher the closer the two proteins are. FRET efficiency can then be used to calculate distances between proteins and prove interaction [34]

calculated from the maximum FRET-efficiency using the formula $d = R_0[(1/E) - 1]^{1/6}$, in which R_0 means the Förster distance and E the FRET-efficiency [27]. The Förster distance, i.e., the distance at which the energy transfer efficiency is 50 %, is specific for the donor acceptor pair and is 4.92 nm for the ECFP and EYFP pair. Of note, FRET efficiency was found to strongly depend on the acceptor/donor ratio [34]. It was therefore important to scan many cells with a range of acceptor/donor ratios and to determine FRET efficiency until it reached its maximum at a plateau (Fig. 17.4). Here FRET efficiency is independent of the acceptor/donor ratio and

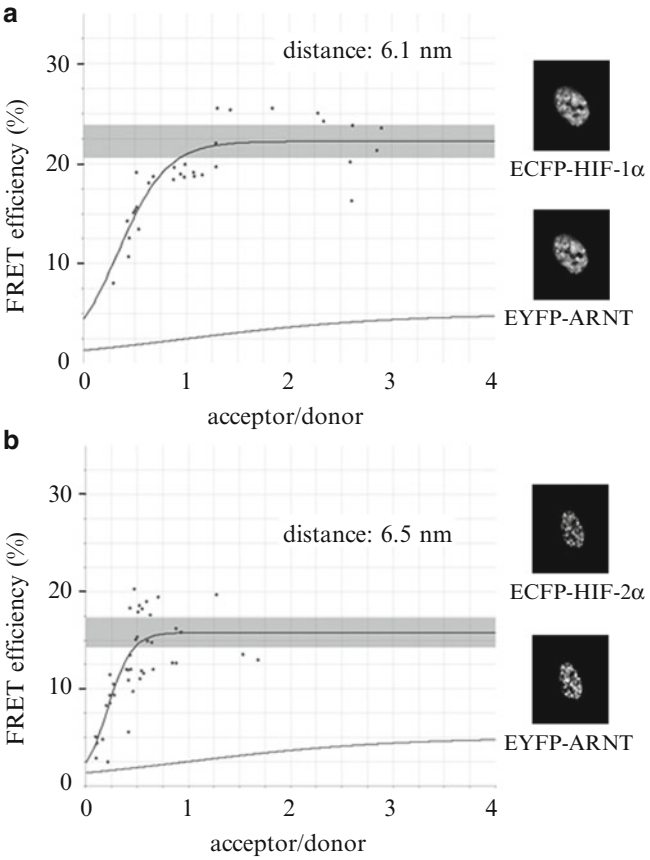

Fig. 17.4 Imaging of HIF-1α/ARNT and HIF-2α/ARNT assembly. U2OS cells were co-transfected with ECFP-HIF-αs and EYFP-ARNT. FRET signals were recorded and FRET efficiency was determined. FRET efficiency is shown over the acceptor/donor ratio and reaches a plateau at maximum FRET efficiency [34]. This efficiency is taken for calculating distances using the formula $d = R_0[(1/E) - 1]^{1/6}$. Each data point represents a single cell. Distances were calculated for HIF-1α/ARNT (**a**) and HIF-2α/ARNT (**b**) respectively. The *lower curve* provides 5 % random FRET efficiency measured with empty vectors for ECFP and EYFP where unspecific FRET signal (or false-positive FRET signal) results from random collision of the dyes

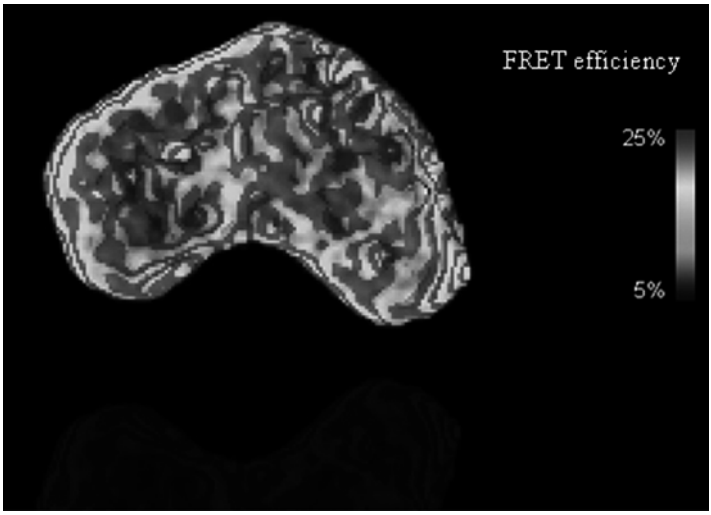

Fig. 17.5 Three-dimensional FRET reconstruction of the HIF-1 complex. U2OS cells were co-transfected with ECFP-HIF-αs and EYFP-ARNT. A single nucleus is shown which was then scanned through multiple planes in *z*-axis. Using Image J software confocal FRET images were used to generate a three-dimensional view of FRET-efficiency inside the nucleus as described [4]. The right bar provides different FRET-efficiency values in grey scale from 5 to 25 %

may be used for calculating the distance between HIF-1α or HIF-2α and ARNT, respectively.

Both of the HIF-α subunits HIF-1α and HIF-2α could interact and form a tight complex with ARNT. Distances of 6.1 nm and 6.5 nm were calculated for HIF-1α/ARNT and HIF-2α/ARNT respectively (Fig. 17.4a, b). Thus, the assembly of HIF-1α/ARNT appeared to be closer than that of HIF-2α/ARNT. These values were independent of the oxygenation status of the cells which confirms earlier results (data not shown). Furthermore, using a spinning disk imaging system [4] we were able to scan multiple image planes along the *z*-axis. Subsequent three-dimensional reconstruction of these confocal images provided a three-dimensional FRET-scan on the assembly of HIF-1α/ARNT within the nucleus of a single U2OS cell (Fig. 17.5). Sites of interaction were heterogeneously distributed confirming the speckle-like distribution of HIF-1α which was described for Fig.17.1 but also in earlier work using 2-photon laser microscopy [2].

17.6 Conclusions and Outlook

Although substantial progress has been made in understanding cellular oxygen sensing and activation of hypoxia-inducible gene expression by HIF, many open questions remain. Although HIF was discovered by deciphering hypoxia-induced erythropoietin production, it is still unclear how hypoxia-inducible and tissue-specific expression of

the erythropoietin gene is coordinated. Moreover, there is growing evidence that hypoxia-inducible genes may be categorized into predominantly HIF-1 or HIF-2 regulated genes and, probably, some regulated by either HIF complex [15, 31]. Still, both HIF-1 and HIF-2, bind to the same conserved DNA sequence called hypoxia-responsive element and it is thus unclear how gene specificity with respect to the HIF complexes is achieved. We have therefore used confocal microscopy combined with FRET to determine differences in complex formation between HIF-1 and HIF-2. Preceding these differences, we also found that mobility of HIF-1α and HIF-2α differs considerably. Initially we thought that this might be due to co-activator or adapter proteins binding to the α-subunit. However, preliminary evidence from studies with deletion constructs in which the binding domain for co-activators in HIF-1α was deleted showed no difference in mobility within the nucleus of hypoxic cells [35].

In this respect, it is of interest that Scheuermann et al. [26] reported binding of small ligands to HIF-1α PAS-B domain but not HIF-1α. Currently, the role for hypoxia-inducible signaling is not clear but since PAS domains are important dimerization interfaces for HIF-αs and ARNT it may be speculated that our observed differences in HIF complex assembly are at least partly caused by additional binding of small molecules to the PAS domains. This may also account for different mobility of HIF-α subunits and also ARNT as observed in our FRAP analyses. Collectively, confocal imaging combined with FRET and FRAP provides a powerful tool for analyzing HIF complex assembly. Future studies are aimed at elucidating different mobility of HIF subunits as well as understanding different HIF-1 vs. HIF-2 complex formation.

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Chapter 18
Modulation of the Hypoxic Response

Christopher W. Pugh

Abstract Hypoxia stimulates a variety of adaptive responses, many mediated via the hypoxia inducible factors (HIF) family of transcriptional complexes. The balance of HIF-1, -2 and -3 controls a variety of genes, directly up-regulating transcription of genes involved in erythropoiesis, angiogenesis, vasomotor tone, metabolic pathways and processes related to cell multiplication and survival, and indirectly reducing the transcription of genes with other effects. HIF transcription factors are heterodimers consisting of an oxygen-regulated alpha chain bound to the constitutive aryl hydrocarbon receptor nuclear translocator. Under circumstances where oxygen is abundant the activity of the alpha chain is blocked by the actions of members of a family of oxygen-, iron- and oxoglutarate-dependent dioxygenase enzymes. Hydroxylation of two critical prolyl residues by the HIF prolyl hydroxylases (PHD1-3) leads to recognition by the von Hippel–Lindau E3 ubiquitin ligase complex, polyubiquitylation of the alpha chain and its consequent destruction by the proteasome. Hydroxylation of an asparaginyl residue by Factor Inhibiting HIF prevents any surviving HIF alpha chains from recruiting p300-CBP proteins, important for maximal transcriptional activation. Under conditions of acute hypoxia enzyme activity is suppressed, the HIF alpha chains are allowed to exist in their active form and target gene transcription is enhanced. In sustained hypoxia, adaptive responses mediated by the HIF pathway reduce oxygen demand and increase oxygen supply and thus ultimately down-regulate the pathway. However, a number of other processes also modulate HIF signalling and the balance between HIF-1 and HIF-2 actions. These include the generation of antisense HIF-1 and micro RNAs, up-regulation of HIF-3 alpha, antagonism of the HIF-p300 interaction by CITED2, increased PHD2 and PHD3 levels and effects on the pool of ankyrins within the cell which compete with HIF for the action of FIH. Additionally, effects on intermediary metabolism, reactive oxygen species, iron availability, nitric oxide levels and redox status within the cell may modulate HIF activity. Together, these effects lead to a reduction in the magnitude of the HIF response even if oxygenation is not restored and are predicted to alter the responsiveness of the system when oxygenation is restored.

Keywords HIF • Hypoxia • Feedback • Prolyl hydroxylase • Asparaginyl hydroxylase • Chuvash • von Hippel–Lindau

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18.1 Introduction

Systemic hypoxia is an inevitable consequence of ascent to altitude, and also occurs in malignant, respiratory and circulatory disease states. Even in health, cells in different parts of the body are exposed to widely differing ambient oxygen tensions. Cells need to be defended against both excessive and inadequate provision of oxygen and this is achieved by a complex network of homeostatic responses which influence heart rate, ventilation, haematocrit and capillary density as well as oxygen consumption. The ventilatory responses are controlled by a system of peripheral and central chemoceptors whereas the haematocrit response is modulated through the regulated production of erythropoietin by specialised cells within the kidney and other tissues. Whilst these effects have long been considered separately, there is increasing evidence that at least at some levels the underlying molecular mechanisms share a degree of commonality.

Turning to the regulation of erythropoietin it is clear that on ascent to altitude there is a rapid initial response, which attenuates when altitude is maintained. On descent back to sea level erythropoietin levels are actually suppressed below those in people who have remained resident at sea level [32, 46]. At first sight these responses could be explained by the changes in haematocrit and therefore consequent tissue oxygenation but close examination of the data indicates that the erythropoietin response begins to attenuate before the haematocrit has really risen. Furthermore, it is debatable whether polycythaemia actually improves tissue oxygenation at sea level. Attenuation of the erythropoietin protein and mRNA response is seen within 32 h during sustained hypoxia in rodents [65]. Taken together these observations suggest that the off response may be more complex and increasing evidence is now accruing that this is indeed the case. In this Chapter the mechanisms leading to the induction of erythropoietin by hypoxia will be outlined. Molecular insights into how this response is modulated when hypoxia is maintained will be described along with their implications for rebound effects when oxygenation increases.

18.2 Up Regulation of Erythropoietin Gene Expression by Hypoxia

In health erythropoietin is predominantly expressed by interstitial fibroblasts that sit between the tubules in the cortico-medullary region of the kidney [3, 14, 43]. As oxygen tensions fall extra interstitial cells, sitting both in the cortical and medullary regions of the kidney, are recruited to produce erythropoietin. At the molecular level the erythropoietin gene is controlled by interactions between its promoter and

an oxygen-sensitive enhancer element situated 3′ to the coding region, just beyond the region encoding the polyadenylation signal [4, 56, 60]. This enhancer element is the binding site for Hypoxia Inducible Factor (HIF) transcription factor complexes. HIFs are dimeric complexes consisting of an oxygen-regulated alpha chain bound to the aryl hydrocarbon receptor nuclear translocator (ARNT) [71]. Three distinct alpha chains are encoded within the human genome [17, 22, 67]. HIF-1 alpha and HIF-2 alpha are generally regarded as agonists of the system whereas HIF-3 alpha, at least in some circumstances, is an antagonist [42].

Subsequent to the discovery of the erythropoietin enhancer it was shown this enhancer element was widely operative in different cell types [44]. In keeping with this it transpired that HIF alpha isoforms were also widely expressed, although it is now clear that the HIF-1 alpha and HIF-2 alpha isoforms have different tissue distributions. For example careful histological examination of the kidney has shown that in hypoxia HIF-1 alpha is predominantly expressed in the nuclei of tubular cells, whereas HIF-2 alpha is the predominant isoform within the interstitial cells [58]. In keeping with this, the current balance of evidence suggests that HIF-2 alpha is in fact the dominant physiological agonist for the erythropoietin enhancer element although HIF-1 alpha was originally cloned because of its ability to interact with this enhancer.

A wide range of genes, subserving diverse physiological functions, are directly regulated by the HIF transcription factors binding to a core RCGTG motif [13, 20, 21, 23, 41]. A further spectrum of genes is indirectly regulated [48]. Further investigation has shown that different genes are predominantly regulated by HIF-1 alpha or HIF-2 alpha in different circumstances. This does not appear to be due to differences in the core sequence of the enhancers to which they bind—although the precise molecular basis for this is still under active investigation [24, 40, 57].

The molecular mechanisms underlying regulation of the HIF transcriptional complex by oxygen have been elucidated over the last two decades. For a functional transcriptional complex to form the alpha chains have to enter the nucleus, heterodimerize with ARNT, bind DNA and supplement their endogenous co-activation potential by recruitment of transcriptional co-activators of the p300/CBP family. Oxygen affects both the abundance of the HIF alpha chains and their ability to recruit co-activators, and in both cases these effects are orchestrated via enzymatic post-transcriptional modification of the HIF alpha proteins. In humans HIF alpha chains are degraded as a result of oxygen-sensitive hydroxylation of either of two critical prolyl residues within the mid portion of the HIF alpha chains, known as the oxygen-dependent degradation domain [30, 31]. This is mediated by one of a family of three iron- and oxoglutarate dependent enzymes known as PHD1-3 [18]. In the presence of adequate amounts of oxygen, iron and 2-oxoglutarate prolyl hydroxylation occurs. The hydroxyprolyl residues produced allow binding of the von Hippel–Lindau E3 ubiquitin ligase complex which causes polyubiquitylation of the HIF alpha chain at a number of lysyl residues which in turn leads to its complete destruction via the proteasome. The hydroxyprolyl residues allow the formation of

two extra hydrogen bonds between HIF and the von Hippel–Lindau complex, increasing the affinity of the interaction between these proteins a thousand fold [28]. Co-activator recruitment is also regulated by an iron- and oxoglutarate-dependent dioxygenase. In this case the enzyme is called Factor Inhibiting HIF (FIH) and its target residue is an asparaginyl residue located in the carboxy-terminus of the HIF molecule. Asparaginyl hydroxylation occurs when oxygen, iron and oxoglutarate are available in adequate amounts and blocks recruitment of p300/CBP [15, 38, 39]. To date there is no compelling evidence for reversal of the processes these HIF hydroxylase enzymes catalyse, and in the presence of appropriate cofactors the net forward reaction depends on the amount of enzyme present. Under hypoxic conditions, the activity of all these enzymes is reduced; HIF alpha chains escape from hydroxylation and are able to form active transcriptional complexes, rapidly activating transcription of genes such as erythropoietin.

Knockout mice lacking the individual HIF hydroxylases have been created and their phenotypes studied. The most striking phenotype is that of embryonic lethality in homozygous PHD2−/− animals [64]. This is due to a placentation defect. Conditional knockout of PHD2 is associated with polycythaemia and heart failure [47]. PHD1−/− animals appear phenotypically fairly normal under non stressed conditions but have been shown to have an improved ability to survive hind-limb ischaemia [2]. PHD3−/− animals have a hyperplastic, but hypofunctional, sympathetic nervous system as young adults [6] and go on to develop cardiac failure in later life. The phenotype of FIH knockout animals is currently under investigation in several laboratories.

In sustained hypoxia, adaptive responses mediated by the HIF pathway reduce oxygen demand and increase oxygen supply and thus ultimately down-regulate the pathway. However, this system is also subject to negative feedback loops which are discussed below. These attenuate the response during sustained hypoxia and potentially alter the balance between HIF-1 and HIF-2 actions and change the poise of the system when oxygen supplies are restored.

18.3 Natural Mutations in Components of the HIF System

Insights into the effects of chronic activation of the HIF pathway come from studies of naturally occurring mutations.

von Hippel–Lindau (VHL) disease is a multi-organ familial cancer syndrome. The organs affected in VHL disease include the central nervous system, retina, kidney and adrenal medulla, and less commonly the pancreas, endolymphatic sac, epididymis and broad ligament [33]. Mutations in the von Hippel–Lindau protein (pVHL) associated with human cancer syndromes generally reduce the interaction between pVHL and HIF alpha chains leading to increased HIF alpha survival and activity in normoxic conditions. Although the genotype-phenotype correlations are not absolute the spectrum of organs affected broadly depends on the VHL mutation present. Type 1 VHL disease mutations cause gross disruption to, or total loss of,

pVHL and are associated with a low incidence of phaeochromocytomas. Missense mutations of the VHL gene are associated with Type 2 disease which is subdivided into Type 2A, in which there is a low risk of renal cell cancer, Type 2B, in which the risk of renal cell cancer is high, and Type 2C, in which only phaeochromocytomas occur. Mutations in Type 1, 2A and 2B disease have profound effects on HIF regulation, although effects on HIF-1 alpha and HIF-2 alpha may not be absolutely equivalent. In Type 2C disease significant, but probably not completely normal, oxygen-dependent regulation of the HIF pathway persists [8].

The HIF pathway is known to be activated at a very early stage in the development of renal cell cancer both in VHL disease and sporadic renal cell cancer, but in contrast in sporadic phaeochromocytomas this is a relatively rare event and a variety of alternative signalling pathways have been implicated in the development of these tumours. Taken together this suggests that the link between tumourigenesis and complete inactivation of the HIF pathway is not absolute. The role of non-HIF-related functions for pVHL, for example in determining the extracellular matrix, is debated [25, 35, 36].

As a cancer syndrome VHL disease behaves as an autosomal dominant disorder. Affected individuals inherit a defective allele from one of their parents but have a wild type allele from the other and cancers arise following somatic inactivation of the wild type allele in the affected organs. At the cellular level the disease is generally described as being recessive in that both copies of the VHL gene have to be inactivated to abrogate HIF pathway regulation. However, some data suggests that quantitative changes may occur in haplo-insufficient neutrophils from people with VHL disease [70] and this raises the possibility that people bearing one abnormal VHL gene may have other abnormal physiological responses to hypoxia when these are measured very carefully.

A second disease associated with a mutation in pVHL is Chuvash polycythaemia. This is a autosomal recessive condition in which a change in VHL nucleotide 598 from cytidine to thymidine converts amino acid 200 from arginine to tryptophan. Patients with these mutations do not develop the tumours typically seen in the classical VHL syndrome but exhibit polycythaemia, varicose veins, low blood pressure, arterial thrombosis and premature mortality. This mutation does not completely abrogate the HIF-VHL interaction but leads to a relative excess of HIF and consequent increased expression of target genes, including erythropoietin and VEGF [1]. Intriguingly, in addition to these molecular events patients with this condition have been shown to have an exaggerated ventilatory response to hypoxia, as is also seen in people acclimatising to high altitude [63]. This has provided an intriguing link between the HIF pathway and ventilatory control in humans that had previously not been recognised and is in keeping with evidence that HIF-1 alpha chain deficiency reduces the ventilatory and pulmonary vascular response to hypoxia in mice [62].

Mutations in PHD2 have been recognised in the context of other patients with previously unexplained erythrocytosis. Interestingly, these mutations (P317R and R371H) sit close to the active site of the enzyme, but in contrast to artificial mutations that completely abrogate hydroxylase activity, these mutations only par-

tially disable the system [50, 52]. Similarly activating mutations of HIF-2 alpha, but not HIF-1 alpha, have also been defined in this context. Again the mutations identified in HIF-2 alpha (G537W, G537R, M535V and P534L) are close to, but do not actually involve, the prolyl residue critical for the HIF-VHL interaction [51, 53]. Presumably mutations with more complete effects on HIF activity are not tolerated, or result in more complex phenotypes not represented in the populations of patients with relatively pure erythrocytosis who have thus far been subjected to detailed analysis. Similar ascertainment biases may explain the absence to date of mutations affecting the amino-terminal oxygen dependent degradation domain or the carboxy-terminal activation domain of HIF-2 alpha or significantly affecting the function of HIF-1 alpha.

It has been suggested that the HIF-2 alpha genotype also influences athletic performance. In an association study two haplotypes of SNPs at this locus associated positively with brief high intensity performance and sustained endurance activities respectively and a third haplotype was negatively associated with high intensity maximal exercise [27].

18.4 Regulatory Mechanisms and Feedback Loops in the HIF System

As one would expect with such a complicated homeostatic mechanism as the HIF pathway a variety of feedback loops have been defined, and more may yet be discovered.

HIF itself induces an antisense HIF transcript from the HIF-1 alpha gene locus [66]. An equivalent system does not appear to exist for HIF-2 alpha. Thus, in sustained hypoxia HIF-1 activity is ameliorated with time, leading to a distortion in the balance between HIF-1 and HIF-2 driven gene expression. Additionally, it has been shown that hypoxia induces a selection of micro RNAs, small non-protein-coding RNAs that negatively regulate the expression of a variety of genes, again shaping the overall hypoxic response [7]. A further transcriptional target of HIF is CITED2, previously known as p35srj. This protein binds to the SH-1 domain of p300/CBP, thereby inhibiting HIF transactivation by competing the HIF alpha-p300 interaction [5].

The prolyl hydroxylase domain proteins PHD2 and PHD3 are also transcriptional targets of the HIF system. Thus, when HIF is active the enzymes that drive the destruction of HIF alpha chains increase in abundance, which in turn mitigates their reduced activity in hypoxic conditions and will tend to blunt the HIF response. Interestingly, at least in some cellular backgrounds PHD3 is both preferentially responsive to HIF-2 alpha and preferentially able to degrade HIF-2 alpha, providing another mechanism for modulating the balance between HIF-1 alpha and HIF-2 alpha responsive genes. Regulation of PHD abundance is not confined to this mechanism. PHD1 is up-regulated by oestrogen [61]. Furthermore, it has been reported that the stability of PHD1 and PHD3 is decreased in hypoxia by mechanisms involving SIAH1A/2 ubiquitin ligases [26, 49]. Thus there are complex overall

effects on hypoxic levels and potential hypoxic activity of these critical enzymes. Studies in murine embryonic fibroblasts show that in hypoxia there is a net accumulation of all three proteins despite the conflicting effects of transcriptional up-regulation and up-regulation of destabilising processes (A. Grosfeld—unpublished).

HIF can also be affected by reactive oxygen species (ROS) [19, 68] and many of these effects may occur because of actions of ROS on hydroxylase activity. Additionally, nitric oxide levels which are themselves in part determined by iNOS, itself a HIF-responsive gene, also influence hydroxylase function [45]. A further level of complexity in the activity of these enzymes comes from the interplay between these enzymes and intermediary metabolism discussed above. It is clear that both fumarate and succinate can competitively inhibit HIF hydroxylases in vitro. Furthermore, mutations in succinate dehydrogenase and fumarate hydratase both lead to the development of tumours with high HIF levels [29, 54]. It is also clear that HIF hydroxylase activity can be suppressed by minor perturbations in iron availability and the balance between Fe^{2+} and Fe^{3+} [34]. What is less clear is what happens to the balance of ROS, nitric oxide, iron, 2-oxoglutarate, succinate and fumarate levels in sustained hypoxia, and on reoxygenation, but effects on these parameters will undoubtedly alter the responsiveness of the HIF system.

A further mechanism by which HIF hydroxylase activity may be modulated is if there is competition between substrates for these enzymes. Various alternate substrates for the PHD enzymes have been proposed, including the RNA-binding protein 1 subunit of RNA polymerase 2 [37] and also inhibitory κB kinase β [12]. In the case of FIH there is clear evidence that this enzyme can hydroxylate ankyrin repeat domains of a variety of proteins [9–11]. These are abundant proteins often with long half lives. In many cases in vitro studies suggest that the ankyrin substrate is the preferred substrate for FIH over HIF [11, 72]. Thus, given the abundance of the ankyrins compared with HIF it would seem likely that on reoxygenation following conditions where hydroxylation has been suppressed FIH will preferentially hydroxylate the ankyrins over HIF, leaving HIF in a disproportionately active state. Therefore, after a period of prolonged hypoxia FIH would be less potent in suppressing HIF than would be the case after a brief period of hypoxia which had not really caused effects on basal ankyrin hydroxylation.

HIF-3 alpha provides another mechanism for modulating the hypoxic response. At least some splice variants of this HIF alpha isoform lack the carboxy-terminal transactivation domain and are therefore relatively impotent activators of gene expression [42]. HIF-3 alpha has been reported to both heterodimerize with HIF-1 alpha, thereby reducing its ability to heterodimerize with ARNT, and with ARNT itself, thereby competing with HIF-1 alpha and HIF-2 alpha for hypoxic response elements.

These various mechanisms, summarised in Fig. 18.1, all have the potential to modulate HIF activity during prolonged hypoxia. However, since some are expected to potentiate HIF actions and others ameliorate them the overall direction of effect and its magnitude is hard to predict. However, analysis of ischaemic myocardium has shown up-regulation of PHD2 and PHD3 protein abundance but not adequate to completely suppress up-regulation of the HIF alpha proteins or their downstream targets [73].

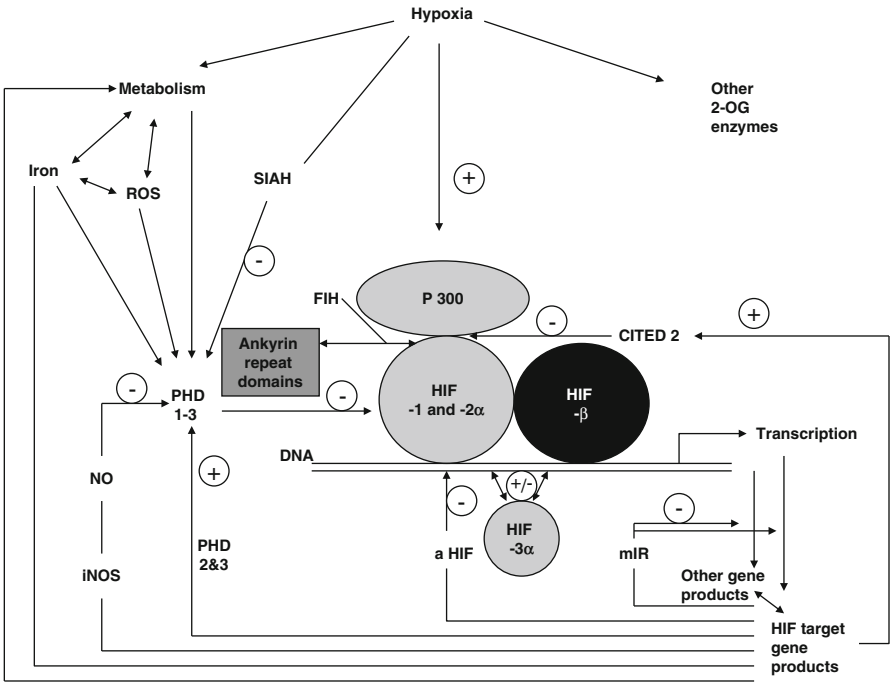

Fig. 18.1 Diagram summarising feedback loops that contribute to the regulation of the HIF pathway during sustained hypoxia described in this Chapter

It is important to recognise that at the cellular level sustained hypoxia is a part of normal life. In large animals oxygen transfer occurs down a gradient from the air to the mitochondria. Thus partial pressures of oxygen are in the range 100–140 mm of mercury in the pulmonary system, 10–100 mm of mercury in various tissues, with particularly low levels in places like the renal medulla, and lower partial pressures still at the cellular level close to mitochondria. In consequence it is necessary for the HIF system and the oxygen-sensing hydroxylases to be tuned to respond to changes in the oxygen tension starting from these diverse levels. It is likely that the feedback loops discussed above contribute to this process.

18.5 Implications for Therapeutic Manipulation of the System and Re-acclimatisation After Exposure to Altitude

Huge interest exists in the potential of manipulation of the HIF system for therapeutic benefit, be that in the fields of cancer, ischaemia or altitude exposure. Benefit might accrue in ischaemic disease and prior to rapid ascent to altitude simply from

enhancing the activity of this pathway and from suppressing it in malignant disease [55, 59]. However, current data suggest that the actual balance of HIF-1 and HIF-2 is critical for the outcome and that more subtle manipulations may be required to obtain optimal effects.

Although there has been interest in gene therapy approaches to altering HIF levels [16, 69], the discovery of the HIF hydroxylases has opened the way to use of small molecule enzyme inhibitors to up-regulate the pathway or manipulation of iron and oxoglutarate availability to suppress it. These enzymes are all members of a large multi-gene family of oxoglutarate-dependent dioxygenases. In the human genome there are about 60 members of this family sub-serving a wide range of different functions relating to collagen biosynthesis, metabolic balance, phytanic acid metabolism, carnitine biosynthesis, DNA repair and epigenetic control. Therefore precise specificity will be required to minimise the risks of untoward consequences from actions on other enzymes in this family.

The complexity of the regulatory networks described above will also need to be borne in mind in establishing optimal dosing strategies. For example, in early experimental studies alternate day dosing regimens were adopted in an attempt to mitigate any attenuation of the response from activation of negative feedback mechanisms. Care will also be required in interfacing use of HIF hydroxylase inhibitors with conventional therapies such as thrombolysis and revascularisation. For example, the high levels of HIF hydroxylase proteins in ischaemic tissues, after hypoxic exposure or after the induction of the HIF system by enzyme inhibition means that these tissues will be particularly well poised to rapidly inactivate HIF should reoxygenation occur or enzyme inhibitors be withdrawn, perhaps leading to a subnormal level of HIF subsequently.

Overall, studies to date on the HIF pathway have opened exciting possibilities for therapy but further information is required to understand the processes fully and maximise the benefits to be gained.

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Part V
Physiological Responses to Hypoxia

Chapter 19
Central Sleep Apnea at High Altitude

Keith R. Burgess and Philip N. Ainslie

Abstract The discovery of central sleep apnea (CSA) at high altitude is usually attributed to Angelo Mosso who published in 1898. It can occur in susceptible individuals at altitude above 2000 m, but at very high altitude, say above 5000 m, it will occur in most subjects. Severity is correlated with ventilatory responsiveness, particularly to hypoxia. Theoretically, it should spontaneously improve with time and acclimatization. Although the time course of resolution is not well described, it appears to persist for more than a month at 5000 m.

It occurs due to the interaction of hypocapnia with stages 1 and 2 NREM sleep, in the presence of increased loop-gain. The hypocapnia is secondary to hypoxic ventilatory drive. With acclimatization, one might expect that the increase in PaO_2 and cerebral blood flow (CBF) would mitigate the CSA. However, over time, both the hypoxic and hypercapnic ventilatory responses increase, causing an increase in loop gain which is a counteracting force.

The severity of the CSA can be reduced by descent, supplemental oxygen therapy, oral or intravenous acetazolamide. Recent studies suggest that acute further increases in cerebral blood flow will substantially, but temporarily, reduce central sleep apnea, without altering acid based balance. Very recently, bi-level noninvasive ventilation has also been shown to help (mechanism unknown). Sleep quality can be improved independent of the presence of CSA by the use of benzodiazepine sedation.

Keywords Central sleep apnea • Cerebral blood flow • Loop gain • Sleep quality

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19.1 Introduction

Central sleep apnea (CSA) at high altitude typically consists of 2–4 breaths, separated by an apnea from the next burst of 2–4 breaths, which in appearance closely resembles the periodic breathing of the premature infant [34]. It is different from the typical waxing and waning of tidal volume that one sees in the periodic breathing of heart failure [6], or the somewhat chaotic or irregular appearance of apneas associated with opiate use [29]. The description of CSA at high altitude is usually attributed to Angelo Mosso who published his description in 1898 and included an illustration of the periodic breathing recorded on his brother [21]. Typically it occurs at altitudes above 2000 m of varying severity, depending on characteristics of the individuals, but above 5000 m altitude it occurs in most people [4, 33]. The bursts of breathing (hyperpneas) are associated with arousal from sleep and sometimes full wakefulness, which causes tiredness during the day and cognitive impairment [1], similar to that seen from other causes of sleep disruption. The severity of CSA has been correlated with ventilatory responsiveness, particularly to hypoxia [17, 18, 32]. Intuitively, one might expect it to improve with acclimatization; however, the time course of resolution is not well described. Our experiments, of up to 2 weeks duration at 5000 m, have shown worsening of the CSA with acclimatization [3, 4]. Salvaggio et al. [24], over a period of 1 month at the same altitude, but in only five subjects, showed no diminution in the severity of CSA over that period.

19.2 Mechanisms

Although severity of CSA has been traditionally linked to hypoxic ventilatory responsiveness, there are other concepts that are also probably important to our understanding of the mechanisms of OSA at high altitude: The concept that CSA is caused by a disproportionate elevation of either hypoxic or hypercapnic ventilation response compared to the other is a relatively new and plausible theory [28]. The engineering concept of "loop gain" has been around since the 1980s in the respiratory control literature as a key cause of CSA [16]. More recently, alterations of cerebral blood flow have been proposed as a potential key factor in CSA [35] (see Fig. 19.1). Figure 19.1 shows a very tight correlation between the degree of fall in cerebral blood flow (CBF) at sleep onset and the subsequent degree of CSA during sleep. This suggests that a fall in CBF during sleep promotes CSA.

CSA occurs in light sleep, typically Stages 1 and 2 of non-rapid eye movement (NREM) sleep, when the patient has crossed the "apnea threshold" [7], which often means in practice that their arterial PCO_2, [and hence brain PCO_2, ($PbCO_2$)] has fallen a few millimeters lower than the resting $PbCO_2$ when they go into light sleep. The acute event is often triggered by a sigh or arousal from sleep, which causes a sudden drop in $PaCO_2$. The baseline hypocapnia at altitude is secondary to hypoxic ventilatory drive [17].

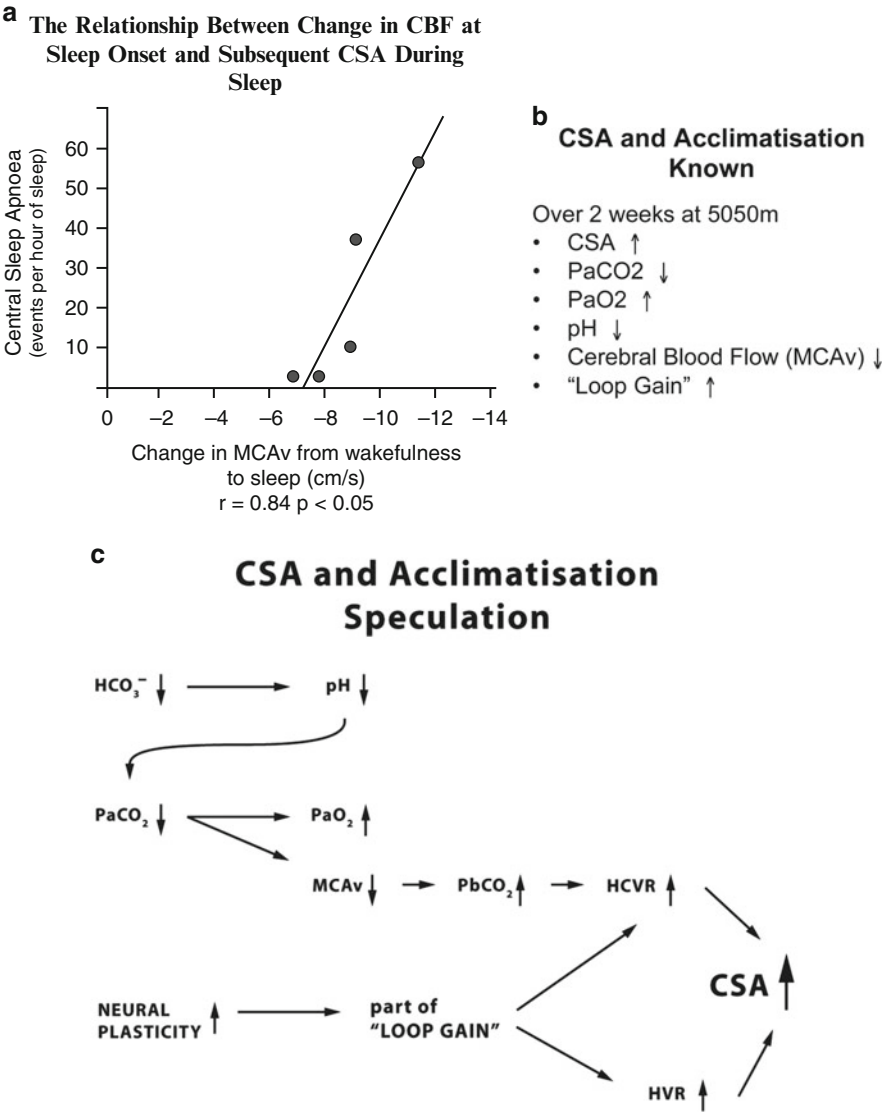

Fig. 19.1 (**a**) The relationship between change in CBF at sleep onset and subsequent CSA during sleep. (**b**) Summary of known effects of acclimatization at 5050 m relevant to central sleep apnea. (**c**) A summary of our speculation as to the mechanisms of worsening CSA during 2 weeks acclimatization

For the initial apnea to develop into sustained periodic breathing or CSA, there must be an increase in the "loop gain" of the feedback control system above 1 [15]. The calculation of absolute loop gain is problematic, although Edwards et al. [11] believe they have discovered a mathematical formula that is applicable to the periodic breathing of newborns and premature animals, and possibly to the clinical

context of high altitude CSA. Commonly one uses a surrogate for absolute loop gain, which can be derived from the polysomnogram and compared from subject to subject and from time period to time period. It is the relationship between the lengths of the hyperpneas and apneas. Put simply, "loop gain" is the response of a feedback control system divided by the stimulus [30]. Different groups have regarded the hyperpneic phase of CSA as either the stimulus [11] or the response [30], and vice versa for the apnea. In practice, it does not matter: Let us assume that hyperpnea is the stimulus, in which case, if a subject has a long apnea after a short hyperpnea, then by definition the response, (the length of the apnea), over the stimulus, (the length of the hyperpnea), is large, whereas if the hyperpnea was long and the apnea short, then loop gain must be much smaller in that situation. We have previously shown an increase in loop gain with acclimatization at 5000 m [3].

It is generally accepted that the severity of CSA at high altitude is strongly correlated with the ventilatory response to hypoxia. Kellogg [17] was one of the first to show a correlation between the slope of the ventilatory response to hypoxia and the severity of CSA. Supporting that view, Hackett and Roach have shown that almitrine, (a stimulus to hypoxic ventilatory response), will increase CSA in normal volunteers, whereas acetazolamide, (which among other actions acutely inactivates carotid chemoreceptors), would suppress CSA [12]. But that may be only part of the explanation. Studies of over 2 weeks duration in 2005 at 3840 m and in 2008 at 5050 m, have found an approximate doubling of both the hypoxic and hypercapnic ventilatory responses in normal volunteers in the transition between low altitude and high altitude [2]. They confirm a similar observation by White et al. [31]. Topor and Remmers have shown, in a computer model, that unstable breathing, due to high loop gain, is more likely to occur at high altitude if there is a disproportion between the hypoxic and hypercapnic ventilatory responses [28]. So a high hypercapnic response coupled with a low hypoxic ventilatory response, could also cause CSA.

It is somewhat surprising that CSA would increase in severity over 2 weeks of acclimatization at 5000 m, and yet not begin to improve by 4 weeks at the same altitude, because by then arterial blood gas values have started to return towards sea level values.

Upon arrival at high altitude, normal subjects will already have established hypocapnia, secondary to increased minute ventilation, due to the hypoxic stimulus to breathe. However, their $PaCO_2$ will not have reached its optimal and lowest level initially because of "hypocapnic braking" [20], which is an effect of the acute respiratory alkalosis affecting the central chemoreceptors inhibiting ventilation. Over time, renal excretion of bicarbonate starts to restore the arterial (and presumably brain) pH from alkaline towards neutral values [8]. This reduces the braking effects of the alkalosis and allows minute ventilation to increase further, with a further fall in $PaCO_2$ and (through the alveolar gas equation) a rise in PaO_2.

Initially, the sympathetic system is activated, so that cardiac output and mean arterial pressures (and CBF) are higher than at sea level [13]. Over a period of 2 weeks at 5000 m, cerebral blood flow returns to, or close to, sea level values [19], although sympathetic activation remains high [13]. Ventilatory responses to hypoxia and hypercapnia will approximately double for a group of subjects over that 2 week

period [2]. Many of these changes generate counteracting forces; the fall in $PaCO_2$ and presumably $PbCO_2$, could be expected to increase the propensity to CSA, however the increase in PaO_2 could tend to counteract that. The initial high cerebral blood flow could be expected to wash out CO_2 from around the brain stem central chemoreceptors and so initially reduce the ventilatory response to CO_2 and hence perhaps reduce periodic breathing. Over time, as cerebral blood flow returns to sea level values [19], $PbCO_2$ may rise despite a lower arterial $PaCO_2$, although that is a speculation.

Experiments designed to tease out the relative importance of changes in $PaCO_2$, ventilatory responses and cerebral blood flow, have not so far clarified this complex issue. Intravenous acetazolamide has been shown to increase cerebral blood flow by approximately 30 % at high altitude and this has been associated with a significant fall in central apnea-hypopnea index (AHI) [3]. Oral indomethacin has been shown to reduce cerebral blood flow by approximately 30 %, but this has been associated with an insignificant increase in central AHI [3]. There was no significant difference in ventilatory responses to hypercapnia between the two post drug conditions and yet there was a strong negative correlation between change in CBF and change in CSA severity. The issue, however, is clouded by the effect of the acetazolamide on arterial PCO_2, which caused an acute rise of 3 mmHg. An acute rise of that size could be expected to inhibit CSA by itself.

19.3 Treatments

Since there is a strong correlation between absolute altitude and severity of CSA [4, 33] the obvious treatment would be to reverse that process and descend. If that were not feasible, or desirable, then Lahiri has shown elegantly the curative effects of supplemental oxygen therapy on a subject with sustained CSA at 5300 m [18] (see Fig. 19.2). We have shown similar transient benefits in the artificial situation of normobaric hypoxia, created using a nitrogen tent, in which a patient with established obstructive sleep apnea (OSA) could be converted, after several hours' exposure, to a simulated altitude of 2750 m, (approximately 15 % oxygen environment), to sustained CSA [5]. Introduction of supplemental oxygen into the subject's face mask quickly terminated the CSA and allowed the underlying OSA to reemerge.

Oral acetazolamide has been shown by a number of authors to effectively suppress CSA at high altitude [12, 25, 27]. This has been attributed to the development of a metabolic acidosis, rather than the effect that one sees with rapid intravenous infusion of acetazolamide. In the acute intravenous administration situation, acid based balance does not change, nor ventilatory responses, but cerebral blood flow increases [35] due to paralysis of vasoconstriction in the cerebral arteries and there is a step up of $PaCO_2$ [3], presumably due to paralysis of carbonic anhydrase in the subject's red cells [26]. Regular oral administration, on the other hand, causes metabolic acidosis, which moves the subjects away from their apnea threshold and has a similar effect to adding CO_2 to the subject's breathing mix.

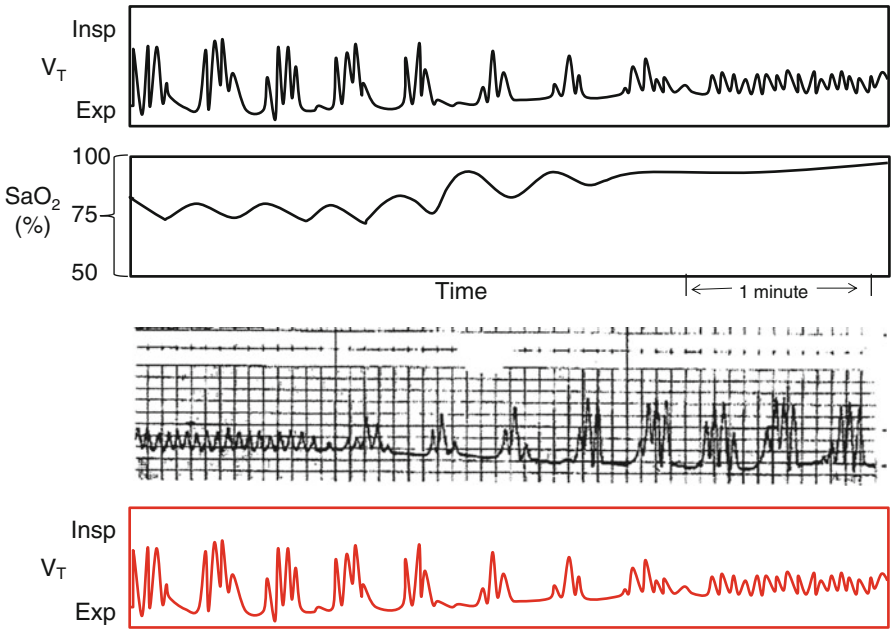

Fig. 19.2 The effect of oxygen breathing upon periodic breathing (*above*) and arterial oxygen saturation (*below*) during sleep at 17,700 ft (5400 m). Periodic breathing is replaced by shallow, continuous breathing as arterial oxygen saturation is increased. From Lahiri et al. [18]

A very different treatment (bi-level ventilation) has recently been shown in a pilot study to halve the severity of CSA in seven volunteers at 3800 m at White Mountain. The author [14] unfortunately did not collect arterial blood gases, nor measure ventilatory responses or cerebral blood flow, so the mechanism of that effect is uncertain. One could speculate that the ventilation further reduced $PaCO_2$ and raised PaO_2, however, one would expect the further fall in CO_2, would favor CSA. Noninvasive positive pressure ventilation (NIPPV), like continuous positive airway pressure (CPAP), raises functional residual capacity (FRC), which would increase oxygen stores and hence lower loop gain. That may be the main mechanism with NIPPV because Edwards et al. have shown a reduction in loop gain in premature lambs by the application of CPAP, with resolution of CSA [10]. In the context of chronic severe heart failure with CSA, CPAP has been shown to have a sympatholytic effect [22], which may also have been a factor.

Separate from treatments that affect the severity of CSA, other treatments for the sleep disturbances associated with the CSA, have been used with varying results (see Table 19.1). Dubowitz [9] and Nickol et al. [23] have used temazepam at 5400 m. Both have shown a subjective improvement in sleep quality, but with varying effects on saturation and CSA severity. Dubowitz, in a group of 11 subjects, showed no change in mean arterial saturation, but appeared to show a reduction in

Table 19.1 Effects of hypnotics on sleep and CSA

Author	Sleep quality	CSAI or ODI	SpO_2
Dubowitz	Subjectively improved	"Reduced episodes"	Mean saturation unchanged
Nickol et al.	Subjectively improved	16 h → 9/h ODI	Mean saturation slightly lower
	Reduced AMS scores		
Beaumont et al.	Subjectively improved	No change (approx 10/h)	No change
	Improved sleep architecture		
	Reduced AMS scores.		

AMS acute mountain sickness. ODI oxygen desaturation index. CSAI central sleep apnea index.

"desaturation events", probably indicating a reduction in CSA severity linked to arousal from sleep, although no measurements of sleep state were recorded [9].

Nickol et al. [23], on the other hand, showed a modest but significant reduction in CSA index, from 16/h to 9/h, in a group of 33 healthy volunteers. There was a small reduction in mean saturation from 78 to 76%. They claim to have found a reduction in acute mountain sickness scores.

New non-benzodiazepine sedative hypnotics have also been studied at high altitude [1]. Sleep quality was improved, but no direct data were provided about effects on CSA, although there was no change in oxygen desaturation index (see Table 19.1).

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Chapter 20
Multigenerational Effects of Rearing Atmospheric Oxygen Level on the Tracheal Dimensions and Diffusing Capacities of Pupal and Adult *Drosophila melanogaster*

C. Jaco Klok, Alexander Kaiser, John J. Socha, Wah-Keat Lee, and Jon F. Harrison

Abstract Insects are small relative to vertebrates, and were larger in the Paleozoic when atmospheric oxygen levels were higher. The safety margin for oxygen delivery does not increase in larger insects, due to an increased mass-specific investment in the tracheal system and a greater use of convection in larger insects. Prior studies have shown that the dimensions and number of tracheal system branches varies inversely with rearing oxygen in embryonic and larval insects. Here we tested whether rearing in 10, 21, or 40 kPa atmospheric oxygen atmospheres for 5–7 generations affected the tracheal dimensions and diffusing capacities of pupal and adult *Drosophila*. Abdominal tracheae and pupal snorkel tracheae showed weak responses to oxygen, while leg tracheae showed strong, but imperfect compensatory changes. The diffusing capacity of leg tracheae appears closely matched to predicted oxygen transport needs by diffusion, perhaps explaining the consistent and significant responses of these tracheae to rearing oxygen. The reduced investment in tracheal structure in insects reared in higher oxygen levels may be important for conserving tissue PO_2 and may provide an important mechanism for insects to invest only the space and materials necessary into respiratory structure.

Keywords Insect • Evolution • Tracheal system • Gigantism

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20.1 Introduction

The correlation between apparent late-Paleozoic hyperoxia and gigantic insect fossils has galvanized interest in the hypothesis that possession of a blind-ended tracheal system limits insect body size, and that historical variation in atmospheric oxygen partial pressure (aPO_2) has controlled the maximal body size of insects. Such evolutionary questions are inherently challenging to address since we cannot yet rear Paleozoic insects in various oxygen levels, and even if this becomes possible through a "Jurassic Park" miracle of molecular genetics, we will be unable to directly test ecological hypotheses for insect gigantism (such as the idea that insect giants were made possible by the lack of large flying vertebrates) without a time machine and some alternative universes at our disposal. Nonetheless, if the physiological characteristics of extant insects, including their developmental and evolutionary responses to aPO_2, do not suggest plausible mechanisms by which hyperoxia might have facilitated gigantism, this hypothesis would be greatly weakened. In addition, there are many reasons for investigating the responses of modern insects to aPO_2, including biomedical interest in the remarkable hypoxia-tolerance of insects, and the need to understand the effects of environmental variation on the ecology and physiology of this important group of animals.

20.2 Hypotheses and Approaches for Testing Links Between aPO_2 and Insect Size

The first hypothesis for why hyperoxia might have allowed evolution of giant insects revolved around the concept that hyperoxia would allow maintenance of an adequate PO_2 for tissues deeper into a larger insect. This argument rests on the concept that calculations suggest that diffusive gas exchange is possible in most insects [25]. All insects tested to date (we have tested insects up to 20 g) can recover from complete short-duration anoxia. Since most insects appear to lack appreciable anaerobic capacity and are therefore completely paralyzed by anoxia, it appears that all insects can achieve the minimal oxygen delivery necessary to jump-start life by diffusion. However, most insects that have been examined utilize some type of convection, including tiny *Drosophila* [27, 38, 39]. Large active insects such as grasshoppers and scarab beetles are highly dependent on convective gas exchange [9, 33]. Second, a simple diffusion-limited system would predict that larger insects would tend to have smaller safety margins for oxygen delivery (higher critical PO_2); this does not appear to be the case [10]. Thus, even though diffusion is clearly important in the blind-ended tracheoles, and perhaps in the distal regions of the tracheal system in general, simple diffusive models do not seem to explain links between aPO_2 and insect size.

Oxygen can affect many aspects of animal performance and development, and thus there are many possible avenues by which aPO_2 might affect the evolution of insect size [11]. At the individual level, on an acute timescale, higher aPO_2 might enhance maximal locomotory capacity, improving survival and fecundity. In support of this idea, hyperoxia sometimes increases locomotory performance of dragonflies and grasshoppers [13, 21]. Reduced ventilation in response to higher aPO_2 would reduce water loss rates. Over a longer, developmental timescale, higher internal PO_2 might increase body size via direct effects on cell size or proliferation [15, 35], or allow insects to grow for a longer period within an instar, since oxygen becomes more limiting as insects grow without molting [7, 8]. Most insects are smaller (and have smaller cells) when reared in hypoxia, but to date there is little evidence for hyperoxia increasing body size developmentally [12, 23].

Large body sizes likely evolve over many generations in response to natural or sexual selection. Thus a key question is whether there are mechanisms by which higher oxygen might facilitate evolution of larger insect size. *Drosophila* evolves larger body sizes in hyperoxia [23]. The mechanism for this effect is unclear; and experiments are needed to determine whether hyperoxia preferentially enhances survival or fecundity of larger *Drosophila*. Hyperoxia does not interact synergistically with selection for large body size; animals evolve the same larger sizes in 21 and 40 kPa aPO_2 [22]. However, hypoxia clearly limits body size in flies during artificial selection for large body mass [22].

Studies of laboratory evolution using model organisms like *Drosophila* are useful for demonstrating the potential for body size evolution in response to aPO_2, but can be criticized on the grounds that patterns of selection in artificial laboratory environments may differ from natural systems. In contrast, comparative studies are potentially useful for capturing macro-evolutionary patterns such as body size differences among clades. To determine whether atmospheric hyperoxia might explain Paleozoic insect gigantism, we need evidence for proximate mechanisms by which aPO_2 might influence the fitness of different-sized insects.

20.2.1 Tracheal System Structure: Scaling and Response to aPO_2

Respiratory structure and function appear to scale differently in vertebrates. An intensive series of studies from many labs over many years have examined respiratory structure allometry and function in vertebrates [6, 17, 18, 42, 43]. In both mammals and other vertebrates, convective ventilation, cardiac output, and tissue oxygen diffusion capacity scale in parallel with metabolic rate across body size, with larger animals having lower mass-specific values (mass scaling exponents in the range of 0.75). Respiratory organ sizes and volumes (lungs, tidal volume, heart, stroke volume) tend to scale isometrically, while frequencies (breathing frequency, heart rate) tend to scale with $mass^{-0.25}$ [2, 37].

In insects, metabolic rate scales with body mass to the 0.6–0.85 in most interspecific studies, so the metabolic scaling pattern is very similar to that observed in vertebrates [4]. However, in contrast to vertebrates, the limited data available suggest that larger insects have a greater mass-specific investment in respiratory structures, and have greater mass-specific ventilation. The first evidence for this trend was presented by Miller in a comparative study of large beetles' respiratory mechanisms [33]. He showed that mass-specific ventilation during flight increases with size in large beetles, leading him to suggest that "*the capacity of the thoracic pump may therefore impose a limitation on the size of flying beetles*". We documented tracheal hypermetry in the leg muscle of grasshoppers (tibia extensor) using histology and electron microscopy [14]. Using an inert gas technique, we showed that whole-body mass-specific tracheal volumes scaled with mass$^{1.3}$ (significantly greater than the isometrically predicted value of 1) in this same species [26]. We then conducted a comparative study of tracheal investment in tenebrionid beetles, using synchrotron X-ray phase contrast imaging. We developed a point-counting method for estimating tracheal system volumes from two orthogonally directed, digitized X-ray images to calculate tracheal volumes [20]. Tracheal volumes scaled with mass$^{1.29}$, significantly above the isometrically predicted value of 1. The percent body volume occupied by tracheae increased from 0.5 % in the smallest beetle (2 mg *Tribolium castaneum*) to 5 % in the largest beetle measured (2 g *Eleodes obscura*). We have recently completed a synchrotron imaging study of tracheal dimensions and function in developing grasshoppers, and shown that tracheal hypermetry occurs in all segments, and that convection scales hypermetrically for inactive animals, again with a scaling exponent of 1.3 [9]. This pattern of increased mass-specific investment in tracheal structure is a surprising finding, especially when contrasted with the patterns documented for vertebrates.

20.2.2 Potential Trade-Offs Associated with Tracheal Hypermetry in Large Insects

> Trends in compensation by differential increase of area-dependent structures cannot be continued indefinitely without producing structural absurdities.—S.J. Gould. Biol. Rev. 1986.

Hypermetric scaling of a structure imposes costs and possibly constraints on larger animals. For example, larger vertebrates must have more upright postures, proportionally larger and thicker skeletons and reduced agility [1]. Tracheal hypermetry may similarly constrain insect size via negative selection effects due to associated trade-offs such as decreased body density, increased respiratory system costs, displacement of other tissues, and exhaustion of internal space available for tracheae.

Increasing animal volume per unit mass could affect many performance aspects, including increasing drag, lever arms for locomotion, or niche space required. Based on tracheal system scaling in grasshoppers, a 1 kg grasshopper would have a 3.7 l volume (<30 % of the density of a 10 mg hopper), greatly increasing the mass-specific need for nutrient investment in the exoskeleton and

tracheal system and likely the susceptibility to breakage [9]. Tracheal system hypermetry could also lead to displacement of other tissues, reducing the animals' performance relative to those with a similar size. Percentage body volume occupied by nonrespiratory tissues falls exponentially with mass, from 92 % in 10 mg grasshoppers to 59 % in 10 g animals, and extrapolated to 27 % in theoretical 1 kg grasshoppers [9]. Potentially, larger grasshoppers' survival or reproduction could decrease relative to smaller individuals due to reduced locomotory, digestive, or reproductive capacities associated with such decreases in relative functional tissue content.

Increasing tracheal hypermetry could also directly limit maximal insect size by filling all available space within key body regions that cannot be expanded for biomechanical reasons. In interspecific comparisons of beetles, the most dramatic example of hypermetry occurred at the connection between legs and body [20]. Across four species of beetles, the leg orifice cross-sectional area scaled with $mass^{0.77}$, but the orifice-penetrating tracheae scaled with $mass^{1.02}$, occupying increasingly larger exoskeleton fractions in larger animals. Extrapolating these trends of tracheal hypermetry to the largest extant beetle, the leg would be 90 % full of tracheae, suggesting a spatial limitation [20]. Studies of the scaling of respiratory and nonrespiratory structures in the largest extant insects are needed to test these extrapolations.

20.2.3 Developmental and Evolutionary Responses of the Insect Tracheal System to aPO_2

The need for tracheal hypermetry in larger insects provides a plausible mechanism by which possession of a tracheal respiratory system might limit insect size, and also has implications for the intriguing question of Paleozoic insect gigantism. The morphology of animal respiratory systems often responds both developmentally and evolutionarily to compensate for changes in aPO_2 [34, 41]. Insects compensate for hypoxia by developmentally increasing the diameters of major tracheae and tracheole numbers; the converse occurs in hyperoxia [16, 19]. As in vertebrates, changes in tracheal dimensions with changing aPO_2 are controlled by HIF (hypoxia-inducible factor) pathway responses. Hypoxia increases the expression of fibroblast growth factor receptors on the tracheae, in response to stabilization of HIF by hypoxia, leading to enhanced tracheal growth [3]. *Drosophila melanogaster* larvae evolve larger tracheae when reared for multiple generations in hypoxia, and smaller tracheae when reared in hyperoxia [16], suggesting that tracheal investment has significant costs (materials, energy, or space) that result in selection against excess tracheal structure. Thus, the higher aPO_2 in the late Paleozoic (*c*. 31 kPa) may have reduced tracheal system investment, and reduced the negative fitness consequences of excessive tracheal investment in giant insects, providing a plausible mechanism by which elevation in atmospheric oxygen level could enable insect gigantism.

Several important questions remain about the pattern of compensatory responses of the insect tracheal system to aPO_2. One is whether the effects transfer across developmental stage. The prior studies that have quantitatively documented compensatory effects of rearing aPO_2 on tracheal dimensions have all utilized embryos or larvae [16, 19, 30], and the tracheal system of the one adult insect studied to date (the grasshopper, *Schistocerca americana*) did not change with aPO_2 [44]. Locke did report that hypoxic rearing of larval *Rhodnius prolixus* resulted in adults with increased and more "robust" wing tracheae; however, metrics of such changes were not provided [29]. Secondly, there is variation in reported responses of tracheal morphology to aPO_2 [10], and the source of this variation is unclear. One possibility is that the magnitude of responses of tracheal dimensions might vary spatially within the insect. In larval *D. melanogaster* there were detectable changes in the diameter of the posterior portion of the dorsal longitudinal tracheae (near the spiracle) but not in the anterior section [16]. Certain tissues, such as the leg muscles and antennal cells, are served by particularly long tracheae, which might be more challenged by decreasing aPO_2, leading to a greater effect on tracheal morphology. Tracheae through which substantial convection occurs might have less need to change dimensions in response to aPO_2; this may explain the lack of response of the grasshopper transverse tracheae to aPO_2 [10].

In this study, we examine the effect of multigenerational rearing in hypoxic (10 kPa), normoxic (21 kPa) and hyperoxic (40 kPa) aPO_2 on the morphology and diffusing capacities of adult and pupal tracheae of *D. melanogaster*. The flies used in this study were from the same lines as a prior study that demonstrated that changes in these aPO_2 environments result in changes in body size [23]. Within the adults, we compared the effect of aPO_2 on the abdominal and femoral tracheae of both males and females.

20.3 Material and Methods

20.3.1 Drosophila *Rearing, Laboratory Natural Selection Protocols, and Atmospheric Oxygen Control*

Wild type *Drosophila melanogaster* cultures (Oregon R strain) were acquired from Carolina Biological Supplies (www.carolina.com) and were reared as previously described [23]. We reared three replicate lines for seven generations in either hypoxia (10 kPa aPO_2) or normoxia (21 kPa aPO_2) or hyperoxia (40 kPa aPO_2) in a laboratory natural selection experiment to investigate the effects of multigenerational rearing on adult body size [23]. Adult flies, taken from one of the three replicate populations during generations 6–7 were used for this experiment.

20.3.2 X-ray Synchrotron Imaging and Image Analyses of Tracheae

Adult flies from generation 6 were shipped from our laboratory to the Advanced Photon Source (APS) at Argonne National Laboratory (ANL) for X-ray examination. Due to rapid pupal developmental rates in *D. melanogaster* mature third instar larvae from generation 7 were allowed to pupate at Argonne to permit imaging of flies during the pupal stage. The fly cultures were packed in gas impermeable containers, sealed and perfused with their rearing aPO_2 level prior to shipping. Prior tests done in the ASU lab showed that the perfused containers could maintain a steady PO_2 for at least 4 days. Upon arrival at the APS laboratories we restored the experimental aPO_2 by perfusing the 10 and 40 kPa containers with air from prepared gas cylinders, so flies were maintained in their treatment aPO_2 until they were imaged.

Flies were imaged at the APS 32-ID beam line using X-ray imaging methods as previously described [20, 40]. Selected flies were killed in an ethyl-acetate bottle and groups of ten individuals per sex were mounted, heads facing up, on narrow strips of Kapton (X-ray transparent polyimide film, DuPont) taking care to position the wings backward so as not to obscure the body, and the legs were straightened ventrally to provide the best view of the leg tracheae. The Kapton strips were then clamped onto an electronic manipulation stage (horizontal, vertical, rotation) to orientate the specimens for examination in the X-ray beam. A 90° side view orientation provided the best view of both leg and abdominal tracheae. Puparia, emerging within a 24 h window, were mounted in rows on small sturdy Kapton sheets (2 cm × 2.5 cm) and positioned in a dorsoventral position in the beam. The X-rays created projection images of the adult flies and puparia on a scintillation screen and the images were then reflected with a prism into a CCD Sensicam recording system for the capturing of high quality still images.

In the adult flies we focused on the metathoracic femoral tracheae and the dorsal tracheal branches coming off the sixth abdominal spiracles of female and male flies (see Fig. 20.1 and also [31] for detailed tracheal layouts). For the pupal-stage flies, we focused our attention on the major thoracic puparium tracheal branches that connect the developing pupa inside the puparium casing with the outside atmosphere for gas exchange (Fig. 20.2). Very early in the pupal stage, within the first few hours after pupation, these tracheal trunks are still connected to the tracheal system of the recently pupated larval tissues, but as the new pupae starts to develop, the tracheal trunks detach from the new pupa while the new pupa starts to develop a tracheal and spiracular configuration similar to that of an adult fly. The two tracheal branches form two ‘snorkels’ that connect the developing pupa to the outside atmosphere.

X-ray images of the flies and puparia were captured in tiff format and converted into jpg format. The converted and resized jpg files were imported in Matlab and a custom programmed Matlab M-file was used to determine the average tracheal diameters for each trachea; the tracheae was manually traced and the average diameter was calculated from the area and length of the polygon and the length of the

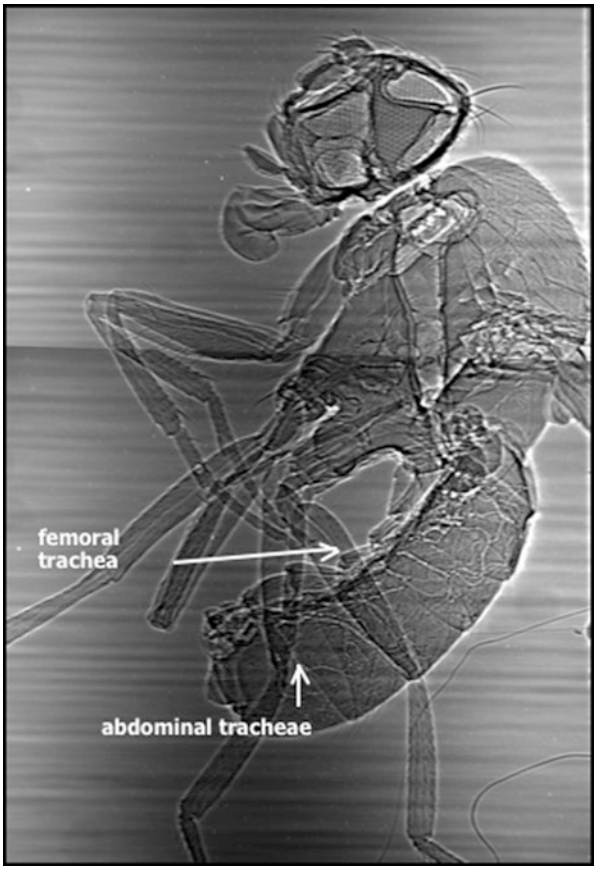

Fig. 20.1 An X-ray image of a male *Drosophila melanogaster* fly reared for six generations at 10 kPa aPO_2. The *arrows* indicate the sixth abdominal and meta-thoracic femoral tracheae measured for this study

tracheae. The lengths of the meta-thoracic femora of the flies and of the puparial thoracic snorkels were additionally measured with Able Image Analysis software (www.mulabs.com).

20.3.3 Tracheal Oxygen Diffusing Capacities

Calculation of oxygen diffusing capacity of an individual trachea requires measurement of both length and cross-sectional area. X-ray images of the adult femoral and puparium snorkel tracheae were well defined and we could get reliable diameter and length measures for those tubes for each adult or pupal individual. However, this was more difficult to do for the adult abdominal tracheae, which curved and branched irregularly. In this case diameters of the adult abdominal tracheae were measured along the best defined lengths available in the X-ray images for the various flies and a mean value of all these lengths (94 μm) was used to calculate a

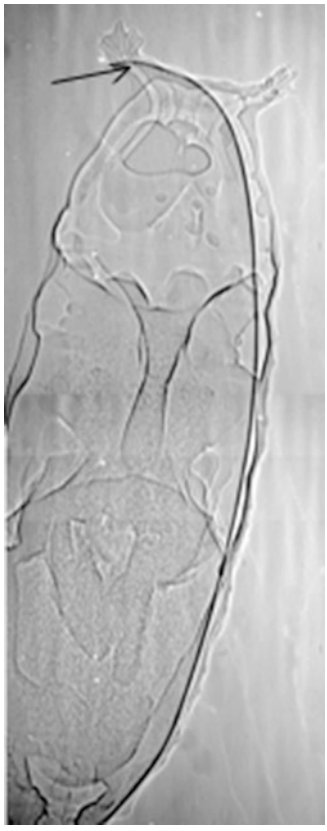

Fig. 20.2 An X-ray image of *Drosophila melanogaster* pupae reared for seven generations at 10 kPa aPO_2. The *arrows* indicate the "snorkel tracheae" whose diameters were measured in this study

standardized estimate of abdominal tracheal oxygen diffusing capacities across all individuals. Oxygen diffusing capacities (GT in nmol kPa^{-1} s^{-1}) along the lengths of tracheae were calculated as:

$$GT = \pi r^2 \times DO_2 \times \beta gO_2 L^{-1} \tag{20.1}$$

where r is the mean tracheal radius, L is the tracheal length, DO_2 is the oxygen diffusion coefficient at 25 °C (0.178 cm^2 s^{-1}, [28]), and βgO_2 is the capacitance coefficient of oxygen in air (4.04×10^5 nmol l^{-1} kPa^{-1}, [36]).

20.3.4 Statistical Analyses

Differences in tracheal diameters and diffusing capacities between the various fly groups were determined either with one way analyses of variance (ANOVA) or with analyses of covariance (ANCOVA). For the femoral tracheae we used ANCOVA, femur length as a covariate since oxygen affected femur size (see below). Male and

female flies were analyzed separately due to significant sexual dimorphic differences in body size. Linear regression analyses of body size indicators on experimental aPO_2 were done to indicate positive, negative or neutral trends in body sizes. All analyses conducted with Statistica 8 (www.StatSoft.com, Tulsa OK).

20.4 Results

20.4.1 Effect of Multigenerational Rearing at Different aPO_2 on Fly Size

After 5–7 generations' rearing at differential aPO_2 the adult body masses and femur lengths were positively related to atmospheric aPO_2 (Table 20.1; see [23] for comprehensive discussion of aPO_2 effects on adult sizes).

20.4.2 Tracheal Dimensions in Adult Flies and Pupae

The aPO_2 differentially affected adult abdominal, adult femoral, and pupal snorkel tracheae (Table 20.2). The diameters of the abdominal tracheae of female flies were statistically unaffected by aPO_2. In males, there was a significant effect of aPO_2, with a significant decrease in diameter for flies reared in 40 kPa aPO_2, but no effect of hypoxic rearing (Table 20.2). In contrast, the diameters of the femoral tracheae were affected by aPO_2 in both males and females, with diameters increasing in hypoxia and decreasing in hyperoxia (Table 20.2).

In the puparia, the thoracic tracheal trunk lengths showed no relationship with aPO_2 ($F_{(1, 29)} = 0.68$, $p = 0.52$—Overall mean length—481.1 ± 33.8 μm) and were therefore not used as a covariate to correct for the effects of body size. Rearing significantly affected diameters of the snorkel tracheae (Table 20.2). Puparia reared in 40 kPa had significantly narrower tracheae than those reared in either 10 or 21 kPa (Table 20.2).

Table 20.1 Linear regression analyses for the effect of oxygen level on body size parameters for flies reared for 5–7 generations at 10, 21, or 40 kPa aPO_2

Intercept ± SE	Slope ± SE	F	df	p	R^2
Meta-femur length (μm) on body mass (mg)—♀ and ♂ combined					
484.5 ± 38.71	131.7 ± 39.66	11.02	1, 4	<0.03	0.73
Body mass on aPO_2 at generation 6					
♀ 0.950 ± 0.012	0.008 ± 0.0005	269.38	1, 268	<0.0001	0.50
♂ 0.636 ± 0.010	0.006 ± 0.0004	260.38	1, 178	<0.0001	0.59
Meta-femur length (μm) on aPO_2 at generations 5–7					
♀ 597.9 ± 3.91	1.36 ± 0.15	88.06	1, 115	<0.0001	0.434
♂ 543.6 ± 5.34	1.98 ± 0.20	97.62	1, 118	<0.0001	0.453

Table 20.2 Summary statistics of adult and puparium tracheal dimensions (in μm) after 5–7 generations of laboratory natural selection at variable atmospheric oxygen concentrations

aPO_2	Mean ± SE	Minimum	Maximum	*n*
Oxygen selection group—30♀ + 20♂ per line (3) per generation				
Abdominal tracheal diameters (μm)				
10 kPa ♀	17.05 ± 0.46^{A}	14.38	18.97	10
21 kPa ♀	17.51 ± 0.70^{A}	14.08	20.47	10
40 kPa ♀	16.23 ± 0.42^{A}	14.71	18.86	10
10 kPa ♂	15.47 ± 0.28^{A}	14.78	17.61	10
21 kPa ♂	15.82 ± 0.57^{A}	13.65	18.80	10
40 kPa ♂	13.88 ± 0.27^{B}	13.09	15.61	10
ANCOVA ♀: $F_{(2, 26)} = 1.35$, $p = 0.276$				
ANCOVA ♂: $F_{(2, 26)} = 5.42$, $p = 0.01$				
Femoral tracheal diameters (μm)				
10 kPa ♀	14.17 ± 0.33^{A}	12.79	16.35	10
21 kPa ♀	13.38 ± 0.40^{AB}	11.65	15.40	10
40 kPa ♀	11.88 ± 0.37^{B}	10.35	13.59	10
10 kPa ♂	13.73 ± 0.42^{A}	12.08	15.83	10
21 kPa ♂	13.00 ± 0.23^{B}	11.99	14.25	10
40 kPa ♂	11.20 ± 0.25^{C}	9.51	12.10	10
ANCOVA ♀: $F_{(2, 26)} = 6.20$, $p = 0.0063$				
ANCOVA ♂: $F_{(2, 25)} = 11.34$, $p = 0.0003$				
Puparium tracheal diameters (μm)				
10 kPa ♂	148.37 ± 5.67^{A}	126.22	190.46	11
21 kPa ♂	149.49 ± 4.55^{A}	127.08	175.65	10
40 kPa ♂	130.14 ± 3.79^{B}	104.93	145.54	11
ANCOVA: $F_{(2, 26)} = 5.30$, $p < 0.011$				

Superscripts denote significant differences in tracheal diameters. Meta-thoracic femur lengths were used as covariates correcting for body size differences. The puparium tracheal lengths showed no significant differences (ANOVA: $F_{(2, 29)} = 0.676$, $p = 0.516$)

20.4.3 Tracheal Oxygen Diffusion Capacities

As for tracheal diameters, the responses of diffusing capacities to aPO_2 varied among the tracheae studied (Table 20.3). Diffusing capacities of the abdominal tracheae of females and the puparium snorkel tracheae were not affected by aPO_2 (Table 20.3). For males, the diffusing capacities of the abdominal tracheae were reduced by 14 % in hyperoxia, but were unaffected by hypoxic-rearing (Table 20.3). Femoral tracheal diffusing capacities were strongly affected by both hypoxia and hyperoxia (18–25 % compensatory change; Table 20.3).

Table 20.3 Summary statistics of tracheal diffusion capacities (in nmol kPa^{-1} s^{-1}) for representative adults and puparia after 5–7 generations of laboratory natural selection at variable atmospheric oxygen concentrations

aPO_2	Mean ± SE	Minimum	Maximum	*n*
Abdominal tracheal diffusion capacities (nmol kPa^{-1} s^{-1})				
10 kPa ♀	0.0178 ± 0.0009^{A}	0.0128	0.0225	10
21 kPa ♀	0.0187 ± 0.0015^{A}	0.0119	0.0254	10
40 kPa ♀	0.0160 ± 0.0008^{A}	0.0131	0.0215	10
ANCOVA ♀: $F_{(2, 26)} = 1.48$, $p = 0.246$				
10 kPa ♂	0.0145 ± 0.0006^{A}	0.0131	0.0189	10
21 kPa ♂	0.0152 ± 0.0011^{A}	0.0112	0.0212	10
40 kPa ♂	0.0116 ± 0.0005^{B}	0.0103	0.0146	10
ANCOVA ♂: $F_{(2, 26)} = 4.85$, $p < 0.016$				
Femoral tracheal diffusion capacities (nmol kPa^{-1} s^{-1})				
10 kPa ♀	0.0019 ± 0.0001^{A}	0.0014	0.0024	10
21 kPa ♀	0.0016 ± 0.0001^{A}	0.0012	0.0021	10
40 kPa ♀	0.0013 ± 0.0001^{B}	0.0010	0.0017	10
ANCOVA ♀: $F_{(2, 26)} = 5.67$, $p = 0.009$				
10 kPa ♂	0.0020 ± 0.0001^{A}	0.0015	0.0026	10
21 kPa ♂	0.0016 ± 0.0001^{B}	0.0014	0.0019	10
40 kPa ♂	0.0012 ± 0.0001^{C}	0.0008	0.0014	10
ANCOVA ♂: $F_{(2, 25)} = 8.54$, $p < 0.0015$				
Puparium snorkel tracheal diffusion capacities (nmol kPa^{-1} s^{-1})				
10 kPa ♀	0.29 ± 0.05^{A}	0.13	0.76	11
21 kPa ♀	0.31 ± 0.03^{A}	0.20	0.46	10
40 kPa ♀	0.24 ± 0.02^{A}	0.15	0.39	11
ANCOVA: $F_{(2, 29)} = 0.91$, $p = 0.414$				

Superscripts denote significant differences in tracheal diffusion capacities

20.5 Discussion

20.5.1 aPO_2 and Tracheal Dimensions

In general, our data support the general conclusion that the morphology of the insect tracheal system responds in a compensatory manner to aPO_2. However, responses varied among the various tracheae, and to some degree, among the sexes. Even after 5–7 generations of rearing, the magnitude of these compensatory changes in diffusive capacities were relatively small (Table 20.3), suggesting that either compensation is incomplete (perhaps explaining why aPO_2 affects body size), that the primary sites of compensation occur at other locations (e.g., the tracheoles) or that convection is important in at least some Drosophila tracheae.

At present it is unclear whether the changes in tracheal morphology observed here are due to developmental plasticity or evolution. Tracheal diameters of the major longitudinal tracheae of larvae do evolve in a compensatory manner in response to

aPO_2, showing 8–15 % compensatory changes in diameters and diffusing capacities in response to 6–7 generations of rearing in 10 or 40 kPa aPO_2. To test for evolution of adult tracheal dimensions in response to oxygen, it will be necessary to test animals reared for multiple generations in hypoxia or hyperoxia, and then returned for at least two generations to normoxia to control for developmental and parental effects.

Why do different tracheae respond differently to aPO_2? One hypothesis is that a greater fraction of gas exchange through abdominal tracheae occurs via convection, while transport through the leg may be more diffusive-based. Responses of the fifth and sixth abdominal tracheae to aPO_2 were statistically identical, as were those of the mesothoracic and metathoracic femoral tracheae (data not shown for brevity), supporting the hypothesis that there are consistent differences in the response of abdominal and femoral tracheae to aPO_2. *Drosophila* exhibit abdominal pumping during flight, though it is of small amplitude [27]. *Drosophila* also exhibit ventilation due to proboscis-pumping during flight [27], and it is plausible that some of these pressure gradients carry through to the abdomen. In addition, the abdominal tracheae appeared flexible and were in contact with the digestive tract, and so it is plausible that convection in these tracheae occur due to digestive peristalsis. One can also imagine that convection might occur in the leg tracheae due to compression of the tracheae by contracting leg muscles. However, we did not observe any compressions of these leg tracheae when flies struggled, and the tracheae appear relatively straight and rigid, supporting the possibility that gas transport in the leg tracheae might be primarily diffusive. Are the diffusing capacities of the femoral tracheae sufficient to support adequate gas exchange by diffusion? Resting oxygen consumption rates of these flies averaged about 4 $\mu l\ h^{-1}$ [24] or about 0.05 nmol s^{-1}. While the metabolic rates of walking *Drosophila* have not been measured, we know that walking increases the metabolic rates of insects by 2–5 fold [5], thus the oxygen consumption of a walking fly is unlikely to be higher than 0.25 nmol s^{-1}. It seems reasonable to guess that half of that oxygen consumption would occur in the legs (brain, respiratory and cardiovascular functions will also increase), suggesting that 0.125 nmol s^{-1} might be divided among the six legs; thus oxygen consumption of a single metathoracic leg of *Drosophila* is unlikely to exceed 0.02 nmol s^{-1}. Dividing this predicted oxygen consumption by the tracheal conductance of the femoral tracheae yields a predicted PO_2 gradient down the tracheae for completely diffusive gas exchange of 10 kPa, 12.5 kPa and 15 kPa for the 10 kPa, 21 kPa, and 40 kPa to aPO_2 reared flies, respectively. These calculations suggest that diffusive gas exchange should be able to support aerobic metabolism in the legs of walking flies in normoxia, though perhaps not in 10 kPa aPO_2.

The snorkel tracheae of pupae did not show compensatory changes in diffusing capacities, though diameters were smaller for flies reared in hyperoxia. One plausible explanation for the lack of an effect of to aPO_2 on these tracheae is that they are so large that the PO_2 gradient down them is minimal. The maximal oxygen consumption rates of *Drosophila* pupae are reported to be 0.04 nmol s^{-1} [32]. Dividing half of this amount by the diffusing capacity of a single snorkel tracheae yields a predicted PO_2 gradient down these tracheae of only 0.06 kPa for the normoxic-reared flies. These calculations suggest that compensatory changes in the dimensions of these tracheae are not necessary to compensate for hypoxia.

Hyperoxia had a more consistent effect on tracheal morphology than hypoxia (Tables 20.2 and 20.3). Perhaps this occurs to help regulate internal PO_2, preventing oxidative damage due to excessive tissue PO_2. Alternatively this effect could occur because tracheae have a significant cost (in materials and/or space occupied).

20.5.2 Implications for Insect Gigantism

These results support prior studies showing a generally negative relationship between insect tracheal investment and aPO_2 [10], extending these results by showing that these morphological changes also occur in pupal and adult stages. This response provides a plausible mechanism for how Paleozoic hyperoxia might have facilitated larger body sizes in insects. A reduction in the need for tracheal investment might facilitate larger insects by allowing greater investment in nonrespiratory tissues [11]. The negative relationship between tracheal dimensions and oxygen might also facilitate insect gigantism by relieving spatial constraints. As noted above, larger insects invest proportionally more in their tracheal systems, leading to an increasing fraction of the body being occupied by tracheae. In the leg coxae, extrapolations suggest that the entire available space might be filled by tracheae in the largest extant insects [19]; with such a scenario, hyperoxia might allow insects to achieve larger sizes with the same-sized tracheae.

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Chapter 21
Hypoxia and Its Acid–Base Consequences: From Mountains to Malignancy

Erik R. Swenson

Abstract Hypoxia, depending upon its magnitude and circumstances, evokes a spectrum of mild to severe acid–base changes ranging from alkalosis to acidosis, which can alter many responses to hypoxia at both non-genomic and genomic levels, in part via altered hypoxia-inducible factor (HIF) metabolism. Healthy people at high altitude and persons hyperventilating to non-hypoxic stimuli can become alkalotic and alkalemic with arterial pH acutely rising as high as 7.7. Hypoxia-mediated respiratory alkalosis reduces sympathetic tone, blunts hypoxic pulmonary vasoconstriction and hypoxic cerebral vasodilation, and increases hemoglobin oxygen affinity. These effects and others can be salutary or counterproductive to tissue oxygen delivery and utilization, based upon magnitude of each effect and summation. With severe hypoxia either in the setting of profound arterial hemoglobin desaturation and reduced O_2 content or poor perfusion (ischemia) at the global or local level, metabolic and hypercapnic acidosis develop along with considerable lactate formation and pH falling to below 6.8. Although conventionally considered to be injurious and deleterious to cell function and survival, both acidoses may be cytoprotective by various anti-inflammatory, antioxidant, and anti-apoptotic mechanisms which limit total hypoxic or ischemic–reperfusion injury. Attempts to correct acidosis by giving bicarbonate or other alkaline agents under these circumstances ahead of or concurrent with reoxygenation efforts may be ill advised. Better understanding of this so-called "pH paradox" or permissive acidosis may offer therapeutic possibilities. Rapidly growing cancers often outstrip their vascular supply compromising both oxygen and nutrient delivery and metabolic waste disposal, thus limiting their growth and metastatic potential. However, their excessive glycolysis and lactate formation may not necessarily represent oxygen insufficiency, but rather the Warburg effect—an attempt to provide a large amount of small carbon intermediates to supply the many synthetic pathways of proliferative cell growth. In either case, there is expression and upregulation of many genes involved in acid–base homeostasis, in part by HIF-1 signaling. These include a unique isoform of carbonic anhydrase (CA-IX) and numerous membrane acid–base transporters engaged to maintain an optimal intracellular and extracellular pH for maximal growth. Inhibition of these proteins or gene suppression may have important therapeutic application in cancer chemotherapy.

Keywords Hypoxia • HIF • pH regulation • Alkalosis • Acidosis • Exercise • Cancer • Shock • Ischemia • Altitude • Lactate • Carbonic anhydrase

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21.1 Introduction

Depending upon its magnitude and the circumstances leading to its occurrence, hypoxia can cause considerable changes in local and systemic acid–base status ranging from mild to severe alkalosis to acidosis. In this review, I will discuss association of hypoxia with such a spectrum of different acid–base consequences, how these acid–base changes can alter the magnitude and dynamic aspects of hypoxic responses, how HIF metabolism many be affected, how the altered acid–base conditions may be both deleterious and beneficial, and how the acid–base milieu might be manipulated to better treat cancer and hypoxic–ischemic injuries.

21.2 Hypoxia and Respiratory Alkalosis at High Altitude

In the main, the hypoxia of high altitude provokes sufficient hyperventilation (hypoxic ventilatory response or HVR) to lower arterial and tissue PCO_2 and increase pH. This augmentation of ventilation acts to limit the magnitude of arterial hypoxemia occurring with the reduction in inspired PO_2 and represents one of the most important compensatory responses for high altitude success and survival. Healthy people at high altitude [54] or persons hyperventilating to non-hypoxic stimuli (drugs with respiratory stimulant activity, severe anxiety, hepatic encephalopathy, brain lesions) can become quite alkalotic and alkalemic with arterial pH acutely rising as high as 7.7 and $PaCO_2$ falling to as low 10 mmHg (reviewed in Ref. [43]). Respiratory alkalosis alters, both negatively and positively, many hypoxic responses involved in enhancing tissue O_2 delivery and utilization.

The effects of acid–base changes are mediated in large part by accompanying changes in hydrogen ion (H^+) concentration and reaction of protons with titratable groups on many proteins. Proton binding or dissociation alters protein function through changes in charge and secondary and tertiary structure (the Bohr and Haldane effects of hemoglobin are a classic example of this important biochemistry). The result is an almost limitless range of alteration in membrane receptor and channel protein activity, enzyme catalysis, cell polarization, intracellular signaling events, and gene transcription and translation. With respect to one of the most important signaling mechanisms in hypoxia, it is emerging that acid–base changes themselves can alter HIF metabolism, both in hypoxia and even in the absence of hypoxia as will be discussed in the following sections. Thus any dissection and understanding of responses to hypoxia without an appreciation for the magnitude and direction of accompanying acid–base changes will be less rigorous and revealing.

In general, respiratory alkalosis has more positive than negative consequences in high altitude adaptation, independently of the increases in arterial oxygenation arising with hyperventilation. The neural and CNS effects of hypoxia include increased sympathetic nervous activity (SNA), increased hypoxic ventilatory responsiveness (HVR), and increased cerebral blood flow (CBF). All are blunted by respiratory alkalosis and augmented by hypercapnic acidosis [44, 50, 116]. The reduction in SNA with alkalosis is likely advantageous in decreasing hypoxic pulmonary vasoconstriction (HPV) and pulmonary vascular resistance [35], and renal vasoconstriction and sodium retention [37]. Both responses may help to mitigate high altitude pulmonary edema (HAPE) and limit fluid retention. HAPE-susceptible subjects have a stronger sympathetic efferent activity as measured by skeletal muscle microneurography [40] and it is known that they have a lower HVR and thus less respiratory alkalosis than HAPE-resistant subjects [61]. Lesser sympathetic tone acts to reduce pulmonary artery pressure [35] and as does hypocapnia itself [5]. Further evidence that high sympathetic tone plays a role in HAPE was shown in a study of dexamethasone for HAPE prophylaxis, in which the drug was very effective in prevention and lowered heart rates in the treated group more than in the placebo arm or in subjects treated with tadalafil, a known HPV inhibitor [89]. Because respiratory alkalosis reduces cerebral blood flow and blunts the increase in CBF with hypoxia [44], it may be deleterious to the brain if it too greatly opposes hypoxic cerebrovasodilation. Despite the reduction in CBF with alkalosis in hypoxia, hypocapnia does mitigate the impairment of dynamic cerebral autoregulation that occurs with normocapnic hypoxia [105]. The findings by Hornbein et al. [63] that Mt Everest summiteers with higher HVR and thus likely more respiratory alkalosis showed more subtle CNS deficits (memory and fine motor activity) upon descent that those with lesser HVR, suggests nonetheless that chronic hypocapnia may impair cerebral oxygenation. Drawing upon the lessons from comparative physiology it is revealing that migrating birds such as the Bar-headed goose, which must fly over the Himalayas in their yearly migration from India to the Tibetan Plateau, have very virtually no cerebral blood flow reduction with hypocapnia [19]; a physiological response that may help to better preserve CBF in these highest flyers.

In the lung, alveolar and arterial hypocapnia reduce pulmonary artery pressure and inhibit hypoxic pulmonary vasoconstriction [5, 35, 136]. This salutary effect on pulmonary vascular pressures likely adds additionally to the main benefit of a strong HVR, that of maintaining higher alveolar and arterial PO_2. The lesser ability to lower alveolar PCO_2 in HAPE-susceptibles by virtue of weaker HVR may act to increase the strong and disadvantageous HPV that these individuals have as a constitutional feature at high altitude. However, as will be discussed later, hypercapnia and metabolic acidosis increases the efficiency of VA/Q matching, in part by augmenting HPV [16, 39, 46, 129, 141]. However, at high altitude any loss

of VA/Q matching from hypocapnia is likely minimal and more than offset by its contribution in lowering pulmonary vascular pressures. Hypoxia reduces alveolar epithelial sodium and fluid reabsorption by downregulation of apical Na^+-K^+ ATPase and basolateral epithelial sodium channels (ENaC) and this may play a role in the development of HAPE [28, 171]. Respiratory alkalosis may impair alveolar fluid reabsorption [98] by reversible downregulation of basolateral membrane Na^+-K^+ ATPase abundance. In addition, alkalosis may also impair ENaC gating leading to less sodium flux across the apical membrane [30].

The responses of the pulmonary circulation and control of ventilation to hypoxia discussed above have only begun to be considered in the light of changes in HIF production at the vascular smooth muscle, brain and chemoreceptors. The limited evidence from studies in mice with heterozygous HIF-1 deficiency reveals that HPV [155] and HVR [75] are blunted. If this is the case, then it is conceivable that some of the blunting of many hypoxic responses by alkalosis in normal animals and humans may be related to alkalotic suppression of HIF-1 levels and activity [65]. Paradoxically, heterozygous HIF-2 deficiency increases HVR [112], thus pointing out the complexity of HIF expression with respect to HIF subtype, organ, and function studied. Although alkalosis has not been studied directly, acidosis independent of hypoxia increases HIF-1 levels and its gene transcriptional and protein translation [97, 161, 165] in part, by inducing nucleolar sequestration of von Hippel–Lindau (VHL) protein, a factor critical to the targeting of HIF-1 for destruction. No studies to date have examined whether HIF deficiency alters alveolar epithelial sodium and fluid reabsorption [28] or whether any of the important alveolar epithelial transport proteins are HIF-inducible or -suppressible, either by direct binding to hypoxia response elements (HRE) in the promoter regions of their genes or via secondary effects of other intracellular signaling molecules such as is the case for the cystic fibrosis transmembrane regulator (CFTR) in other tissues [170].

Respiratory alkalosis has several relevant effects on hemoglobin and blood oxygen carrying capacity, delivery, and release. Hypocapnia increases hemoglobin–oxygen affinity (the Bohr effect) allowing greater oxygen uptake at the lung for any given PAO_2. Thus in terms of O_2 delivery to tissues the increased O_2 content is clearly favorable and again comparative physiology bears this out in the fact that many high altitude resident birds and mammals have left-shifted hemoglobins [113]. Those humans with genetically variant left shifted hemoglobins have less reduction in exercise capacity at high altitude than those with normal adult hemoglobin [58]. Countering the hypocapnic left shift of the hemoglobin–O_2 dissociation curve, is a rise in 2,3 diphosphoglycerate (2,3-DPG) elicited by alkalosis that reduces hemoglobin O_2 affinity [90]. While an increase in hemoglobin–O_2 affinity aids oxygen uptake in the lung, the facility of oxygen off-loading to the tissues may be hindered. Given these two opposing effects, it then becomes a matter of which effect is greater and dominates. Modeling of the question suggests that at altitudes above 15,000–18,000 ft it appears a left-shifted hemoglobin supports better O_2 delivery that a right shifted curve; largely by virtue of the fact that the slope of hemoglobin–O_2 dissociation curve at relevant alveolar oxygen

tensions is steeper than the slope of the curve at prevailing tissue PO_2s [149]. This, in effect, means that the gain in O_2 uptake at the lung is greater than the smaller impairment in O_2 release in tissue capillary blood. Although the natural adaptation and fitness of intrinsically left shifted hemoglobins in native high altitude nonhuman mammals and birds is not disputed, the question still remains unanswered in humans owing to the opposing changes in 2,3 DPG and other unmeasured effects [90, 153]. Lastly, does respiratory alkalosis, alter renal erythropoietin (EPO) production and release with hypoxemia? While it is very clear that hypercapnic and metabolic acidosis inhibits EPO formation [42], there does not appear to be a potentiation of EPO production by respiratory alkalosis directly [74] or in those with a high HVR [22]. The renal PO_2 in the vicinity of the proximal tubular EPO producing fibroblasts is set by the balance of oxygen consumption of the proximal tubules and their O_2 delivery. In the event acid–base status can affect both, so it is not just a simple matter of O_2 delivery determinants such as hemoglobin–O_2 affinity or arterial oxygen content.

Respiratory alkalosis enhances glycolysis and lactate formation by increasing the activity of phosphofructokinase (PFK), the rate limiting enzyme in glycolytic metabolism [120]. The increase in circulating lactate is rather small with alkalosis at rest (roughly 0.3 mEq per 10 mmHg fall in PCO_2) with acute normoxic hyperventilation at sea level [88] and hypoxic hyperventilation at high altitude [122]. Hypoxia in addition may further increase lactate formation via the disinhibition of PFK by falling ATP and rising ADP and AMP, as well as reducing the NAD^+–NADPH ratio and increasing the reduction of pyruvate to lactate. Altogether these effects are small, and their impact in exercise and the lactate paradox at high altitude is uncertain. Under some circumstances such as severe hypoxia or ischemia, lactate may be a preferred fuel by critical cells such as neurons [17], but whether this extends to more physiological state of hypocapnic hypoxia (for instance in normal brain metabolism at high altitude) again is unknown.

Finally, respiratory alkalosis induces sodium and potassium bicarbonate diuresis that contributes in part to the early diuretic phase of persons adapting well at high altitude [140]. This renal response (a compensatory metabolic acidosis) has the important effect of returning arterial pH back toward normal and mitigating the effect of alkalosis on reducing the full strength of HVR in defense of arterial oxygenation [142].

Although the healthy response to reductions in inspired oxygen tension, whether at high altitude or with reduction in oxygen concentration and with mild–moderate lung dysfunction, is generally respiratory alkalosis and alkalemia, there is ultimately a degree of inspired hypoxia or pulmonary disease that leads to insufficient oxygen delivery and failure of aerobic ATP production to engender a true anaerobic metabolic lactic acidosis. This varies across species, age, underlying health status and acuity of onset, but for healthy mammals it ranges between 5 and 7 % oxygen in inspired air at sea level [52, 59].

21.3 Acidosis and Hypoxia with Exercise

In heavy and maximal exercise "lactic acidosis" does not appear to impose much limitation. For example, in Olympic level gold medal performances, such as rowing, the winner may cross the finish line with pHs as low as 6.74 and lactates as high as 34 mM [102], values much more extreme than in less gifted athletes. Similar values are noted in grand mal seizures, a strenuous muscle effort of a different type, associated with arterial hypoxemia and hypercapnia, but one with no lasting harm if arrested within several minutes. There may even be several benefits to the profound acidosis of heavy exercise, although it is generally thought to be a fatiguing factor. The first is that acidosis, either hypercapnic or metabolic, shifts the Hb–O_2 dissociation curve to the right (Bohr effect) and aids in progressively more O_2 unloading down to a pH of 6.4 [119]. Muscle intracellular acidosis helps to preserve muscle excitability when muscles become more depolarized due to rising extracellular potassium with increasing work intensity by decreasing chloride ion permeability [111]. This enables action potentials to continue to be propagated along the T tubule system despite muscle depolarization. The generation of large amounts of lactate and accompanying acid in heavy exercise, is not driven by any critical hypoxia at the respiring mitochondria [121], but rather by the ever increasing sympathetic activity and rising catecholamine levels, which act to maintain a higher level of arterial blood pressure to support muscle perfusion [126] and increase muscle sarcolemmal membrane Na^+/K^+ ATPase pump activity via local glycolytic production of ATP [70, 84]. This nonoxidative cytosolic ATP production is necessary to partially counter the constant K^+ leak out of muscle with repetitive contractions [95] which can drive interstitial muscle potassium to as high as 13 mM and arterial potassium as high as 8.0 mM.

The generation of large amounts of lactate may also better support skeletal, neuronal and cardiac muscle energetics, because its uptake into mitochondria by monocarboxylate transporter-1 (MCT-1) and oxidation in the Krebs cycle permits better maintenance of redox balance in cytosol and mitochondrial compartments [17, 117]. Brooks [17] has also made the case that lactate itself may be an important signaling molecule for tissues under stress via changes in redox state. Acidosis and catecholamines help to limit the potential negative electrophysiological effects of hyperkalemia on the heart [106], and as a form of acidosis, lactic acidosis is much less inhibitory of myocardial contractility than equivalent hypercapnic or mineral acidoses [10]. Because the reduction of pyruvate to lactate by lactate dehydrogenase is proton consuming [123] it has been suggested in fact that the acidosis of exercise might be worse without lactate formation [71]. However, this was not the case in the ischemic working muscle, where it was found that lactate formation equaled H^+ formation [91]. The idea that lactate formation might reduce acidosis supposes that acid production is the result of other H^+ producing reactions, such as ATP hydrolysis [123]. However, with constancy of cellular ATP largely maintained, even at work rates generating lactate and lactic acidemia, any protons formed with ATP breakdown are consumed with ADP rephosphorylation, making it difficult to

explain acidosis on this basis because it would require stoichiometric accumulation of ADP, which does not occur to any extent. When dichloroacetate (DCA), an activator of pyruvate dehydrogenase that reduces lactate formation by enhancing the formation of acetyl CoA for use in the Krebs cycle was given to exercising humans, it slightly reduced exercise arterial lactate concentrations at submaximal work rates and ventilation, but did not alter maximum oxygen consumption or endurance times [41, 160]. With the failure of DCA to greatly suppress lactate formation in vivo and the complicated bookkeeping of all relevant buffers and H^+ producing/consuming reactions in exercising muscle, the idea that lactate formation reduces the total acid burden and is beneficial must remain speculative. The subject of whether lactate accumulation is an advantage or disadvantage was taken up in a recent Point-Counterpoint exchange [82] and remains a lively matter of debate. Lastly for the psychological benefits of heavy exercise, acidosis stimulates beta-endorphin release, which is prevented with bicarbonate supplementation sufficient to prevent acidosis [147].

21.4 Hypoxia and Metabolic Acidosis in Critical Illness

With severe hypoxia either in the setting of profound arterial hemoglobin desaturation and reduced O_2 content, poor perfusion (ischemia) at the global or local level, or primary mitochondrial dysfunction (as in sepsis), metabolic and hypercapnic acidosis can develop along with impressive lactate formation. Under these situations the pH may fall well considerably below 7.0. Although no doubt exists that the diseases or insults causing severe acidoses can be life-threatening, the conventional wisdom that acidosis itself is harmful does not stand up to scrutiny. It may be only a fellow traveler or more interestingly an attempt by the organism refined by evolution to mitigate injury and enhance survival.

Although it is widely and conventionally taught that acidosis is injurious and deleterious to cell function and survival in disease states, both hypercapnic and metabolic acidoses may be cytoprotective by various anti-inflammatory, antioxidant, anti-apoptotic mechanisms which limit total hypoxic or ischemic reperfusion injury (reviewed in Ref. [144]). Indeed, it has been in the most severe forms of metabolic acidosis with or without arterial hypoxemia, characterized by high rates of H^+ formation that bicarbonate and other alkalinizing agents have shown no efficacy. The traditional reasons given to treat severe acidemia (pH <7.1–7.2) in cardiogenic, hypoxic, and septic shock states are to reverse depressed catecholamine sensitivity both in the heart and peripheral vasculature, raise the threshold for cardiac arrhythmias, increase insulin sensitivity, and provide greater buffer reserve to prevent further pH declines with ongoing acid production [99]. To test these long-held assumptions directly, there have been several randomized trials in patients with arterial pH between 6.8 and 7.10 using sodium bicarbonate against equal amounts of sodium as NaCl in diabetic ketoacidosis and various forms of lactic acidosis. The amounts given ranged from 100 to 200 mEq over 15–30 min, rates

roughly equivalent to or greater than the ongoing rates of acid production in these situations. The data in aggregate are consistent across all studies in showing no meaningful clinical benefit.

Studies in patients with severe diabetic ketoacidosis treated appropriately with volume repletion and insulin show that bicarbonate does not alter the rate of hyperglycemia correction, nor does it speed the rate of normalization of arterial pH or bicarbonate concentration [104]. The findings in patients with lactic acidosis are similar and do not support bicarbonate use even in the worst cases [31]. In these three studies, which randomized patients and used equivalent amounts NaCl in the control groups, in general cardiac output and blood pressure were not improved, and in some instances cardiac output fell with bicarbonate. Any purported benefit to bicarbonate, such as a transient rise in blood pressure, may simply be due to the volume expanding effects of the accompanying sodium administration. Another strategy to limit the purported adverse effects of lactic acidosis in critically ill patients utilized DCA and found statistically significant but clinically unimportant changes in arterial blood lactate concentrations and pH and found no alteration in hemodynamics or mortality [137]. The doses used were ten times higher than that used in the study of normal subjects at maximum exercise [41] suggesting that the lack of effect is not a problem of low dosing.

The failure of sodium bicarbonate and DCA given in severe endogenous organic metabolic acidoses has multiple causes and it would appear that their purported benefits are outweighed by its disadvantages, some of which are better recognized than others. Better known problems include hyperosmolality, hypernatremia, hypokalemia, and post-therapeutic metabolic alkalosis. These, however important they may be, are uncommon or minimally relevant to the acute setting of severe acidemia. Of greater and more compelling concern are the deleterious effects of alkalinization on O_2 delivery, organic acid production, uptake and metabolism by still viable tissues, cytokine and oxidant radical formation, and the fate of cells and tissue near the threshold of death.

In situations where oxygen delivery to the tissues may be marginal, acidemia aids in unloading oxygen from hemoglobin by shifting the hemoglobin–O_2 dissociation curve to the right as described above in heavy exercise. Reduction in the magnitude of the Bohr effect will mean that oxygen release from hemoglobin is hindered and tissue hypoxia may worsen. Acidosis and acidemia reduce organic acid production by virtue of simple mass action effect: that is, accumulation of product will slow the rate of any chemical reaction if all other factors are kept constant. Since the accumulation of lactate and ketoacids is a balance between their production and consumption, anything that reduces their uptake and metabolism will be disadvantageous. There is now much evidence to show that the cell membrane mechanisms (organic acid transporters) for organic acid uptake in the liver and skeletal muscle are stimulated by acidosis and inhibited by alkalosis [57, 62]. This may help to explain the lack of any influence of bicarbonate on plasma organic acid concentration in the above cited studies.

In more severe forms of lactic acidosis, short of circulatory collapse, the ongoing lactic acid formation may be as high as 20 mmol/min, making it virtually impossible

to keep pace quantitatively with bicarbonate repletion. These rates would quickly lead to arterial hypercapnia by reaction with protons if ventilation were not adjusted accordingly, but also hypernatremia, volume overload, and worsened edema, if the added sodium could not be removed by forced natriuresis or hemofiltration. The only animal model to successfully employ this strategy was one in which severely hypoxemic rats (breathing 5 % oxygen) were given large amounts of glucose and bicarbonate to support normal ATP generation rates by anaerobic glycolysis alone. The result of this "bridging therapy" was to reduce mortality by 50 % in the first 40 min, but not at 90 min [59]. Translating this strategy to humans would mean the provision of roughly one half ampoule of bicarbonate per minute and an equivalent rate of normal saline infusion of 20 l/h, clearly an impractical intervention.

The most fascinating and possibly chilling aspect of attempting early simultaneous successful alkalinization and suppression of lactate formation in severe metabolic acidosis ahead of resolution of the primary cause is a potential hastening and increase of cell death in marginal areas [32]. A wealth of cell and isolated organ studies in animals show that reoxygenation (whether by reperfusion or addition of oxygen to formally anoxic media) alter a period of hypoxia is associated with greater cell death when the pH is normal or alkaline. When the extracellular pH is purposefully kept acidic (pH 6.6–6.8), there is much more (>50 %) tissue salvage [12, 51, 167]. This "pH paradox" is not entirely understood, but several mechanisms may be germane. The first is that as cells become sufficiently hypoxic, they are unable to maintain low intracellular calcium [18]. An acidic pH also inhibits calcium entry into cells and thereby slows the eventual critical rise in intracellular calcium. As intracellular calcium increases above its low sub-micromolar concentrations, several metabolic pathways and enzymes are stimulated, including phospholipase A, that activate degradative proteases, lipases, and nucleases, causing cell necrosis [154]. These enzymes, like most enzymes, are inhibited by acidic pH. Other possible benefits of acidosis include blunting of oxidant radical formation [2, 80] including NO and superoxide, inhibition of pro-inflammatory cytokine production and release by leukocytes and vascular endothelial cells [11]. Acidosis blocks many apoptotic pathways and enhances the signaling of several anti-apoptotic mediators [45, 80, 166] and attenuates the opening of mitochondrial permeability transition pores that release cytochrome C3, a pro-apoptotic signaling molecule [107]. Lastly, akin to the situation of heavy exercise and high metabolic demand, in critical illness with compromised perfusion and oxygen delivery, lactate produced in large amounts in skeletal muscle [85] may be a preferred fuel for neuronal and cardiac mitochondrial oxidation [17, 117].

These putative benefits to acidosis have lead to the idea of delaying the correction of acidosis to permit reestablishment of oxygenation first, or the administration of drugs that limit the ability of cells to export protons or carbon dioxide. The first strategy involves initiating reperfusion of an ischemic organ with acidic perfusates as low as 6.4. In small mammal models of myocardial infarction caused by a 30–40 min period of ischemia, reperfusion with the oxygenated acidic fluid for several minutes delayed intracellular pH normalization, improved phosphocreatine recovery, decreased LDH release and reduced total infarct size

by almost 40 % [29, 68]. This concept has also found expression in the strategy of ischemic post-conditioning, whereby in establishing circulation, cycles of intermittent cessation are interposed with almost equivalent results to initial acidic reperfusion [156]. Blocking cellular membrane sodium–hydrogen exchange (NHE) by caroporide, an inhibitor of NHE-1 (which acts to export H^+ in exchange for sodium in response to intracellular acidification), is cardioprotective in ventricular fibrillation by attenuating intracellular calcium overload [4, 47]. The protection by NHE inhibition was enhanced if caroporide was given with an inhibitor of the other critical membrane acid–base transporter activated in acidosis, namely the sodium–bicarbonate symporter (NBS) that facilitates intracellular uptake of extracellular bicarbonate [127]. Caroporide has moved into early clinical trials with promising results in myocardial infarction treated both by coronary artery bypass grafting and percutaneous revascularization [85]. Lastly, acetazolamide and methazolamide, two potent carbonic anhydrase (CA) inhibitors at doses (20 mg/kg) capable of generating a combined metabolic and respiratory acidosis by renal and red cell CA inhibition are protective in a model of ischemic cerebrovascular occlusion [157]. Lacking robust controlled trials in humans, direct acidification strategies must be still considered experimental; nonetheless in the treatment of patients with severe acidosis, the physician should resist the temptation to play the chemist and correct acidosis with alkaline fluids except in a few situations such as hyperkalemia and toxic ingestions in which renal clearance can be accelerated [144].

21.5 Hypoxia and Respiratory Acidosis in Critical Illness

Both local and systemic hypercapnia may also develop with hypoxic or ischemic pathological processes, as well as in isolation with severe ventilatory failure. In the case of poorly perfused areas the CO_2 retention in tissues is the result insufficient clearance by reduced blood flow and CO_2 generation as accumulating H^+ is buffered by interstitial and intracellular bicarbonate stores. As with metabolic acidosis, the reflex response until most recently was to generate greater ventilation and clearance of carbon dioxide. Until the last decade, it was standard practice to correct hypercapnic acidosis with mechanical ventilation using high tidal volumes and pressures, if necessary, owing to the perceived dangers of respiratory acidosis itself. The evidence for harm, however, had always been weak. Indeed, the advent of clinical blood gas analysis in the 1950s revealed surprisingly profound hypercapnia ($PaCO_2 > 150$ mmHg, pH <7.0) without negative consequence in thoracic surgical cases involving one-lung ventilation [9]. A $PaCO_2$ of 501 mmHg from massive grain aspiration in a healthy farmer with complete recovery has been reported [135]. The concept of permissive hypercapnia—purposefully limiting tidal volume and pressure and accepting subsequent hypercapnic acidosis in status asthmaticus was first studied and gained credence in the mid 1980s. Darioli and Perret [33] reported a mortality reduction from 20 % to almost zero largely by

prevention of pneumothorax and hemodynamic compromise, even with initial $PaCO_2$s exceeding 100 mmHg. Acute hypercapnic acidosis is well tolerated as long as perfusion and arterial oxygenation are assured [114]. In rats maintained at two atmospheres pressure (50 % O_2–50 % CO_2) cerebral oxidative metabolism is unaffected even at PCO_2s > 750 mmHg and pH as low as 6.1 [86]. The ability to withstand hypercapnic acidosis depends upon a strong neuro-endocrine response that maintains and/or increases cardiac output and blood pressure despite direct negative inotropic and vasodilating effects of CO_2 [76, 158] and active intracellular pH defense mechanisms, particularly in critical organs such as brain and heart, that transport H^+ out into the extracellular space [79].

With growing acceptance of the permissive hypercapnia and "pH paradox" paradigms, Shibata et al. [130] studied prophylactic hypercapnic acidosis (12 or 25 % inspired carbon dioxide) in isolated perfused rabbit lungs undergoing ischemia–reperfusion injury or oxidant-induced injury. Their favorable results on alveolar edema, compliance, gas exchange and histology were followed by positive results in other models of lung injury and then replicated in live animals even when inspired carbon dioxide as high as 10–12 % was given to drive arterial pH into the 7.00–7.15 range and $PaCO_2$ as high as 80–90 mmHg. These benefits to inspired carbon dioxide are also evident when it is given after the onset of lung injury [79]. Despite the preponderant evidence for salutary anti-inflammatory, immunomodulatory and antioxidant effects of hypercapnic acidosis in various lung injuries [101, 143], there is the concern that therapeutic hypercapnia might blunt those desirable host defense mechanisms necessary for dealing with infection-related ARDS or on infections developing in the course of their need for intensive care and mechanical ventilation [103, 143].

At this juncture, despite the largely favorable results in animals, it must be appreciated that a number of clinically relevant factors have not been incorporated into the animal models of hypercapnic acidosis or metabolic acidosis (a critique equally apposite to all animal models of injury prophylaxis or treatment) that in all likelihood would make therapeutic hypercapnia a tougher proposition. These include a longer maintenance of the hypercapnic state than presently studied (only up to 2 days), older age, co-existing chronic medical conditions, and concurrent endogenous or exogenous immunosuppression. Apart from the concern of increased infectious risk, we still have no certainty that patients with compromised cardiovascular, renal and cerebral function can mount and tolerate the resulting strong neurosympathetic activation and cardiovascular responses evoked by hypercapnic acidosis, which may cause myocardial oxygen supply–demand imbalance, renal vasoconstriction leading to oliguria, and pulmonary hypertension [79, 143].

Trials of lung-protective low tidal volume ventilation shed little light since permissive hypercapnia has neither been a target goal nor enthusiastically embraced. In the large ARDS Net trial of 6 ml/kg vs. 12 ml/kg tidal volumes there was only a mean 5 mmHg greater $PaCO_2$ in the more successful lower tidal volume arm [1]. Analyzing this large database we found that after controlling for other variables predictive of mortality, moderate levels of hypercapnic acidosis (pH 7.15–7.35, $PaCO_2$: 45–65 mmHg) lowered the odds ratio of death significantly, but only in the

12 ml/kg tidal volume group [78]. Although we found no equivalent protection in the low tidal volume arm, the data nonetheless suggest that permissive hypercapnia may limit ventilator-induced lung injury of higher tidal volumes.

Should hypercapnic acidosis be proven therapeutic, do we administer it by hypoventilation or addition of inspired carbon dioxide? Much of the animal work has used inspired carbon dioxide. This may be the wiser choice if the intention is to make lung tissue homogenously acidotic to suppress local inflammatory events. It is clear from computed tomographic imaging that the lung is not homogenously injured in ARDS leaving the few better and more compliant units to be quite over-ventilated with respect to their volume and blood flow. These high ventilation–perfusion units will have lower regional alveolar PCO_2 (<20 mmHg) and higher local tissue pH. Thus, despite deliberate global hypoventilation, these functioning units may sustain on-going high tidal volume injury, made worse by their own local respiratory alkalosis [81]. Inspired carbon dioxide guarantees all lung regions, at a minimum, will always see a PCO_2 equivalent to the inspired value. One could argue for purposes of limiting the immune-modulation to the lung and minimizing the neuro-sympathetic stress of systemic hypercapnic acidosis a technique of achieving selective lung acidosis would make even better sense. We have done this in the normal lung by adding carbon dioxide into the latter half of the inspired breath of anesthetized dogs and showing that we attain the same improvement in ventilation–perfusion matching from acidification of the lung observed with carbon dioxide added into the entire inspired volume, but without the generation of systemic respiratory acidosis [16].

21.6 Hypoxia, Acidosis, and Malignancy

Rapid growth of malignant cancer cells can lead to hypoxia within tumors that upregulates via hypoxia inducible factor (HIF)-mediated gene expression an array of critical survival responses to ensure adequate nutrient and energy provision, in addition to enhancing clearance of metabolic end products to sustain continued growth [128]. The ability to limit accumulation of acidifying waste products, such as carbon dioxide and lactic acid, feature prominently in this regard. However, intracellular pH defense by cancer cells is not simply a response limited to hypoxic cells, it is observed even in areas of tumor that are well oxygenated. Although HIF-dependent gene transcription both drives tumor acidosis, it concurrently upregulates intracellular pH defense regulation as will be discussed below. Interestingly, acidosis-induced HIF-1 upregulation and effective activity [96, 161, 165] does not extend to all genes targeted by HIF such as erythropoietin [96]. The development of acidosis in malignant cells has both negative consequences for growth, but paradoxically also aids them in escaping growth controls imposed by the host [83, 138].

Otto Warburg in his seminal Nobel Prize winning studies more than 80 years ago observed that tumors rely heavily on glycolysis even in the presence of sufficient oxygen and live and grow in a more acidic milieu as a result of increased lactic acid

production not generally tolerated by normal cells [159]. The paradox of why tumor metabolism is weighted so heavily upon glycolysis has been conventionally explained by the paradigm that this represents a shift toward anaerobic metabolism to generate sufficient ATP in hypoxia or the anticipatory threat of it. However, equally compelling and consistent with the high rate of glycolysis in the presence of oxygen, despite its being only 5% efficient in ATP generation from glucose as mitochondrial respiration, is the notion that a high rate of 3-carbon products of glycolysis are necessary as building blocks of all the anabolic aspects of tumor growth; nucleotides, amino acids, and lipids [150]. While acidosis is generally limited to the tumor, in fast growing malignances the rate of tumor metabolism may be great enough to exceed normal muscle and liver lactate clearance and cause systemic lactic acidosis [77, 133]. Measurements of extracellular pH (pHe) and intracellular pH (pHi) have shown that it is the extracellular space of tumors that becomes acidic (~6.0–6.8) vs. 7.2–7.4 in normal cells [72, 152] due to high lactic acid concentration (5–10 mM). Additionally carbon dioxide tensions of 60–80 mmHg have been documented [56] arising from aerobic mitochondrial production, increased pentose-phosphate shunting and buffering of lactic acidosis by bicarbonate. Despite the combined metabolic and respiratory acidoses, tumor pHi is much more alkaline and nearly in the range of normal cells (pHi—7.1–7.3) [48, 49, 53].

Successfully growing and competitive malignant cells maintain pH homeostasis (normal pHi and low pHe) by a number of HIF-dependent gene transcription pathways. Additionally other acid-dependent genomic and non-genomic responses are activated that also facilitate high rates of acid export and/or bicarbonate uptake across the cell membrane [13]. To support high rates of glycolysis, membrane glucose transporters [1, 3] and hexokinase 1/2 are upregulated to increase glucose uptake [94]. The switch to lactic acid production and suppression of aerobic glycolysis is brought about by up-regulation of lactate dehydrogenase (LDH) to increase the flux from pyruvate to lactate, and of pyruvate dehydrogenase kinase (PDK) to block pyruvate uptake into the mitochondria [145]. Many of these are HIF-regulated gene products [128]. To maintain high rates of acidic product export, several monocarboxylate transporters (MCT) particularly HIF-inducible MCT-4 are inserted into the membrane to permit higher rates of lactate plus H^+ efflux [15]. In addition, proton export via NHE-1 is present in malignant cells [20] and may be upregulated by HIF-1 [131]. Membrane bound proton translocating ATPases (vacuolar H^+-ATPase and H^+-K^+-ATPase) are expressed in some tumor cells [92, 93, 97, 132] and are involved in trans-membrane proton efflux.

High glycolytic activity in tumors also generates considerable amounts of carbon dioxide as lactic acid is buffered by intracellular bicarbonate. To maintain favorable intracellular pH, both aerobic and glycolytic CO_2 must be excreted. In part this is facilitated by carbon dioxide diffusion across the cell membrane via aquaporin-1 which can serve as a gas channel [14]. Aquaporin-1 and aquaporins are richly expressed on tumors [100, 151], are up-regulated by HIF-1 [60, 156] and are correlated with worse tumor prognosis and treatment failure [87]. In addition, tumor cells express two isoforms of membrane-bound carbonic anhydrase, CA IX and CA XII. These isoforms are highly expressed in malignant cells and to a much lesser

extent otherwise only in rapidly dividing normal cells such as those in the gastrointestinal mucosa and reproductive organs [69]. CA IX and CA XII have been the focus of much attention owing to their very high expression in cancers, as the only carbonic anhydrases that are upregulated in hypoxia via HIF-1 binding to a promoter region as in the case of CA IX and by other effects of HIF-1 on CA XII [66, 73, 163], as a strong prognostic predictor of poorer outcome in most cancers [162] and as attractive targets in cancer chemotherapy [115].

The pivotal role of membrane-bound CA IX and XII in malignant cell growth and survival is multifaceted in terms of tumor pH homeostasis as well as possibly by alteration of intracellular signaling pathways initiated by CA IX binding to other receptors on the cell membrane independent of pH [38]. Because there has been more structural work and interrogation of its gene, more is known about CA IX than CA XII. Because both are membrane-bound with their catalytic activity facing outwardly, it is likely they share many of the same roles. CA IX consists of a large extracellular part, containing a unique N-terminal proteoglycan-like region (PG), and a carbonic anhydrase domain which accelerates CO_2–HCO_3 interconversion 105–106-fold over the uncatalyzed reactions. This extracellular domain is anchored to the cell membrane by a single pass transmembrane region connected to a short intracellular (IC) domain [36]. The PG domain appears to play some role in cell adhesion characteristics of malignant cells leading them to more easily separate from the primary tumor and travel to attach elsewhere and begin metastatic growth [169]. It also by virtue of its rich basic amino acid content provides buffering capacity for more optimal catalysis in the acidic extracellular environment of tumors [67]. The IC domain appears integral to holding CA IX in an extracellular position, because its absence by mutagenesis leads to a CA activity is internalized to the cytoplasm [64].

CA IX and XII facilitate acid disposal in a variety of ways. Lactic acid efflux from the cell is enhanced by providing catalysis in the extracellular space for HCO_3 buffering of the proton carried by MCT-4 [56, 139]. It has also been shown that CAII by an unknown non-catalytic activity also facilitates transmembrane lactic acid movement by MCT [8]. In this fashion the driving gradient for lactic acid transport is better maintained by limiting buildup of H^+ on the extracellular side. In combination with intracellular cytosolic CA II, both enzymes facilitate CO_2 diffusion by permitting rapid interconversion between CO_2 and bicarbonate [55]. This has the effect of enhancing the gradient for CO_2 diffusion by allowing parallel diffusion across the cell cytoplasm of bicarbonate and small mobile substances such as phosphate and amino acids to carry the proton. This same chemistry occurs in the extracellular space to augment total CO_2 flux, particularly when perfusion is limited as can be the case with rapid tumor growth. Swietach and colleagues [146] have performed elegant biological and modeling studies in tumor cell spheroids of differing size with and without CA IX. With no CA IX expression, spheroids developed very low pHi (6.3) and reduced pHe (6.9) at their core, associated with a diminishing gradient of acidity extending out to the periphery. With CA IX expression, core intracellular acidity was less (pHi 6.6), whereas extracellular acidity was greater (pHe 6.6). These effects were similar when CA IX activity was inhibited by membrane-impermeant CA inhibitors. Similar findings have been shown in tumor cells bearing both CA IX and CA XII [25].

Given the profound changes in acid–base balance in tumors and the role of pH in tumor survival and growth, altering the acid–base milieu has been of interest in treating cancer [110, 118]. The marked extracellular acidosis, generated by the high glycolytic rate of tumors and powerful acid-secreting processes appears to promote migration and metastasis of cancer cells by disrupting normal cell–matrix interactions [138] that act to contain cells in stable or controlled growth patterns. Additionally host defense against malignant cells involving macrophages, lymphocytes, and natural killer cells in general is reduced with acidosis [83] and the above discussion of anti-inflammatory and antioxidative effects of acidosis in critical illness) may extend to tumor immunosurveillance. Sodium bicarbonate therapy in amounts sufficient to increase tumor pHe in vivo can inhibit the metastatic spread of tumors and extend survival times in an animal model of breast carcinoma [125, 134]. Additionally bicarbonate therapy enhances tumor killing by many chemotherapeutic drugs that are weak bases, such as doxorubicin, by increasing their unionized state and thus intracellular penetrance [118].

Pharmacologic therapy directed at both increasing pHe and lowering pHi of tumors has shown promising results in animal models. Acetazolamide and methazolamide, two clinically available nonselective inhibitors of carbonic anhydrase, suppressed growth and invasiveness of tumors in vivo and in culture either alone or in combination with other antimetabolite drugs [23, 24, 27, 34, 109, 148, 164]. The far greater expression of CA IX and CA XII in tumors and virtual lack of expression in normal tissues has generated a number of strategies to target these membrane-bound CAs selectively. Because of their extracellular orientation, both extremely hydrophilic sulfonamide inhibitors [27, 108] and inhibitors linked to large molecules like albumin [3] have shown promise. Small interfering mRNA against CA IX reduced growth of a CA-IX bearing human breast cancer cells [124]. Monoclonal antibodies against CA IX and XII have been developed for treatment [6, 168] as well as linking them to either other potent anticancer drugs [7] or radioactive iodine [26] to achieve greater drug/radiation delivery to the tumor at less total systemic exposure. Lastly, these antibodies have also been radiolabeled for diagnostic imaging and staging [21]. Inhibition of other membrane proteins contributing to pHi defense in malignant cells, such as NHE, bicarbonate transporters, H^+-ATPases, and MCT, give encouraging results in tumor growth and survival models (reviewed in Ref. [110]). Altogether this body of work on proton chemistry and dynamics in cancer suggests that with better knowledge, targeting pH will be synergistic with those aimed at tumor hypoxia.

21.7 Summary

In conclusion, I have attempted to show the complex and inextricable links between hypoxia and acid–base alterations and how a fuller understanding of hypoxia in various circumstances can only be achieved by appreciating the myriad effects of acidosis and alkalosis on hypoxic and ischemic responses as well as on other

cellular functions. This is as relevant to healthy creatures at high altitude and in exercise as it is to patients with critical illnesses and cancer. Although alkalosis helps to blunt some of the deleterious effects of hypoxia, especially on the central nervous system and autonomic activity, it is becoming less clear that acidosis, both lactic acidosis and hypercapnic acidosis in critically ill patients, should be aggressively reversed owing to their cytoprotective effects. Lastly, insights into intracellular pH defense may lead to therapies that control cancer by impairing the impressive acid–base regulating abilities of malignant cells.

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Chapter 22
Ensemble Input of Group III/IV Muscle Afferents to CNS: A Limiting Factor of Central Motor Drive During Endurance Exercise from Normoxia to Moderate Hypoxia

Markus Amann and Jerome A. Dempsey

Abstract We recently hypothesized that across the range of normoxia to severe hypoxia the major determinant of central motor drive (CMD) during endurance exercise switches from a predominantly peripheral origin to a hypoxic-sensitive central component of fatigue. We found that peripheral locomotor muscle fatigue (pLMF) is the prevailing factor limiting central motor drive and therefore exercise performance from normoxia to moderate hypoxia ($S_aO_2 > 75\,\%$). In these levels of arterial hypoxemia, the development of pLMF is confined to a certain limit which varies between humans—pLMF does not develop to this limit in more severe hypoxia ($S_aO_2 < 70\,\%$) and exercise is prematurely terminated presumably to protect the brain from insufficient O_2 supply. Based on the observations from normoxia to moderate hypoxia, we outlined a model suggesting that group III/IV muscle afferents impose inhibitory influences on the determination of CMD of working humans during high-intensity endurance exercise with the purpose to regulate and restrict the level of exercise-induced pLMF to an "individual critical threshold." To experimentally test this model, we pharmacologically blocked somatosensory pathways originating in the working limbs during cycling exercise in normoxia. After initial difficulties with a local anesthetic (epidural lidocaine, L_3–L_4) and associated loss of locomotor muscle strength we switched to an intrathecally applied opioid analgesic (fentanyl, L_3–L_4). These experiments were the first ever to selectively block locomotor muscle afferents during high-intensity cycling exercise without affecting maximal locomotor muscle strength. In the absence of opioid-mediated neural feedback from the working limbs, CMD was increased and end-exercise pLMF substantially exceeded, for the first time, the individual critical threshold of peripheral fatigue. The outcome of these studies confirm our hypothesis claiming that afferent feedback inhibits CMD and restricts the development of pLMF to an individual critical threshold as observed from normoxia up to moderate hypoxia.

Keywords Performance • Neural feedback • Brain • Arterial oxygenation • Altitude

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22.1 Introduction

It is critical to recognize that this manuscript was written in 2009 and therefore does not consider the more recent literature. Whole-body endurance exercise is impaired in hypoxia. The reduction in aerobic performance of humans stems from the diminished alveolar partial pressure of O_2 and the resulting curtailment in arterial oxygenation affecting O_2 transport to various organ systems including two of the key determinants of endurance performance—the skeletal muscles and the brain. The heart has also been identified as a significant contributor to compromise aerobic exercise in hypoxia. It was proposed that the myocardium is sensitive to arterial oxygenation and that exercise intensity—and the associated pumping activity of the heart—is regulated/limited to ensure proper myocardial oxygenation and to avoid severe myocardial hypoxia [51]. However, not only the lack of evidence supporting this hypothesis, but especially the finding that the myocardium appears to work adequately even in very severe hypoxia equivalent to the summit of Mt. Everest [53, 63], speak against the theory of hypoxia-induced myocardial dysfunctions limiting endurance exercise.

Aerobic exercise capacity is highly sensitive to even small reductions in arterial oxygenation. This statement is supported by observations made at sea level, namely, exercise-induced arterial hypoxemia [23] deteriorates whole-body endurance exercise [6, 36] and accelerates the rate of development of locomotor muscle fatigue [56] of trained humans. These phenomenon are exacerbated in hypoxia [2, 25, 41, 42], but the extent of the effects of hypoxia on aerobic capacity are not uniform amongst humans. Well-trained endurance athletes seem to be affected at lower degrees of hypoxia and the decrease is more rapid at more severe levels of arterial hypoxemia as compared to untrained or moderately trained humans whose aerobic capacity suffers by about ~1 % for each 100 m ascended above ~1500 m above sea level [16, 25, 27, 31, 54, 69]. In contrast, anaerobic exercise capacity (i.e., Wingate performance, sprint efforts, maximal muscle contractile force) is not, or much less, affected by hypoxia [2, 18, 42, 54].

Of fundamental importance in the discussion on hypoxia limiting endurance exercise is not only the degree of arterial hypoxemia, but also whether certain observations were made during acute (<1–2 days) or chronic (>2 days) altitude exposure since the latter is known to evoke adaptations, like increases in hemoglobin concentration promoting convective O_2 transport, which partially compensate for exercise impairments as observed in acute hypoxia. The focus of this evaluation is on acute normobaric hypoxia and I discuss, based on data from experiments in normoxia and hypoxia, the role of locomotor muscles and brain in limiting endurance exercise under conditions of reduced convective O_2 transport.

22.2 Effects of Hypoxia-Exposure on the Development of Peripheral Locomotor Muscle Fatigue

Alterations in O_2 delivery to the working muscles affect the development of peripheral fatigue during whole body endurance exercise. Its rate of development is mediated by the rate of accumulation of intracellular metabolites known to cause failure of excitation–contraction coupling within the muscle fiber (e.g., H^+, phosphates) [26, 47, 70]. Since the rate of accumulation of protons [1, 39] and the hydrolysis of phosphocreatine and concomitant cytoplasmic inorganic phosphate (P_i) accumulation [37, 39] are faster under conditions of reduced—and slower under conditions of increased—O_2 delivery to the working muscle, both metabolites are considered as major determinant of the rate of development of peripheral fatigue in conditions of altered muscle O_2 delivery [61].

The effect of altitude-exposure on oxygen delivery to the locomotor muscles is twofold. First, reductions in the inspiratory partial pressure of oxygen (P_IO_2; hypobaric hypoxia, i.e., exposure to altitude) or inspiratory oxygen fraction (F_IO_2) (normobaric hypoxia) cause a reduction in arterial hemoglobin saturation (S_aO_2) and arterial partial pressure of oxygen (P_aO_2) which combined result in a lower arterial oxygen content (C_aO_2) compared to sea level conditions. Although various compensatory mechanisms—like increases in limb blood flow and/or oxygen extraction fraction [14, 44, 45]—are available to reduce these forfeits in limb O_2 transport at *lower* exercise intensities, cardiac output and leg blood flow at *high* intensities of exercise approach their peak values and are no longer capable of compensating for the reduced C_aO_2. And second, hyperventilation of heavy sustained exercise (>85 % VO_{2max}) causes substantial increases in respiratory muscle work leading to respiratory muscle fatigue [22]. Accumulation of metabolites in these muscles activates unmyelinated group IV phrenic afferents which, in turn, increase sympathetic vasoconstrictor activity in the working limb via a supra-spinal reflex [22]. The result is a work of breathing-induced reduction in limb blood flow and a corresponding reduction in O_2 delivery to the working muscles—even at sea level [35]. Now, hypoxia not only reduces C_aO_2 compared to normoxia but also substantially increases respiratory muscle work during exercise compared to the identical exercise performed in normoxia [8, 21]. These two factors lead to a faster and more severe development of respiratory muscle fatigue [66] which potentiates the metaboreflex described above. Amann et al. have previously shown that both of these factors, namely the reduction in C_aO_2 and the increased respiratory muscle work, significantly and independently contribute to the accelerated rate of development of peripheral locomotor muscle fatigue in hypoxia (Fig. 22.1) [8].

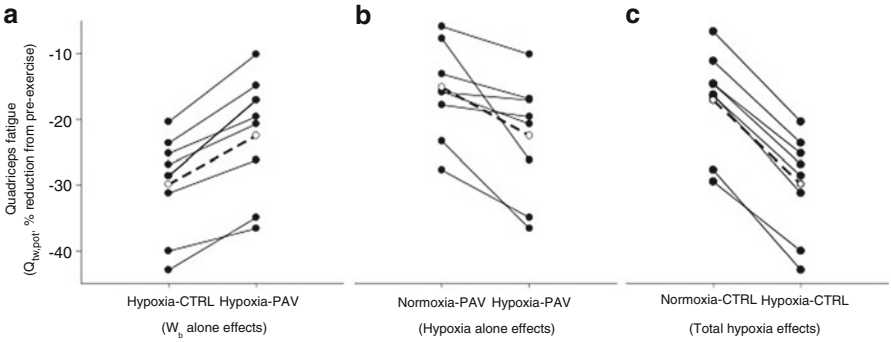

Fig. 22.1 Individual (*solid symbols*) and group mean (*open symbols*) effects of cycling exercise in acute moderate hypoxia (F_IO_2 0.15) on quadriceps muscle fatigue expressed as a percent reduction in 1 Hz potentiated twitch force ($Q_{tw,pot}$) from pre- to 2-min post-exercise. All trials were conducted at identical work rate (273 ± 6 W) and identical duration (8.6 ± 0.2 min). The figure illustrates the independent effects of inspiratory muscle work (W_b) and hemoglobin saturation (S_aO_2) on peripheral locomotor muscle fatigue. A proportional assist ventilator (PAV) was used to reduce W_b. By comparing conditions of (1) identical levels of reduced S_aO_2 in combination with significantly different levels of W_b (Hypoxia-CTRL vs Hypoxia-PAV, Panel **a**) and (2) identical levels of reduced W_b in combination with significantly different levels of S_aO_2 (Normoxia-PAV vs Hypoxia-PAV, Panel **b**), we were able to isolate the two main effects of hypoxia on peripheral locomotor muscle fatigue. Panel (**a**) shows the isolated effects of W_b in hypoxia on quadriceps fatigue. W_b was reduced by about 70 % whereas S_aO_2 (~82 %) was unchanged from Hypoxia-CTRL to Hypoxia-PAV. Panel (**b**) shows the isolated effects of S_aO_2 on quadriceps fatigue. S_aO_2 was reduced from ~95 % to ~82 % whereas W_b was unchanged from Normoxia-PAV to Hypoxia-PAV. Panel (**c**) shows the exacerbating effects of reductions in S_aO_2 (~14 %) combined with increases in W_b (~36 %) on peripheral fatigue when exercising in hypoxia vs normoxia. From Amann et al. [8]

22.3 Effects of Peripheral Locomotor Muscle Fatigue and Hypoxia on Muscle Afferent Activity

With the onset of exercise, but especially when peripheral fatigue develops, mechanical and chemical stimuli change the activity of various intramuscular receptors (metaboceptive/nociceptive) which is reflected in the firing frequency of the respective large- and small diameter afferents (group I/II and group III/IV, respectively) [30]. Group III/IV muscle afferents have also been shown to be stimulated by intra-arterial injections of metabolic byproducts of muscle contraction/peripheral muscle fatigue [30, 43, 48, 49, 57, 58, 62]. Furthermore, during prolonged maximal efforts and especially during contractile fatigue characterized by the intramuscular accumulation of metabolic byproducts, muscle afferent feedback is thought to cause a reflex inhibition of the α-motoneuron pool of the working muscles at spinal, but especially via supraspinal levels [28, 29].

In addition, small diameter muscle afferents are also sensitive to reductions in arterial oxygenation, per se. Acute arterial hypoxemia increases baseline discharge frequency of group III, and especially of group IV muscle afferents in resting cats

[38, 46] and rabbits [12]. Furthermore, during muscle contraction, the *net* firing frequency of group III/IV muscle afferents is higher in hypoxia compared to normoxia. Hill et al. directly recorded from group III and IV fibers of triceps surae muscles of barbiturate-anesthetized cats and found potentiated responses of these afferents to electrically induced static muscle contraction in acute hypoxia vs normoxia (P_aO_2 ~23 mmHg vs. ~108 mmHg, respectively) [38]. This increased *net* discharge results from the higher resting firing frequency plus the increase in discharge frequency evoked by the hypoxia-induced exaggerated accumulation of metabolites [39] known to stimulate small diameter afferents.

22.4 Effects of Hypoxia-Exposure on Brain

Insufficient O_2 transport to the brain depresses electrical activity of cerebral neurons. Blunted activity and excitability of cortical neurons result not only from a hypoxia-induced disturbance of ionic homeostasis of cerebral nerve cells [33], but also from the extracellular release of neurotransmitter affecting the postsynaptic membrane [32]. This derangement of the turnover of several CNS neurotransmitters (i.e., dopamine, norepinephrine, serotonin) might affect the limbic-to-motor link within the basal ganglia [50], motivation [60], preparation and execution of movement [20], and cognitive functions [34] involving both the prefrontal cortex and the basal ganglia [20].

Indirect evidence suggests that cerebral oxygenation as seen during high intensity whole body endurance exercise is unlikely to play an important limiting role for endurance exercise performance in normoxia and acute moderate hypoxia ($S_aO_2 > 80\,\%$) [11, 67], whereas severe hypoxia might impose cerebral hypoxia of sufficient severity to limit whole body exercise [11]. This postulate is based on various classical "reoxygenation" studies. For example, Amann et al. had subjects perform the identical strenuous constant-load cycling exercise to task failure (pedal frequency drops below 70 % of self selected cadence for ≥5 s) in normoxia (F_IO_2 0.21, $S_aO_2 \sim 93\,\%$), acute moderate hypoxia (F_IO_2 0.15, $S_aO_2 \sim 82\,\%$), and acute severe hypoxia (F_IO_2 0.10, $S_aO_2 \sim 66\,\%$) [11]. At task failure, arterial oxygenation was surreptitiously increased via switching the inspirate to a hyperoxic gas mixture (F_IO_2 0.30) and the subjects, unaware of the gas switch, continued to exercise to exhaustion (pedal frequency drops below 60 % of self selected cadence for ≥5 s). Frontal cerebral cortex oxygenation was continuously measured throughout the trials via near-infrared spectroscopy. At task failure, just prior to the increase in F_IO_2 to 0.30, cerebral oxygenation in the normoxic trial was slightly below resting values (~6 %, not significant), whereas in acute moderate and severe hypoxia, cerebral cortex oxygenation was substantially reduced below resting normoxic baseline levels (by 18 % and 24 %, respectively). Following the increase in F_IO_2 to 0.30, C_aO_2 in the normoxic and moderate hypoxic trial increased towards resting normoxic levels within seconds and cerebral tissue oxygenation substantially increased. However, despite the increase in brain O_2 delivery, the subjects were not able to maintain or

further increase central neural drive to continue the exercise suggesting that cerebral oxygenation might not have limited performance [11]. In contrast, following reoxygenation at task failure in severe hypoxia, C_aO_2 increased to resting normoxic baseline levels within seconds, cerebral oxygenation immediately and significantly rose, and the subjects were able to continue the exercise for an additional ~3.4 min until exhaustion was reached in hyperoxia (Fig. 22.2a). These findings are supported by other experiments in acute and chronic hypoxia [15, 17, 42, 64, 65].

22.5 What Determines the Regulation of Central Motor Drive and the Voluntary Cessation of Endurance Exercise in Normoxia and Hypoxia? The Development of a Hypothesis

Previous findings in humans showed that during incremental cycling exercise to voluntary exhaustion in normoxia and moderate normobaric hypoxia (F_IO_2 0.14, $S_aO_2 \sim 81\,\%$), changes in sarcoplasmic reticulum Ca^{2+} cycling [24] and Na^+-K^+-ATPase [59] determined from muscle biopsies obtained at voluntary exhaustion were identical, despite marked differences in time to exhaustion (~13 %) and peak work rate (~19 %). Furthermore, magnetic resonance imaging measurements during incremental plantar flexion exercise to voluntary exhaustion showed hypoxia-induced (F_IO_2 0.10, $P_aO_2 \sim 45$ mmHg) acceleration and hyperoxia-induced (F_IO_2 1.00, $P_aO_2 \sim 600$ mmHg) slowing of intra-cellular P_i and H^+ accumulation in calf muscle and identical levels of these muscle metabolites, which are known to cause peripheral fatigue, were achieved at end-exercise—despite markedly different peak work rates and exercise times [39]. In a different study, Hogan and Welch [40] had subjects cycling at the same absolute workload and for the same duration but breathing different F_IO_2s (0.16 or 0.60) during exercise. By doing so, the authors induced different levels of $[H^+]$ and $[La^-]$ and investigated these effects on subsequent (4 min break) cycling performance trials in normoxia. Blood $[H^+]$ and $[La^-]$ at the beginning of these constant workload performance trials were significantly higher following the hypoxic vs hyperoxic "pretreatment" and resulted in a significantly shorter time to exhaustion for the subsequent constant load exercise trial. Nevertheless, $[H^+]$ and $[La^-]$ at exhaustion were not significantly different between

Fig. 22.2 (continued) twitch force ($Q_{tw,pot}$) as measured pre- vs post-exercise in response to supramaximal femoral nerve stimulation. On separate days, peripheral fatigue was assessed following task failure (*left bar* of the respective condition) and following exhaustion (*right bar*). Note that peripheral fatigue at task failure and exhaustion were identical in normoxia and moderate hypoxia; whereas at task failure in severe hypoxia, the locomotor muscles were significantly less fatigued. Following reoxygenation, peripheral fatigue continued to increase during the additional 205 s of cycling to reach the similar level at exhaustion as seen in normoxia and moderate hypoxia. #$P < 0.05$. Modified from Amann et al. [11]

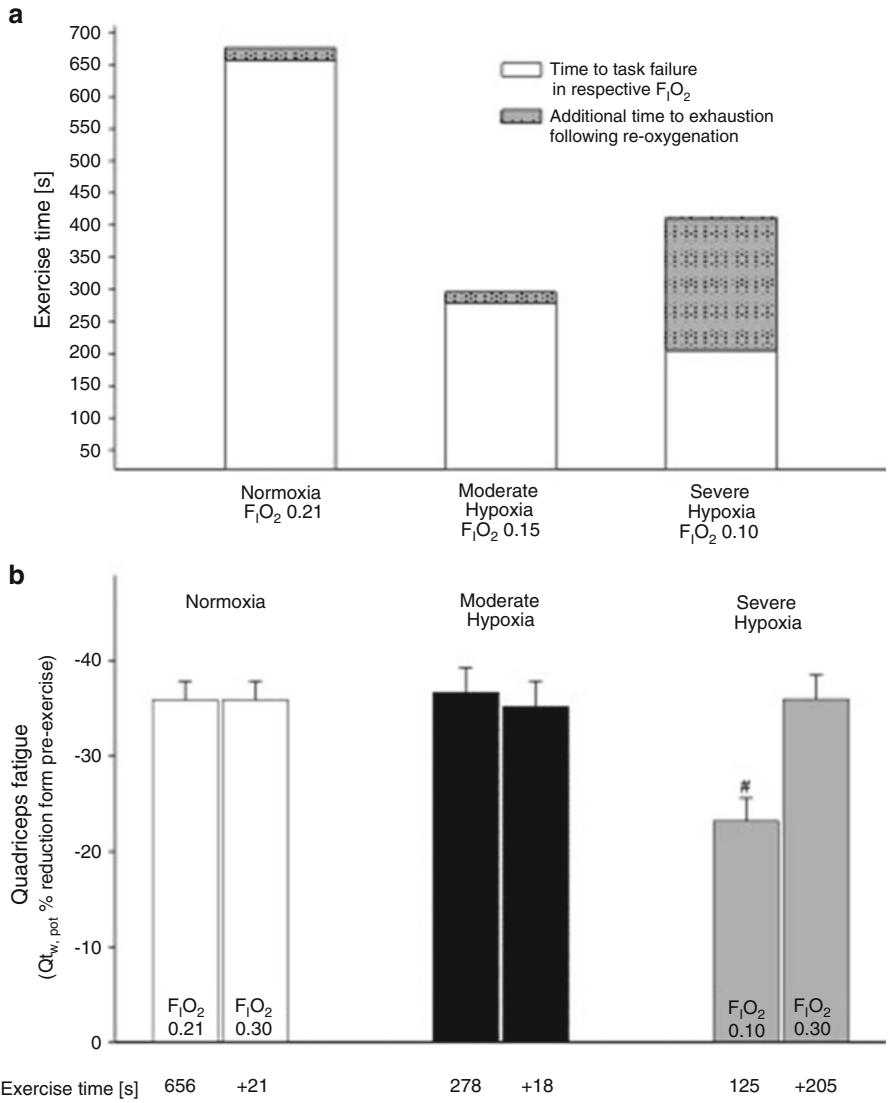

Fig. 22.2 The subjects performed constant-load cycling exercise (333 ± 9 W) in normoxia (F_IO_2 0.21, S_aO_2 ~ 93 %) and two levels of hypoxia (F_IO_2 0.15/S_aO_2 ~ 82 % and F_IO_2 0.10/S_aO_2 ~ 66 %). The target pedal frequency was individually chosen but had to be held constant throughout exercise. At task failure (pedal frequency drops below 70 % of self selected cadence for ≥5 s), arterial and cerebral oxygenation was surreptitiously increased via switching the inspirate to a slightly hyperoxic gas mixture (F_IO_2 0.30) and the subjects, unaware of the gas switch, continued to exercise to exhaustion (pedal frequency drops below 60 % of self selected cadence for ≥5 s). (**a**) Time to task failure and additional time to exhaustion. Note that the subjects were not able to significantly prolong exercise following reoxygenation at task failure in normoxia and moderate hypoxia. In contrast, exercise was significantly prolonged after reoxygenation at task failure in severe hypoxia. (**b**) Exercise-induced quadriceps fatigue assessed via changes in potentiated quadriceps

trials despite the marked differences in exercise performance time [40]. Combined, these experiments suggest that exercise is regulated to preserve a certain intramuscular metabolic milieu.

Based on these findings and knowing that endurance exercise performance and the rate of exercise-induced fatigue development critically depend on convective O_2 transport [2] (see above), Amann et al. conducted a study in which four different 5-km cycling time trials (power output voluntarily adjustable, [7]) were performed at different levels of C_aO_2—from moderate hypoxia (F_IO_2 0.15, S_aO_2 ~ 78 %) to hyperoxia (F_IO_2 1.00, S_aO_2 100 %) [6]. Central motor drive and power output were upregulated with increased and downregulated with reduced C_aO_2; however, the magnitude of peripheral locomotor muscle fatigue developed at end-exercise was identical. Since the rate of accumulation of peripheral fatigue (i.e., fatigue causing metabolites) is enhanced with reduced C_aO_2 and slowed with increased C_aO_2, the downregulation of central neural drive and consequently power output in the presence of reduced C_aO_2 ensured that the rate of development of peripheral locomotor muscle fatigue was slowed and prevented from exceeding a certain limit. Hence, end-exercise peripheral locomotor muscle fatigue was identical between the time trials of various levels of C_aO_2 and limited to an "individual critical threshold" [6]. The existence of this individual critical threshold of peripheral fatigue that is never exceeded during high intensity whole body endurance exercise was confirmed in various other investigations [4, 8, 11, 55, 59] and the idea of peripheral locomotor muscle fatigue as a sensed and regulated variable in normoxia and moderate hypoxia was introduced [3, 5, 19].

The postulate claims that peripheral locomotor muscle fatigue develops only up to a threshold unique for each individual and endurance exercise is either voluntarily terminated once peripheral fatigue has reached this critical threshold (in case of constant workload trials) or the exercise intensity is drastically reduced once a critical rate of fatigue development is reached (in case of time trial exercise where power output is voluntarily adjustable). We interpreted existing correlative evidence to mean that the rate of development of peripheral fatigue is associated with increasing sensory feedback (i.e., group III/IV muscle afferents which are sensitive to fatigue metabolites) from locomotor muscles to the central nervous system (CNS). In turn this feedback influences the regulation of central motor drive—and consequently locomotor muscle power output—in order to limit the level of peripheral fatigue development and thereby avoid intolerable levels of effort/"pain" perception and/or excessive muscle dysfunction as presumably associated with peripheral fatigue beyond the individual critical threshold (Fig. 22.3).

In other words, our fatigue theorem claims that neural afferent feedback associated with peripheral locomotor muscle fatigue exerts an inhibitory influence on the central motor drive resulting in a centrally mediated limitation of exercise in normoxic and moderately hypoxic (S_aO_2 > 76 %) exercise [6, 11]. To be very explicit here, I distinctively emphasize that peripheral locomotor muscle fatigue and/or its rate of development might only be one of many potential mechanisms [52] available to consciously and/or subconsciously influence the determination of central motor output and performance during high intensity whole body endurance exercise.

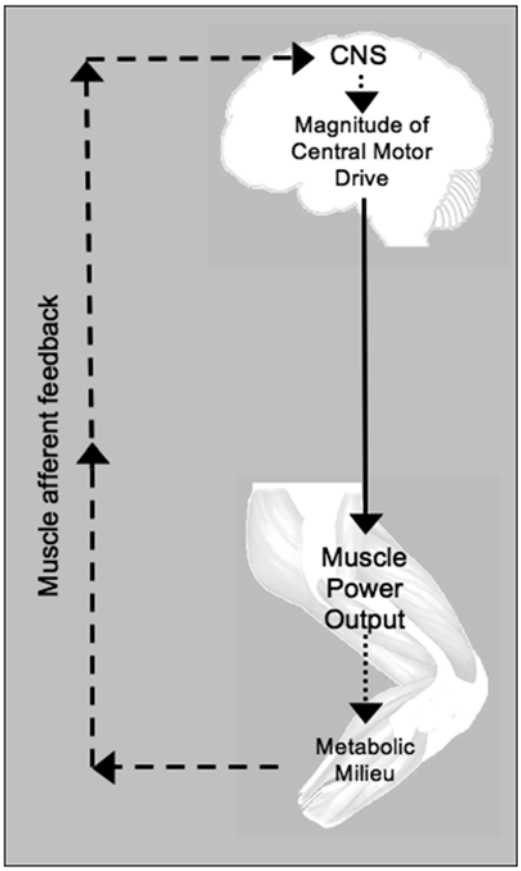

Fig. 22.3 Schematic illustration of our proposed fatigue theorem. The *solid line* indicates efferent nerve activity (central motor drive), the dashed line indicates afferent nerve activity. This regulatory mechanism suggests that the cortical projection of muscle afferents (inhibitory feedback) affects the determination of the magnitude of central motor drive which in turn determines power output of the locomotor muscles. The magnitude of power output determines the metabolic milieu within the working muscles which in turn determines the magnitude of the inhibitory afferent feedback. Based on our data, the purpose of this feedback loop is apparently to restrict peripheral locomotor muscle fatigue to an individual threshold and/or sensory tolerance limit which is never exceeded

However, what happens if humans are exposed to more severe levels of arterial hypoxemia? Is the exercise-induced magnitude of peripheral locomotor muscle fatigue and/or the rate of development of fatigue of these muscles still as important a regulated variable at extreme altitudes/arterial hypoxemia? Or does the priority change and other organ systems, like the brain, take over the hierarchy of regulated variables due to the increased threat associated with severe systemic hypoxemia induced by exercise beyond moderate hypoxia ($S_aO_2 < 76\,\%$)? We have suggested that the relative effects of centrally vs peripherally originating impairments of

central motor drive (and consequently limitations in exercise performance) change with the level of convective O_2 transport as affected by acute hypoxia [2, 11]. In a carefully designed study, subjects were instructed to pedal against a heavy intensity fixed workload to exhaustion in normoxia, and moderate and severe hypoxia (F_IO_2/S_aO_2: 0.21/94 %, 0.15/82 %, and 0.10/67 %, respectively). Clear criteria for task failure (drop in pedal cadence below 70 % of self-selected target cadence for ≥5 s) and exhaustion (drop in pedal cadence below 60 % of self-selected target cadence for ≥5 s) were established prior to the study. When the subjects, unaware of the procedure, reached task failure in each condition, arterial hypoxemia was rapidly removed by surreptitiously switching to an F_IO_2 of 0.3 (reoxygenation). A significant prolongation of exercise time to exhaustion was not achieved following reoxygenation at task failure in normoxia (S_aO_2 ~ 94 %, time to task failure ~660 s) and moderate hypoxia (S_aO_2 ~ 82 %, time to task failure ~280 s). However, in severe hypoxia (S_aO_2 ~ 67 %, time to task failure ~130 s), reoxygenation at task failure elicited a significant prolongation (~170 %) of time to exhaustion (Fig. 22.2a).

Why this difference with severe hypoxia? At task failure in normoxia and moderate hypoxia peripheral locomotor muscle fatigue—assessed via changes in quadriceps twitch force as measured pre- versus post-exercise in response to supramaximal femoral nerve stimulation—was identical (despite different exercise performances) and presumably reached the individual critical threshold. As expected, the magnitude of peripheral fatigue did not change further within the additional few seconds of exercise to exhaustion following reoxygenation in normoxia or moderate hypoxia (Fig. 22.2b) [11]. This is consistent with the literature indicating that reoxygenation has no instant alleviating effect on the already induced magnitude of peripheral muscle fatigue. Interestingly however, at task failure in *severe* hypoxia peripheral muscle fatigue was significant but only about two-thirds of the level of fatigue measured at task failure in normoxia and moderate hypoxia and therefore far below the individual critical threshold. Following reoxygenation in severe hypoxia, subjects continued to exercise and peripheral fatigue continued to develop to the same level (individual critical threshold) as observed at exhaustion in normoxia and moderate hypoxia (Fig. 22.2b) [11].

So, what limits endurance exercise in normoxia and moderate hypoxia vs severe hypoxia and why was there significantly less locomotor muscle fatigue at task failure in severe hypoxia? The data indicate that exercise- and altitude-induced arterial hypoxemia and the associated reduction in cerebral oxygenation as experienced at sea level and up to moderate hypoxia, per se, might not be severe enough to impose an inhibitory influence on central motor drive in healthy humans [2, 11, 64, 65]. The CNS at these levels of hypoxemia/cerebral oxygenation "allows" the development of peripheral locomotor muscle fatigue until the individual critical threshold is reached, which then in turn curtails central motor drive presumably via strong inhibitory neural feedback to the brain as proposed above. The roles seem to be reversed in severe hypoxia (S_aO_2 < 68 %). The level of arterial hypoxemia during exercise at those extreme altitudes might impose a severe threat to cerebral oxygenation. Accordingly, central motor output might be constrained largely independent from

any inhibitory afferent feedback originating in the periphery. This central inhibitory effect of severe hypoxia probably serves to avoid severe cerebral dysfunction far in advance of reaching the individual critical threshold of peripheral muscle fatigue.

22.6 Experimental Challenge and Verification of Hypothesis Under Normoxic Conditions

Again, based on strong correlative evidence as partly outlined above [4, 6, 8, 11, 39, 55], we have formulated a hypothesis. Namely, somatosensory feedback from the fatiguing locomotor muscles exerts inhibitory influence on central motor drive to modulate and/or limit the development of peripheral muscle fatigue during high intensity whole body endurance exercise (S_aO_2 > ~70 %), presumably to avoid a severe disturbance of locomotor muscle homeostasis—and this postulate had to be challenged experimentally. As a reminder, the key component of our proposed "regulatory mechanism" (Fig. 22.3) is the afferent arm consisting of both myelinated (group III) and unmyelinated (group IV) nerve fibers which increase their spontaneous discharge—and therefore their cortical projection—in the presence of metabolic byproducts of fatigue.

As a first step in testing our hypothesis experimentally, we blocked the cortical projection of locomotor muscle afferent feedback during a 5 km cycling time trial via the lumbar epidural injection of a local anesthetic (0.5 % lidocaine, vertebral interspace L_3–L_4) [9]. However, lidocaine also affected efferent motor nerves leading to a significant loss in resting locomotor muscle strength (~22 %). These confounding effects did not allow us to adequately test the role of afferent feedback effects, per se, on exercise performance. And indeed, power output during the time trial performed with the local anesthetic was lower as compared to the control trial. However, several lines of evidence were observed which support a higher central neural drive during the time trial performed with blocked afferent feedback from the locomotor muscles. For example, electromyographic (EMG) activity (relative to the maximal EMG measured during pre-race maximal voluntary muscle contractions—which was lower with vs without epidural lidocaine) obtained from the vastus lateralis suggests that on average and over time the "drive" to race averaged about 9 % stronger when neural feedback was blocked [9]. Furthermore, cardiorespiratory variables (minute ventilation, heart rate, blood pressure) are well known to reliably reflect increases in central motor drive [13, 68]. A substantially increased central command during the time trial with impaired neural feedback was reflected by the similar or even greater cardiorespiratory response to exercise despite the significantly lower workload and metabolic rate during the lidocaine vs the control time trial. In other words, heart rate and mean arterial blood pressure were nearly identical and minute ventilation was even significantly increased despite the lower power output and metabolic rate during lidocaine vs control 5 km time trial [9] (Fig. 22.4).

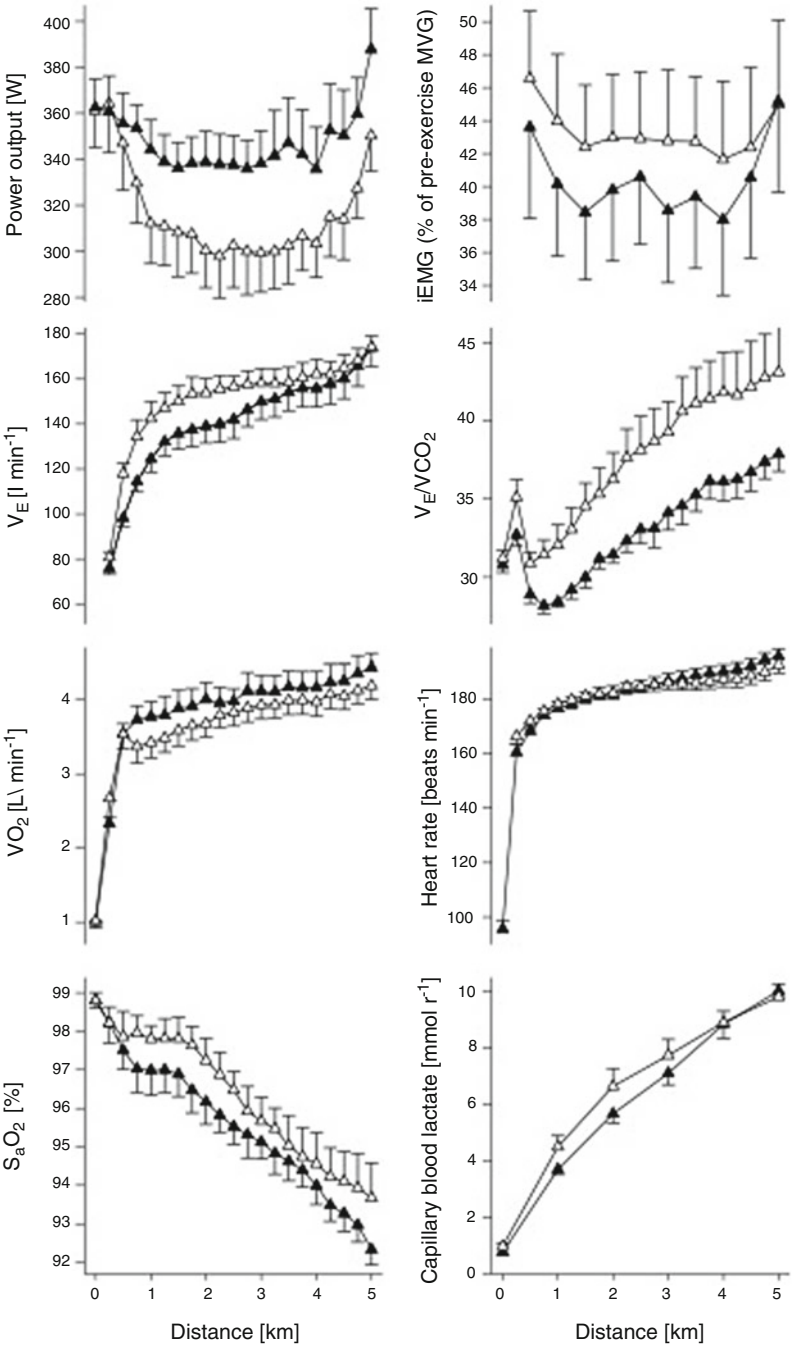

Fig. 22.4 Power output and physiological responses during a 5 km cycling time trial without (control, *solid symbols*) and with (experimental, lumbar epidural anesthesia, *open symbols*) blocked somatosensory neural feedback from the fatiguing locomotor muscles. The subjects were

To circumvent the lidocaine-induced forfeit of locomotor muscle force generating capacity and to adequately determine the effect of neural feedback from exercising muscle on power output and the development of peripheral fatigue during high intensity whole body endurance exercise, Amann et al. then used fentanyl (intrathecally, L_3–L_4) an opioid analgesic, to selectively block the cortical projection of ascending sensory pathways without affecting motor nerve activity or maximal force output [10]. Again, the subjects had to perform a 5 km cycling time trial either with (fentanyl) or without (placebo) opioid-mediated neural feedback from the locomotor muscles. Blocking these fibers attenuated the centrally mediated reflex inhibition and central motor drive during the fentanyl time trial was less restricted and significantly higher as normally chosen by the athlete, i.e., in the placebo time trial (Fig. 22.5). This higher central neural drive resulted in a substantially higher power output during the first half of the race and the CNS "allowed" or "tolerated" the exercise-induced development of peripheral locomotor muscle fatigue drastically beyond levels as observed following the same exercise but with an intact neural feedback system (Figs. 22.5 and 22.6) [10]. In the absence of afferent feedback, the magnitude of central neural drive was thus uncoupled from the intramuscular metabolic milieu of the locomotor muscles. As a consequence, the "naïve" CNS did not limit the development of excessive peripheral fatigue beyond the individual critical threshold which caused ambulatory problems like short-term difficulties with upright standing and walking. Nevertheless, the resulting metabolic and respiratory acidosis eventually prevented the performance to be improved during the fentanyl vs placebo time trial.

22.7 Summary

We have formulated and tested a model with the intent to provide an alternative approach to the "traditional" way of explaining hypoxia-induced endurance exercise limitations in terms of reduced O_2 transport into the muscle cell or changes in relative exercise intensity. Our model suggests that exercise- and hypoxia-induced alterations of the metabolic milieu (and associated peripheral fatigue) of locomotor muscles affect, in a dose-dependent manner, the firing rate—and thus the cortical

Fig. 22.4 (continued) required to reach an individual target power output before the race was launched. Group mean performance was significantly reduced from the control trial (7.35 ± 0.10 min) to the experimental trial (7.66 ± 0.17 min). Mean iEMG of the vastus lateralis was normalized to the iEMG obtained from pre-exercise MVC maneuvers performed either without or with epidural lidocaine. Each point represents the mean iEMG of the preceding 0.5 km section. Despite the substantially lower power output during the experimental trial, central neural drive was higher during the experimental trial vs the control trial. This is indicated by (1) the increased relative integrated EMG (iEMG), (2) ventilation (VE) increased out of proportion to the metabolic rate (VE/VCO_2), and (3) similar heart rate and blood pressure (not shown) despite the higher power output during the control trial. From Amann et al. [9]

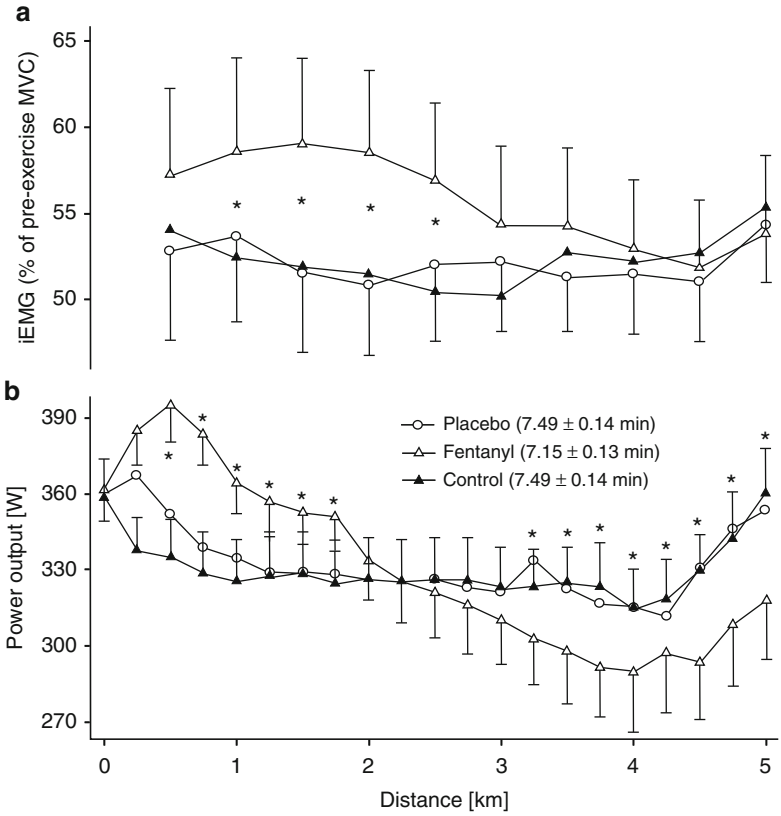

Fig. 22.5 Effect of modified somatosensory feedback on neural drive and power output during a 5 km cycling time trial. All subjects raced with no intervention (Control), with a placebo injection (Placebo; interspinous ligament injection of sterile normal saline, L_3–L_4), and with intrathecal fentanyl (Fentanyl, L_3–L_4). (**a**) Effects of opioid analgesic (fentanyl) on group mean integrated EMG (iEMG) of vastus lateralis. Mean iEMG of the vastus lateralis was normalized to the iEMG obtained from pre-exercise MVC maneuvers performed either without (Placebo and Control) or with (Fentanyl) intrathecal fentanyl. Each point represents the mean iEMG of the preceding 0.5 km section. (**b**) Group mean power output during the 5 km time trial with and without impaired afferent feedback. The subjects were required to reach an individual target power output before the race was launched. *$P<0.05$ (Fentanyl vs Placebo). From Amann et al. [10]

projection—of muscle afferents providing inhibitory feedback to the determination of central motor drive during high intensity whole-body endurance exercise. The purpose of this proposed feedback-loop might be to regulate and restrict the level of exercise-induced peripheral locomotor muscle fatigue to an "individual critical threshold" and ultimately to prevent an excessive disturbance of muscle homeostasis. We believe that this regulatory mechanism is applicable during strenuous endurance exercise at sea level and up to moderate hypoxia ($S_aO_2 > \sim 75\,\%$), whereas in acute severe hypoxia ($S_aO_2 < \sim 70\,\%$), a critical level of CNS hypoxia presumably precedes the development of significant peripheral muscle fatigue and dominates the decision to reduce central motor drive and/or to terminate the exercise.

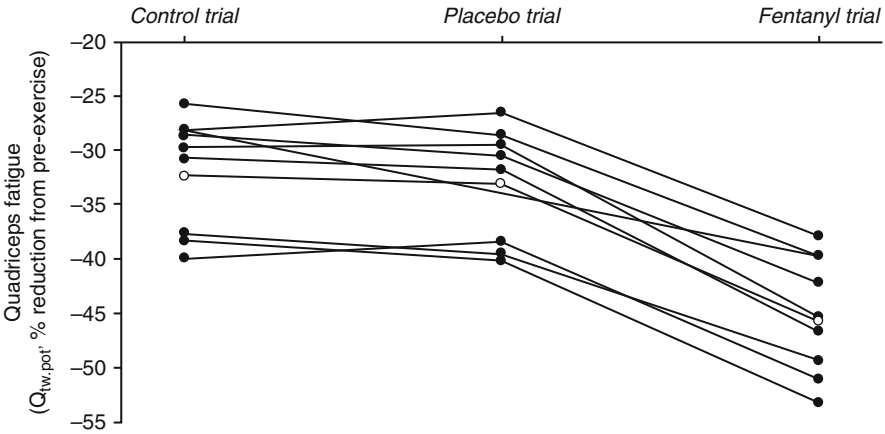

Fig. 22.6 Individual (*solid symbols*) and group mean (*open symbols*) effects of 5 km time trial without (control trial, placebo trial) and with intrathecal fentanyl (fentanyl trial) on quadriceps twitch force ($Q_{tw,pot}$). Exercise performance was similar between Control and Placebo (~7.49 min, $P=0.75$) which was also reflected in similar exercise-induced reductions in $Q_{tw,pot}$ from before to 3-min after the time trial. Despite a similar overall exercise performance (7.51 ± 0.13 min), end-exercise quadriceps fatigue was significantly exacerbated following the Fentanyl vs Placebo trial ($P<0.001$). From Amann et al. [10]

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Chapter 23
Physiological and Clinical Implications of Adrenergic Pathways at High Altitude

Jean-Paul Richalet

Abstract The adrenergic system is part of a full array of mechanisms allowing the human body to adapt to the hypoxic environment. Triggered by the stimulation of peripheral chemoreceptors, the adrenergic centers in the medulla are activated in acute hypoxia and augment the adrenergic drive to the organs, especially to the heart, leading to tachycardia. With prolonged exposure to altitude hypoxia, the adrenergic drive persists, as witnessed by elevated blood concentrations of catecholamines and nerve activity in adrenergic fibers. In response to this persistent stimulation, the pathways leading to the activation of adenylate cyclase are modified. A downregulation of β-adrenergic and adenosinergic receptors is observed, while muscarinic receptors are upregulated. The expression and activity of Gi and Gs proteins are modified, leading to a decreased response of adenylate cyclase activity to adrenergic stimulation. The clinical consequences of these cellular and molecular changes are of importance, especially for exercise performance and protection of heart function. The decrease in maximal exercise heart rate in prolonged hypoxia is fully accounted for the observed changes in adrenergic and muscarinic pathways. The decreased heart rate response to isoproterenol infusion is another marker of the desensitization of adrenergic pathways. These changes can be considered as mechanisms protecting the heart from a too high oxygen consumption in conditions where the oxygen availability is severely reduced. Similarly, intermittent exposure to hypoxia has been shown to protect the heart from an ischemic insult with similar mechanisms involving G proteins and downregulation of β receptors. Other pathways with G proteins are concerned in adaptation to hypoxia, such as lactate release by the muscles and renal handling of calcium. Altogether, the activation of the adrenergic system is useful for the acute physiological response to hypoxia. With prolonged exposure to hypoxia, the autonomous nervous system adapts to protect vital organs, especially the heart, against a too high energetic state, via a purely local autoregulation mechanism necessary for the preservation of overall homeostasis.

Keywords β receptors • G proteins • Autonomic • Muscarinic

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23.1 Introduction

A complex machinery of responses is triggered when an aerobic organism is exposed to hypoxia, involving all cells through the activation of genes presenting a Hypoxia Responsive Element. Factors expressed by the activation of these genes are responsible for initiating physiological processes that, for some of them, will reduce the level of tissue hypoxia [37, 38]. One of the principal response systems to hypoxia is triggered by the stimulation of the peripheral chemoreceptors and involves, from one side the activation of ventilatory control centers, from the other side the activation of the cardiovascular control centers. The stimulation of the adrenergic nervous system is responsible for the acute cardiovascular response to hypoxia, i.e., tachycardia and increase in cardiac output that will try to compensate the acute decrease in blood oxygen content. As one of the most powerful response to the hypoxic stress, the sympathetic system and its counterpart, the parasympathetic system will play a crucial role in the adaptation to acute and chronic hypoxia, especially during exercise [11].

23.2 Desensitization of the β-Adrenergic System in Prolonged Hypoxia

The reduction in maximal cardiac output (Q_{max}) and maximal heart rate (HR_{max}) in prolonged hypoxia is a striking feature of among the various changes observed after exposure to high altitude. It may appear paradoxical and counterproductive for the maintenance of physical performance at high altitude. It has been attributed to several factors [46]: (1) reduction in blood volume and cardiac filling, (2) increased blood viscosity and vascular resistance, (3) alterations in the control by the autonomous nervous system, (4) passive reduction due to a tight coupling between muscle O_2 consumption (which is reduced for metabolic causes) and cardiac output. The third hypothesis is based on the greatest experimental evidence. Since Christensen and Forbes in 1937 [6], the reduction in HR_{max} at high altitude has been observed by many authors (review in Refs. [24, 32]) (Fig. 23.1). Above 4000 m, the reduction in maximal cardiac output and heart rate becomes an important limiting factor. However, the importance of this reduction has been debated since the advantage of rising cardiac output to increase O_2 transport to the periphery can be offset by the disadvantage of increasing diffusion impairment in the lungs [46]. A large, still largely unconcluded, debate about the decrease in HR_{max} being a cause or a consequence of reduced VO_2 max at high altitude developed among scientists [5, 11, 29, 46]. Curiously, the control of chronotropic function by the heart itself, as a defense mechanism, has been scarcely evoked in this debate. The mechanisms of reduction of HR_{max} are to be looked for in the control of the cardiovascular system by the autonomous nervous

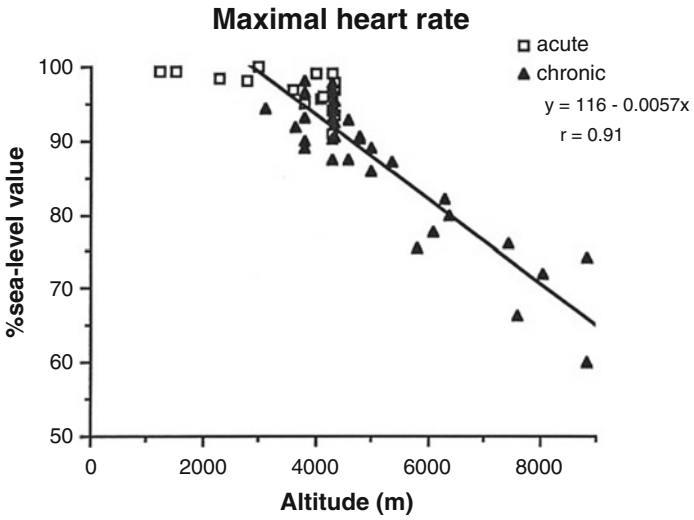

Fig. 23.1 Variation of heart rate at maximal exercise as a function of altitude in acute and chronic hypoxia. From Richalet [32]

system. The activation of the adrenergic system in acute hypoxia has been evidenced by direct and indirect observations. Plasma and urine norepinephrine concentrations have been found elevated in most studies performed in acute and chronic hypoxia, at rest or at a given absolute level of exercise [27, 32]. The direct measurement of the activity of adrenergic fibers has also evidenced an increase in sympathetic activity in hypoxia [42]. The hypoxia-induced tachycardia is responsible for an increase in cardiac output in hypoxia, for a given level of O_2 consumption [12]. The decrease in arterial O_2 content in acute hypoxia cannot be fully compensated by an equivalent decrease in O_2 venous content in the working muscles, so that the arteriovenous O_2 difference decreases and cardiac output increases.

In humans as well as in other mammals, prolonged hypoxia tends to reduce resting and exercise heart rate while circulating catecholamines remain elevated [1]. These results can suggest either a decrease in the responsiveness of the adrenergic system to stimulation or an increase in parasympathetic activity [3, 14, 41]. Both hypotheses can be validated by some experimental evidence.

The responsiveness of the adrenergic system to endogenous (exercise) or exogenous (isoproterenol infusion) stimulation is decreased in prolonged hypoxia [25, 33–35]. For example, for a given increase in norepinephrine plasma concentration from rest to exercise, the corresponding increase in heart rate is lower in prolonged hypoxia than in normoxia [33] (Fig. 23.2). The infusion of increasing doses of isoproterenol leads to a lower increase in heart rate in prolonged hypoxia vs normoxia

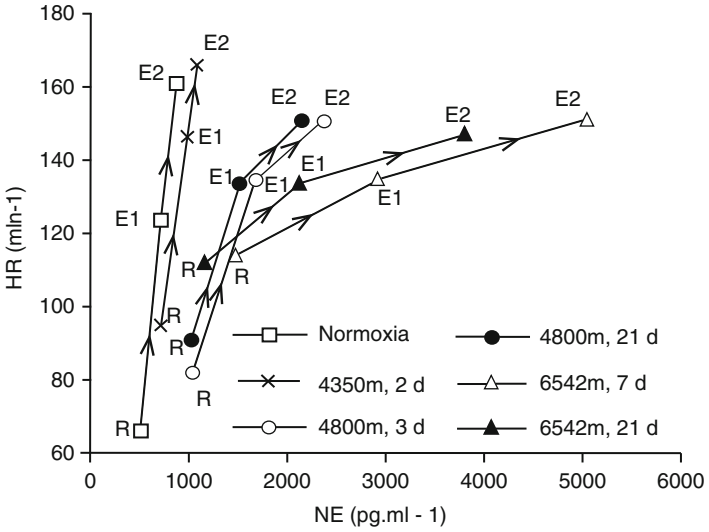

Fig. 23.2 Relationship between heart rate at exercise and corresponding plasma norepinephrine in normoxia and various high altitude conditions. For a given norepinephrine concentration (adrenergic drive), heart rate is lower in hypoxic conditions. From Antezana et al. [1]

in humans [34, 35] or in dogs [25]. This blunted response to adrenergic activation is partly rapidly reversible with reoxygenation [35].

23.3 Molecular Mechanisms of Hypoxia-Induced β-Adrenergic Desensitization

This hypoxia induced blunted responsiveness of the adrenergic system has been explored through the study of signal transduction in the main receptor systems controlling cardiac chronotropic function: β-adrenergic (β-AR), muscarinic (M-Ach-R), and adenosinergic receptors (Fig. 23.3). Acute hypoxia (1–5 days at 4559 m) has been found to reduce HR_{max} and this reduction was fully reversible with O_2 inhalation, which suggests an uncoupling of β-AR, as a result of phosphorylation of G protein or second messenger, rather than a downregulation of the receptor [24]. Chronic hypoxia leads to a downregulation of β-AR in the rat myocardium [17, 45] and in human lymphocytes [1]. This decrease is associated with a decrease in adenylate cyclase activity in rats [16, 17, 26, 30] and in guinea pigs [23]. The effects of hypoxia on the adrenergic pathway could be mediated by the desensitizing effect of permanently increased catecholamine levels or to a direct effect of hypoxia on one or several elements of the transduction pathway. To test this hypothesis, chronically hypoxic rats were compared to rats exposed

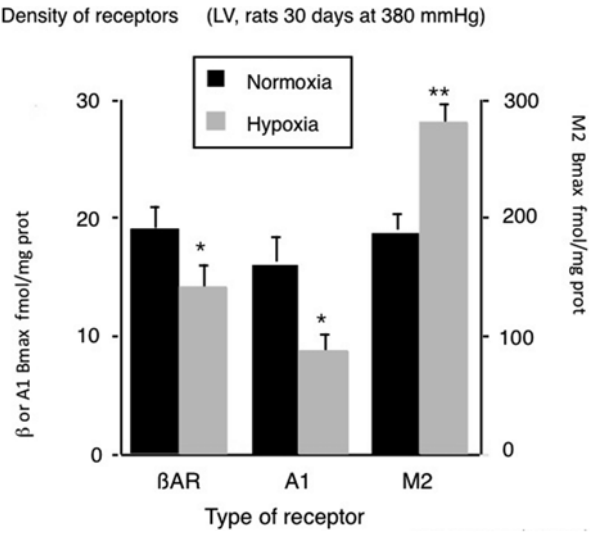

Fig. 23.3 Effect of exposure to hypoxia on the density of β-adrenergic, muscarinic and adenosinergic receptors in the heart of rats exposed for 30 days at low barometric pressure (380 mmHg). β-adrenergic and adenosinergic receptors are downregulated, while muscarinic receptors are upregulated. From Kacimi et al. [18]

to prolonged norepinephrine infusion [22]. There were some clear differences between the two models, especially at the level of Gi inhibitory protein that was more activated in hypoxic rats than in rats exposed to norepinephrine. The changes are also very much chamber dependent, since in rats exposed to chronic hypoxia, the right ventricle is hypertrophied and in the norepinephrine stimulated group the left ventricle is hypertrophied [22]. Several studies have also observed that chronic exposure to hypoxia led to an increase in the density of myocardial M-Ach-R [10, 18, 50]. These results are consistent with the hypothesis that myocardial β-adrenergic receptors as well as muscarinic receptors are involved in the reduction of maximal heart rate after acclimatization to hypoxia. [10]. Favret et al. provided clues of the possible role of myocardial β-AR and M-Ach-R on Q_{max} by studying the time course of acclimatization [10]. They showed a strong correlation between ventricular β-AR density as well as M-Ach-R density and HR_{max}. These results suggest that β-AR downregulation and M-Ach-R upregulation could be considered as a possible mechanism leading to the reduction of HR_{max} and Q_{max}. Boushel et al. [3] have shown, by parasympathetic blockade using glycopyrrolate, that the parasympathetic nervous system was involved in the decrease in HR_{max} in human acclimatized to 9 weeks at 5260 m. In subjects acclimatized for 2 weeks at a much lower altitude (3800 m), glycopyrrolate also increased HR_{max} but failed to significantly increase maximal cardiac output [2]. However, in rats acclimatized for 3 weeks at 5500 m, Clancy et al. [7] did not observe that the M-Ach-R were responsible for the low HR_{max}.

Prolonged exposure to severe hypoxia (3 weeks at 6542 m) leads to a permanent increase in adrenergic activity (although plasma norepinephrine decreases from the first to the third week of exposure), a decrease in the density of β-AR in circulating lymphocytes and in the heart rate response to isoproterenol infusion [1]. The desensitization of the β-adrenergic pathway is not only linked to the decrease in the β-AR density but also to an alteration of the Gs protein coupling to adenylate cyclase [19] (Fig. 23.4). The impaired function of Gs could be due to a reduction in the biologically active form Gsα-small and/or an increase in the biologically inactive form Gsα-large of the Gs protein [30]. Moreover, the bioactivity of the membrane-bound Gsα would be reduced [16]. The stimulation of adenosinergic receptors, by activating the inhibitory Gi protein can also reduce the adenylate cyclase activity, although these receptors are also downregulated in hypoxia [19]. The Gi protein has been found increased in the heart of rats exposed to 5 days of hypoxia [26]. An impairment of norepinephrine intravesicular uptake in hypoxia could also contribute to increase the concentration of norepinephrine in the synaptic space and contribute to the desensitization of the adrenergic pathway [26, 36]. Opioid receptors can also be involved since a cross-talk exists between these receptors and β-AR: the pertussis toxin-sensitive G protein of the opioid pathway inhibits the Gs protein of the adrenergic pathway [51]. The

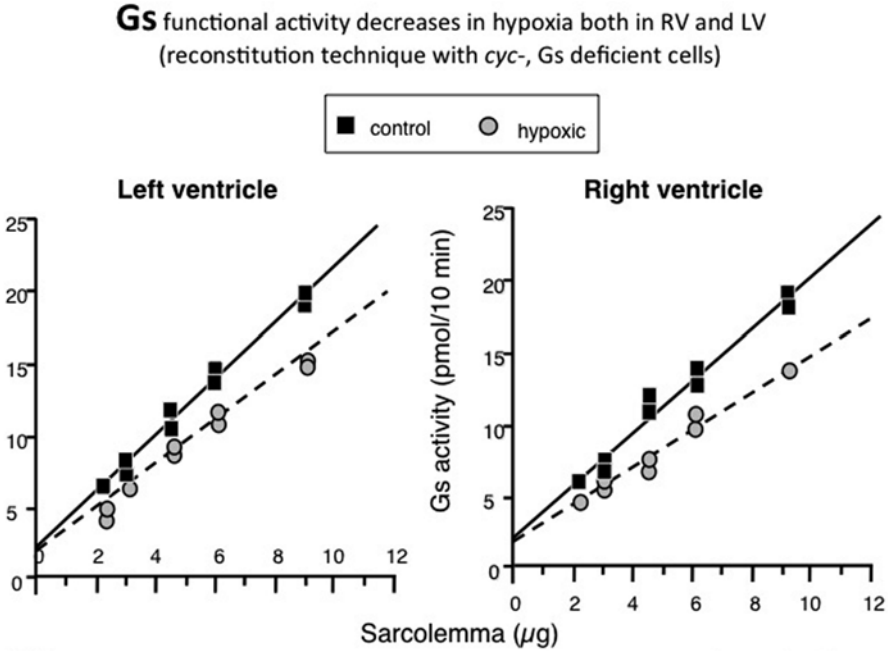

Fig. 23.4 Effect of hypoxia on Gs functional activity in the left and right ventricle of rats exposed to chronic hypoxia. Hypoxia decreases Gs activity in both ventricles. From Kacimi et al. [19]

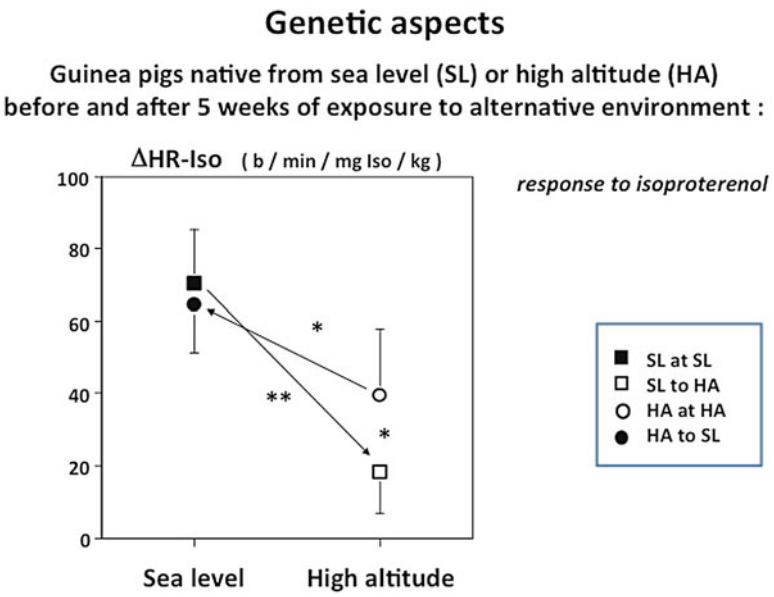

Fig. 23.5 Response to isoproterenol infusion in guinea pigs native from sea level or high altitude when exposed to native or alternative environments. In all cases, response to isoproterenol is lower at high altitude, showing that the hypoxia-induced desensitization process of adrenergic system is persistent in high altitude species. From Leon-Velarde et al. [23]

release of dynorphins from the heart in hypoxia can activate the kappa opioid receptors and blunt the adrenergic pathway, therefore protecting the heart from too high-energy consumption [47]. These adaptations of the autonomic control of the heart in hypoxia seem well established even in species genetically adapted to high altitude, such as the guinea pig living on the Altiplano [23] (Fig. 23.5).

Altogether, it appears that many observations are in favor of an important role of the changes in the autonomic nervous system in mammals acclimatized to hypoxia, as limiting VO_2 max despite increased O_2 carrying capacity [11].

23.4 Adrenergic System and Preservation of the Myocardium in Hypoxia

In a model of oxygen transport in the myocardium, it was clearly shown that the maintenance of a normal myocardial tissue PO_2 at maximal exercise in hypoxia was possible at the only condition that the myocardial energy expenditure decreases, that is to say that HR_{max} decreases [32]. Therefore, the currently observed decrease in HR_{max} in chronic hypoxia would be a homeostatic

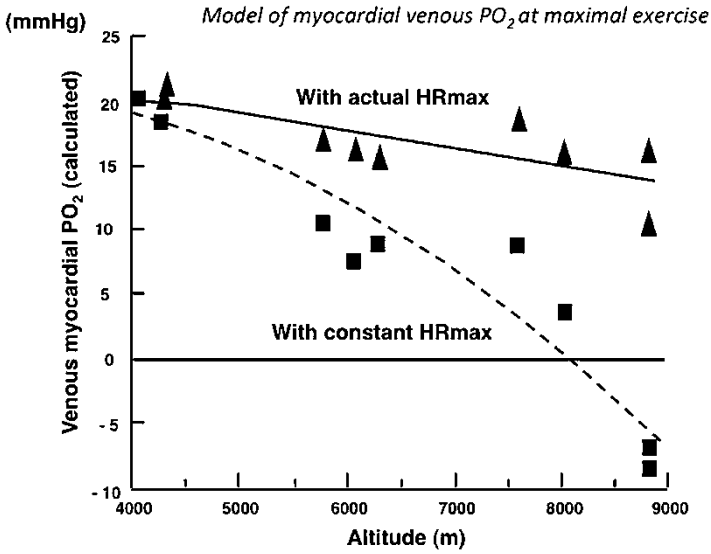

Fig. 23.6 Model of oxygen transfer within the myocardium. Calculated venous myocardial PO_2 decreases in an unrealistic manner when maximal heart rate is maintained constant. With actual values of heart rate, i.e., decreasing with high altitude, venous PO_2 is maintained merely constant. From Richalet [32]

mechanism contributing to the maintenance of myocardium tissue oxygenation, despite a decrease in oxygen supply (Fig. 23.6). By reducing maximal O_2 transport, the decrease in HR_{max} can be considered as a limiting factor of performance at high altitude. However, this mechanism is protective for the heart, a vital organ that is also demanding for a high O_2 supply at exercise. The coronary flow reserve has been shown to be limited to 33 % above what is prevailing during maximal exercise at sea level [20]. Therefore, the compensation of decreased arterial O_2 content by increasing coronary blood flow is not possible above a certain altitude and the only option to preserve cardiac integrity is to decrease myocardial O_2 demand and therefore maximal heart rate. It is important to note that there is no need to involve the brain and a hypothetic central governor to explain the reduction in VO_2 max and HR_{max} in hypoxia [29]. This theory supposes that a tissue sensor alerts the brain and reduces heart rate when the availability of O_2 is low. No such mechanism has ever been evidenced, except in very severe cerebral ischemia, which is not the case at altitudes such as 4000 m where the decrease in HR_{max} is already significant (Fig. 23.1). On the contrary, hypoxia, via the chemoreceptors and the medullar control centers, always induces an activation of the adrenergic system and therefore an increase in heart rate. The downregulation of the β-adrenoceptors and upregulation of the muscarinic receptors within the myocardium are sufficient to explain the decrease in HR_{max} and are purely local homeostatic mechanisms that protect the

myocardium against a too high energy expenditure. It is important to note that all these changes in cardiac chronotropic function are not associated with alterations in the inotropic function. Stroke volume and myocardial contractility, explored by echocardiography up to the simulated altitude of 8848 m are not diminished, at least at rest [4].

23.5 Other G-protein Coupled Receptors Involved in the Response to Hypoxia

Other systems than cardiac β-receptors have been studied with respect to activation and desensitization of the adrenergic system. Moreover, other receptors involving a G-protein coupling pathway may be involved in adaptation to hypoxia.

23.5.1 Adipose Tissue Lipolysis in Hypoxia

During the Operation Everest III, a study was conducted in eight normal sea level natives to examine the effects of prolonged hypoxia (31 days of gradually increasing hypobaric hypoxia up to the equivalent altitude of 8848 m in a decompression chamber) on adipose tissue lipolysis, in relation to the weight loss usually observed at high altitude [8]. A biopsy of subcutaneous adipose tissue was performed before and after hypoxic exposure, to study in vitro changes in adipose tissue sensitivity. The in vitro lipolytic response to epinephrine, isoproterenol, growth hormone (GH), and parathormone (PTH) decreased significantly. The anti-lipolytic effect promoted by α2-adrenergic receptor stimulation (epinephrine with propranolol) was greater after hypoxia ($P<0.05$), while the anti-lipolytic activity of insulin was decreased ($P<0.01$). Therefore, prolonged exposure to hypobaric hypoxia led to a potent reduction in lipid mobilization, through a decrease in the efficiency of β-adrenergic, GH and PTH lipolytic pathways, as well as an increment in the α2-adrenergic-receptor-mediated anti-lipolytic effects. These processes may be considered as a protecting effect against a too high consumption of fat reserves in an extreme condition where body mass is severely reduced, mainly via a reduction in food intake [48, 49].

23.5.2 Calcium Handling by the Kidney

The calcium-sensing receptor is a G protein-coupled receptor that binds and signals in response to extracellular calcium. It is highly expressed on parathyroid and kidney cells, where it participates in the regulation of systemic calcium homeostasis.

The biological response to exogenous parathormone (PTH) was measured in ten healthy male volunteers during a short stay at high altitude (Vallot observatory, 4350 m) [43]. Blood and urine samples were obtained before (basal condition) and after a 200 U synthetic 1-34 PTH infusion bath at sea level (Normoxia: N) and, 1 week later, after a 36 h stay at the Vallot observatory (Hypoxia: H). PTH infusion induced in both N and H a similar increase in serum Ca^{2+} and phosphaturia and a decrease in calciuria. In contrast urinary cAMP responses to PTH was reduced in H. These results argue for a relative resistance to PTH in hypoxia.

23.5.3 Hormonal Response to Hypothalamic Factors

The hypophyseal hormones are also subjected to a hypoxia-induced decrease in their response to hypothalamic factors. Basal levels of hormones and the responses of TSH, thyroid hormones, prolactin, sex hormones, and growth hormone to the injection of TRH, GnRH, and GHRH were studied in eight men in normoxia and on prolonged exposure (3–4 days) to an altitude of 4350 m [39]. The effect of hypoxia on the hypophyseal response to hypothalamic factors was not affected by hypoxia and similar among subjects, except for the GH response to GHRH administration. The decreased response of GH to GHRH observed in several subjects in hypoxia suggests a disturbed influence of this hypothalamic regulator at high altitude. This could be caused by a desensitization of the GHRH receptor or an altered GH production. The GHRH receptor is a seven transmembrane G-protein-linked receptor and, and similarly to other G-protein-linked systems, could be downregulated in prolonged hypoxia [19].

23.6 Adrenergic System and High Altitude-Related Diseases

Acute mountain sickness has been related to cerebral edema secondary at least in part to hypoxic cerebral vasodilation and elevated cerebral capillary hydrostatic pressure. This results in reduced brain compliance with compression of intracranial structures. It is postulated that these primary intracranial events elevate peripheral sympathetic activity that acts neurogenically in the lung possibly in concert with pulmonary capillary stress failure to cause high altitude pulmonary edema (HAPE) and in the kidney to promote salt and water retention [21]. However, the hypothesis of a neurogenic basis for altitude sickness has never been proved by experimental evidence.

β2 adrenoceptors are important in the clearance process of alveolar fluid in the lung. A defect of this mechanism may predispose subjects to HAPE. Based on this hypothesis, a double-blind, randomized, placebo-controlled study, was conducted to explore the effects of prophylactic inhalation of the β2-adrenergic agonist (salmeterol) on the incidence of pulmonary edema during exposure to

high altitude (4559 m) in 37 subjects who were susceptible to HAPE [40]. Prophylactic inhalation of salmeterol decreased the incidence of HAPE in susceptible subjects by more than 50 %, supporting the concept that sodium-driven clearance of alveolar fluid may have a pathogenic role in pulmonary edema in humans and therefore represent an appropriate target for therapy. However, side effects of β2 agonists refrain people from using them in the treatment of HAPE. Interestingly, in a recent case–control study, 110 unrelated HAPE patients and 143 unrelated HAPE-resistant controls matched on age and ethnicity were used to examine the association between polymorphisms of β2-adrenergic receptor (ADRB2) and disease. The haplotypes of ADRB2 consisting of the single nucleotide polymorphisms 46A/G and 79C/G, appeared to have a greater power for predicting HAPE [44].

23.7 Perspectives

Therefore, similarly to what was shown in the β-adrenergic and adenosinergic pathways in the myocardium, the desensitization process of the adrenergic, PTH and GH receptors in the adipose tissue, the hypophysis, and the kidney, as well as the blunted lactate release by the muscle could be attributed to an alteration in the G-protein signal transduction within these receptor systems.

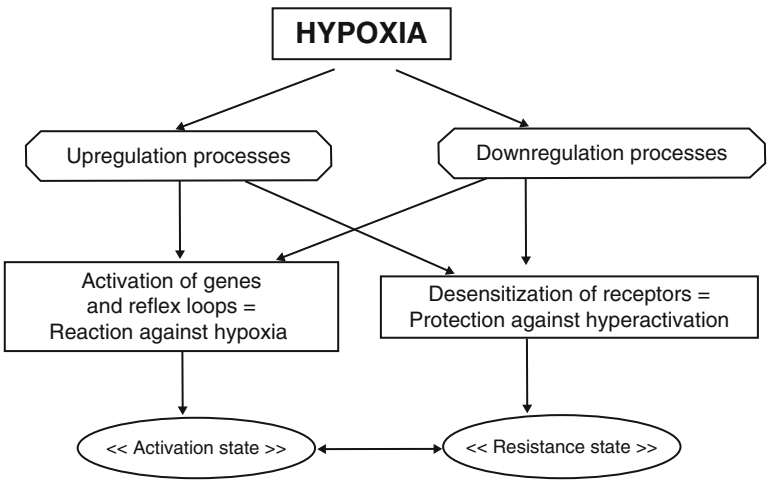

Fig. 23.7 The exposure to hypoxia triggers both upregulation and downregulation processes, activating reflex loops, and on the other side blunting some mechanisms through the desensitization of receptors pathways. An appropriate balance of these two types of processes may be necessary for optimal adaptation to the hypoxic stressor

Mechanisms accounting for the effect of hypoxia on these receptor pathways remain speculative and further studies on the regulation of the signal transduction systems of these hormones are needed, with a special interest for the role of β-arrestin in the hypoxia-induced desensitization of the G-protein linked receptors [31].

The exposure to hypoxia triggers both upregulation and downregulation processes, activating reflex loops, increasing ventilation and heart rate as well as erythropoietin production, and on the other side blunting some mechanisms through the desensitization of receptors pathways. An appropriate balance of these two types of processes may be necessary for optimal adaptation to the hypoxic stressor (Fig. 23.7).

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Chapter 24
Hemoglobin Mass and Aerobic Performance at Moderate Altitude in Elite Athletes

Jon Peter Wehrlin, Bernard Marti, and Jostein Hallén

Abstract Fore more than a decade, the live high–train low (LHTL) approach, developed by Levine and Stray-Gundersen, has been widely used by elite endurance athletes. Originally, it was pointed out, that by living at moderate altitude, athletes should benefit from an increased red cell volume (RCV) and hemoglobin mass (Hb_{mass}), while the training at low altitudes should prevent the disadvantage of reduced training intensity at moderate altitude. VO_{2max} is reduced linearly by about 6–8 % per 1000 m increasing altitude in elite athletes from sea level to 3000 m, with corresponding higher relative training intensities for the same absolute work load. With 2 weeks of acclimatization, this initial deficit can be reduced by about one half. It has been debated during the last years whether sea-level training or exposure to moderate altitude increases RCV and Hb_{mass} in elite endurance athletes. Studies which directly measured Hb_{mass} with the optimized CO-rebreathing technique demonstrated that Hb_{mass} in endurance athletes is not influenced by sea-level training. We documented that Hb_{mass} is not increased after 3 years of training in national team cross-country skiers. When athletes are exposed to moderate altitude, new studies support the argument that it is possible to increase Hb_{mass} temporarily by 5–6 %, provided that athletes spend >400 h at altitudes above 2300–2500 m. However, this effect size is smaller than the reported 10–14 % higher Hb_{mass} values of endurance athletes living permanently at 2600 m. It remains to be investigated whether endurance athletes reach these values with a series of LHTL camps.

Keywords Altitude training • Hypoxia • Red cell volume • VO_{2max}

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24.1 Introduction

For several decades, altitude training has been used by endurance athletes and coaches to enhance sea-level performance. This "classical" altitude training has been performed by living and training at moderate altitude (live high–train high; LHTH). However, the scientific literature about performance effects of LHTH is equivocal since there are studies with improved [10, 14, 16, 27, 52], but also studies with unchanged [1, 5, 11, 18, 42, 43, 81] performance after LHTH. This encouraged the search for alternative strategies to use hypoxia as an additional stimulus for endurance athletes. In 1992, Levine and Stray-Gundersen [51] introduced the altitude training method "live high–train low" (LHTL). With living at moderate altitude, athletes theoretically should acquire the beneficial effects of altitude acclimatization, particularly an increase in hemoglobin mass (Hb_{mass}) and red cell volume (RCV) for maximizing the oxygen transport capacity. At the same time the low altitude or sea-level training would decrease the negative effects of reduced absolute training intensity caused by reduced VO_{2max} at altitude [48].

In 1997, Levine and Stray Gundersen showed in a complex study that the effect of LHTL on sea-level performance is superior to normal sea-level training or classical LHTH altitude training [49]. In elite sport, the LHTL paradigm has been widely used by endurance athletes during the last years. Altitude houses and tents have been developed in order that the LHTL can be conducted even at the home of the elite endurance trained athletes (ETA) [90]. Various studies using the LHTL paradigm have been conducted and the scientific debate has rather been why there are improvements in endurance athletes after LTHL than if there are improvements after LTHL [30, 50]. However, performance after LHTL altitude training is influenced by a wealth of confounding factors like the individual training plans, sickness, timing of performance after LHTL camp, and individual responses. In the last years there has also been a debate if there is an increase in Hb_{mass} and RCV with LHTL altitude training because several studies did not find this expected increase in elite athletes. The aim of this paper is therefore twofold: on one hand to review the expected negative effect of reduced VO_{2max} and associated reduced absolute training intensity when living and training at moderate altitude in elite endurance athletes (Part I) and on the other hand to review the expected beneficial effect of living at moderate altitude on hemoglobin mass and red cell volume (Part II).

24.2 Part I: Performance at Altitude in Elite Endurance Athletes

Optimal endurance performance relies upon frequency, duration, and intensity of training [38]. Especially with endurance performance, maintenance of training intensity appears to be the principle variable in optimizing subsequent endurance performance [39, 40]. Although VO_{2max} is not performance in a strictly physical

way (power per time), it clearly is one of the major characteristics that determine performance in endurance sport. VO_{2max} is generally accepted as the single best measure of the functional limit of the combined respiratory and circulatory systems to deliver oxygen to active muscles and the ability of the muscles to use oxygen [4] and is reproducible [41, 44]. Moreover, VO_{2max} is the most often studied and well-described effect of altitude exposure on exercise performance and is more or less independent of exercise protocol. At altitude, VO_{2max} is mainly physiologically affected by the reduction of air pressure that leads to reduced partial pressure of oxygen and consequently reduced oxygen flux at every step along the oxygen cascade. Consequently, VO_{2max} is reduced at altitude and this reduction is directly related to increased relative training intensity for the same absolute work load. The effect of decreased air density reducing air resistance is primarily relevant for endurance disciplines with high speeds like cycling etc. and will therefore not be discussed. In the performance at altitude part of this review, we first evaluate the "maximal" size effect of reduced VO_{2max} and absolute training intensity at altitude.

Fulco et al. [26] concluded in their review about aerobic performance at altitude one decade ago, that the reduction in VO_{2max} is larger in trained than in untrained subjects, in acute than after chronic hypoxic exposure and in unacclimatized versus acclimatized subjects. We therefore focused on studies which measured VO_{2max} for elite sport at altitudes up to 3000 m in acute hypoxia under laboratory settings (to avoid different acclimatization states) in trained sea level resident athletes ($VO_{2max} > 60$ ml kg^{-1} min^{-1}). Thereafter, we investigated in LHTH and LHTL studies if and how this reduction in VO_{2max} changes with increasing acclimatization of the elite endurance athletes.

24.2.1 Reduction of VO_{2max} in Acute Hypoxia

It was long believed that the sigmoid shape of the O_2–hemoglobin dissociation curve and the increased ventilation (VE) defend a reduction in arterial O_2 saturation (SaO_2) and VO_{2max} at altitudes below 1500 m. Buskirk et al. [11] concluded in 1967 that up to an altitude of 1524 m VO_{2max} is reduced only minimally, but thereafter is about 10.5 % per additional 1000 m. However, several more recent studies have shown that VO_{2max} can be reduced at altitudes even below 1000 m [29, 32, 83] and that there is a substantial individual difference in the reduction of VO_{2max} with increasing altitude [45, 47]. Although the reasons for this individual response are not clear, it seems that fitness level may be an important factor, as endurance-trained athletes (ETA; $VO_{2max} > 60$ ml kg^{-1} min^{-1}) have demonstrated a larger decline in VO_{2max} with increasing altitude compared with untrained individuals [45, 47]. It has been suggested that this is due to the fact that ETA have developed exercise-induced desaturation already at sea-level [12, 29, 83] and operate at the steeper part of the oxygen equilibrium curve at low altitudes [20].

There are only few studies that have tested the reduction of VO_{2max} for ETA in acute hypoxia at altitudes relevant (0–3000 m) for endurance disciplines in the

laboratory [12, 21, 29, 32, 47, 56–58, 80]. Three studies showed that VO_{2max} declines even at altitudes as low as 750–900 m [29, 32, 83] suggesting that the decrease is linear from sea-level to 3000 m.

However, none of these studies tested VO_{2max} from sea-level (0–300 m) to very low (300–1000 m), low (1000–2000 m) and moderate (2000–3000 m) altitude in the same athletes. In addition, the VO_{2max}-tests used in these studies were either incremental step tests to exhaustion or all out tests for a given distance. Under hypoxia, these protocols result in reduced absolute exercise intensity. It has therefore been hypothesized that one reason for the decreased VO_{2max} in hypoxia is the result of reduced maximal absolute intensity [55].

In order to test the hypothesis that there is no threshold altitude for decrement in VO_{2max}, we therefore measured VO_{2max} (Douglas bag system) at simulated altitude (hypobaric chamber) 300, 800, 1300, 1800, 2300, and 2800 m in a randomized and double blind order in endurance athletes with a $VO_{2max} > 60$ ml kg^{-1} min^{-1} [88]. To ensure that the results of reduced VO_{2max} would not be influenced by reduced muscle recruitment associated with reduced exercise intensity, our athletes absolved a preliminary VO_{2max} test from which we calculated individual constant speed to reach VO_{2max} by running at sea level between 2 and 6 min to exhaustion. Athletes thereafter ran at all different altitudes with these same speeds in order to reach VO_{2max}. Before each maximal running test to exhaustion, athletes additionally ran at an individual constant speed of 55 % of sea-level VO_{2max} in order to compare the altitude related effects between submaximal and maximal performance. As expected, we found a quite uniform and highly linear decrease in VO_{2max}, beginning already between 300 and 800 m and extending through 2800 m with a rate of decline of 6.3 % per 1000 m altitude (Fig. 24.1). Individual decreases in VO_{2max} ranged between 4.7 and 7.5 % per 1000 m, a small variation compared with that found in ETA earlier by Gore et al. [32] (+1 to −12 % change from 168 to 748 m above sea-level) or Billat et al. [6] (−8 to −24 % from sea-level to 2400 m). However, none of these studies or the other before mentioned studies included reported test-retest reproducibility. It is therefore not clear how much of the reported variability is methodological variation and how much is biological variation between the subjects. In our study the test-retest reproducibility (coefficient of variation) at 300 m was 1.4 %. The magnitude of the decrease in VO_{2max} was with 6.3 %/1000 m very close to the 7.2 %/1000 m calculated from the other studies which tested athletes with a $VO_{2max} > 60$ ml kg^{-1} min^{-1} in acute hypoxia in a laboratory (Fig. 24.2). The magnitude of this reduction is moreover very similar to the studies measuring the VO_{2max} at real altitudes after 1–2 days of exposure (mean reduction 7 %/1000 m; see next section). SpO_2 reduced also linearly (5.5 %/1000 m) and was strongly associated with the decrease in VO_{2max} with altitude. According to Ferretti et al. [20], the decrease in SpO_2 accounts for about 86 % of the decrease in VO_{2max}, which fits with the present study where approximately 70 % of the decrease in VO_{2max} could be explained by the decrease in SpO_2 at VO_{2max}. These results support the conclusion of Powers et al. [61] that a reduction of 1 % in SpO_2 below 92–93 % causes a decrease of ~1 % of VO_{2max}. Hence, the main mechanism for the hypoxia-induced decrease in VO_{2max} at low and moderate altitude is the decrease in SpO_{2max}. Maximal

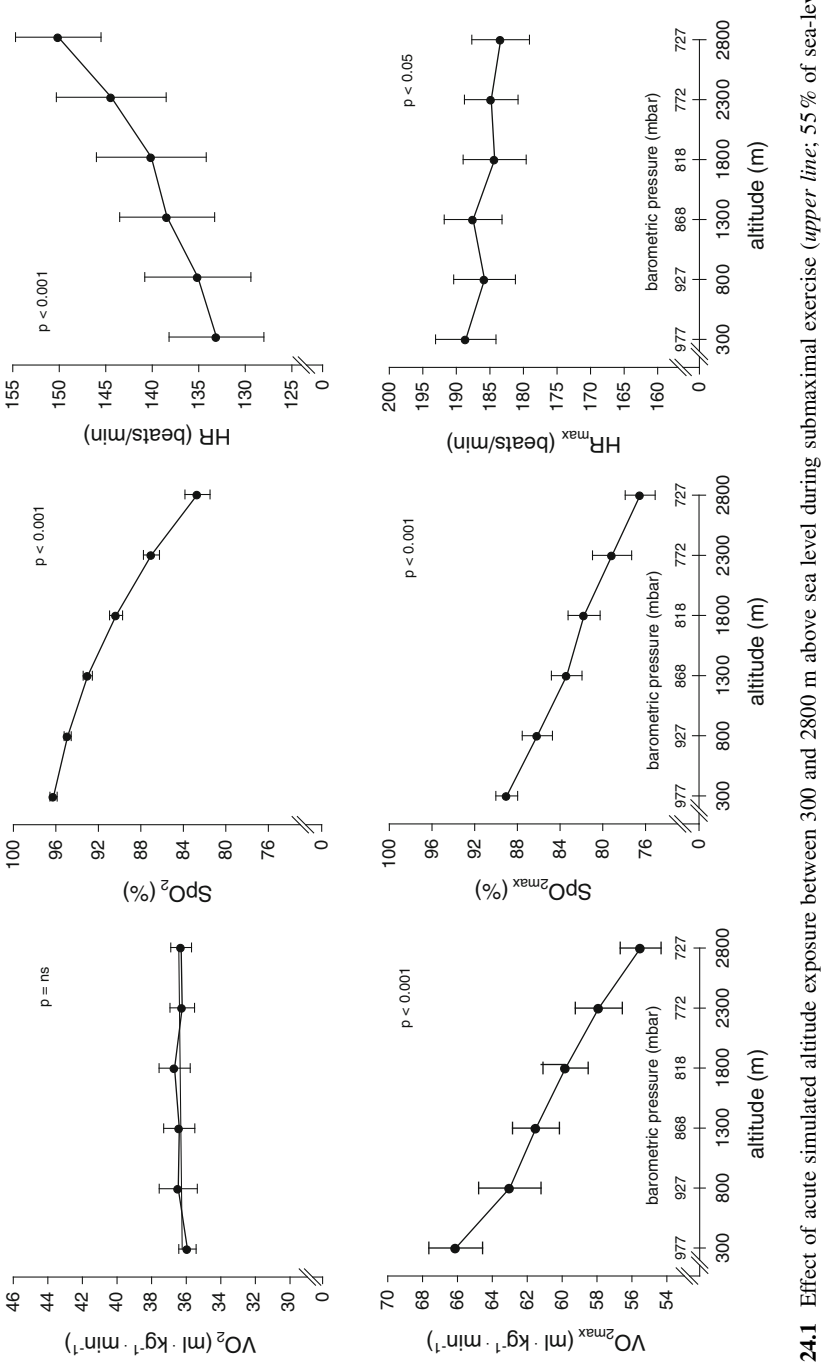

Fig. 24.1 Effect of acute simulated altitude exposure between 300 and 2800 m above sea level during submaximal exercise (*upper line*; 55% of sea-level VO_{2max} identical absolute intensity at all altitudes) and maximal exercise (*lower line*; 107% of sea-level VO_{2max}; identical absolute intensity at all altitudes) on oxygen uptake (VO_2 and VO_{2max}), arterial oxygen saturation (SpO_2 and SpO_{2max}), and heart rate (HR and HR_{max}). Modified after Wehrlin and Hallén [88]

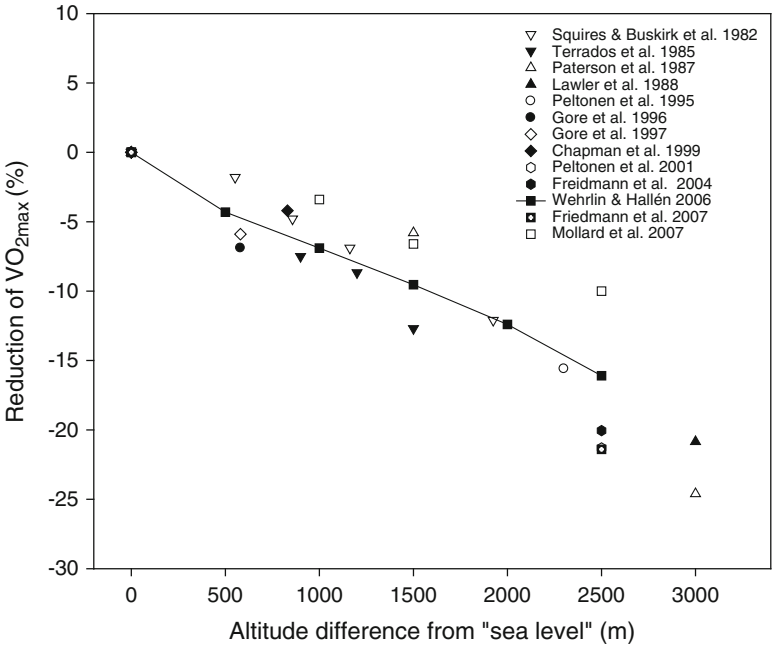

Fig. 24.2 Decline in VO_{2max} with altitude from sea-level values. "Sea-level" in these studies [12, 21, 22, 29, 32, 47, 53, 56, 57, 59, 80, 83, 88] is set at 0 m but varies originally from 0 to 362 m. Only studies which tested male unacclimatized ETA with a mean $VO_{2max} > 60$ ml kg^{-1} min^{-1} under laboratory conditions at acute hypoxia are included. Wehrlin and Hallén [88]

heart rate tended to decrease by about 2 beats per 1000 m increasing altitude (Fig. 24.1). Submaximal values showed as expected, that VO_2 for the same absolute speed is the same independent of altitude. SpO_2 reduced curvilinearly as did heart rate increase to compensate for the reduced oxygen content of the arterial blood.

24.2.2 VO_{2max} at Altitude with Increasing Acclimatization to Moderate Altitude

Surprisingly few studies have investigated the effect of moderate altitude exposure on VO_{2max} with increasing acclimatization in elite endurance athletes (Fig. 24.2). Most of these studies [1, 19, 62, 70] evaluated the effect of a LHTH altitude training camp on VO_{2max} in the run-up to the Olympic Games 1968 carried out in Mexico City at an altitude of 2240 m above sea level. Mean reduction of VO_{2max} in these athletes measured at sea level after 1–2 days at altitudes between 1822 and 2344 m was 7 % per 1000 m increasing altitude (range between 5.4 and 8.3 %). This reduction is, as mentioned, likewise very similar to the results of our laboratory study where the mean reduction of VO_{2max} was 6.3 % per 1000 m increasing altitude, or

the mean of our overview of the laboratory studies with endurance athletes, where VO_{2max} was reduced by 7.2 % per 1000 m increasing altitude. With increasing acclimatization, the reduction of VO_{2max} could be compensated by about 1/3 during 2–3 weeks in these LHTH studies. Only in one group of the legendary LHTH crossover study [1], VO_{2max} was compensated only by 10 %. In all other LHTH studies in Fig. 24.2, the compensation varied between 29 % [71] and 36 % [70]. When athletes do a 3 week LHTH altitude training camp at for instance 2500 m, VO_{2max} will therefore be reduced by about 15–20 % at the beginning and around 10 % at the end of the LHTH camp. In endurance athletes, this reduction in VO_{2max} will be associated with a reduction of absolute training intensity. It is important to note that these estimated values reference to training intensities near VO_{2max}. At lower training intensities, the athlete can profit from the sigmoidal reduction of SaO_2 that results in smaller altitude related effects (Fig. 24.1). However, combined with the recommended reduction of training volume [90] (20 % during the first week, 10 % during the third week) the absolute training stimulus is reduced considerably. To our knowledge, there is only one LHTL study which measured VO_{2max} several times during the altitude training camp. In the interesting study of Schuler et al. [78], elite cyclists lived for 21 days at an altitude of 2340 m above sea level (Sierra Nevada, Spain) and performed all training at altitude below 1100 m (30 min transport time). Mean decrease in VO_{2max} on day 1 at altitude was −12.6 % similar (−5.4 % per 1000 m increasing altitude) to the other LHTH studies (Fig. 24.3). The main point, however, is that the initial decrease in VO_{2max} was compensated by about 50 % after 14 days and by 70 % after 21 days.

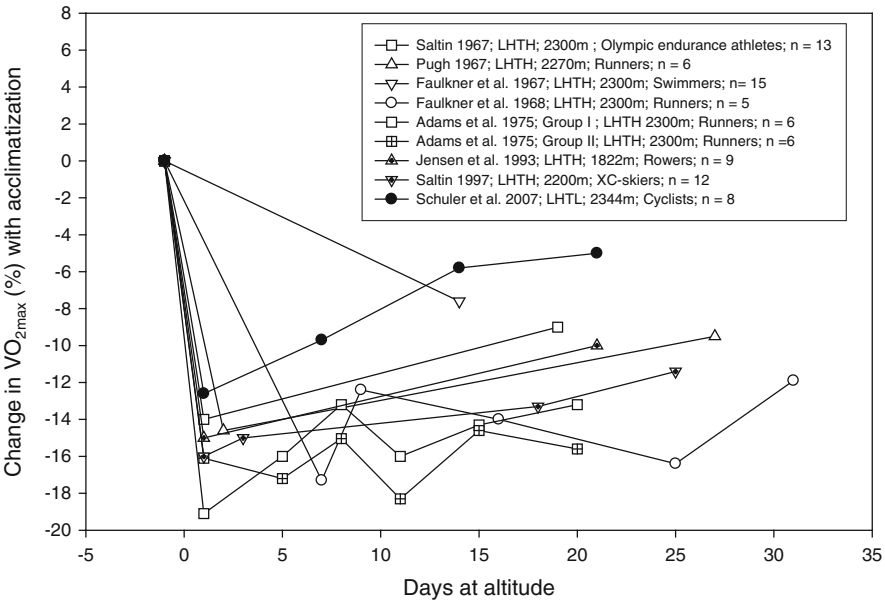

Fig. 24.3 Changes in maximal oxygen uptake (VO_{2max}) in relation to time spent at real moderate altitude in studies with a live high–train high (LHTH) [1, 18, 19, 43, 62, 70, 71] or a live high–train low (LHTL) [78] design in elite endurance-trained athletes

This result is more than the compensated 30 % in the LHTH studies at similar altitudes and raises the question if the LHTL concept should also be used to prepare for competition at altitude? This interesting question remains to be investigated. In summary, the results showed that VO_{2max} in elite endurance athletes in acute altitude exposure is linearly reduced by about 6–8 % per 1000 m increasing altitude from sea level to about 3000 m. This reduction was highly correlated with the decrease in SpO_2 and of course, with the increase in relative training intensity for the same absolute running speed. With living for 2–3 weeks LHTH acclimatization, the initial deficit in VO_{2max} can be reduced by about 1/3, whereas this deficit has been shown to be reduced by about 50–70 % with the LHTL approach.

24.3 Part II: Effect of Training and Altitude Exposure on Hb_{mass} and RCV

24.3.1 Methodological Aspects

By its nature, blood volume compartments can't be measured directly. All the known methods for blood volume compartment determinations are based on the dilution principle and are more or less directly. However, until 1990 only few data on Hb_{mass} and RCV for training and altitude conditions were available because the prevailing direct determination methods at that time were based on radioactive markers like the ^{51}Cr or ^{99}mTc method and were associated with considerable side effects. The T-1824 method (called Evans blue dye) was another method often used to determine plasma volume and then calculate RCV with the help of hematocrit values but this is also an invasive technique. The CO-rebreathing modified by Thomsen et al. [84], Burge and Skinner [9], and Schmidt and Prommer [77] provide the possibility to measure Hb_{mass} noninvasively directly without any side-effects. However, when comparing results of different training studies in normoxia and hypoxia, one has to take into account that not all techniques have the same precision and are suitable to answer training or hypoxic related questions. In their meta-analysis, Gore et al. [31] concluded that the CO-rebreathing method with a mean error of 2.2 % (90 % confidence interval 1.4–3.5 %) and the ^{51}Cr Method with a mean error of 2.8 % (90 % confidence interval 2.4–3.2 %) are the best measures for research on blood-related changes in oxygen transport and research. The T-1824 (Evans Blue) technique with a mean error of 6.7 % (90 % confidence interval 4.9–9.4 %) should only be used with care for clinical applications. Results from earlier studies with the Evans Blue technique should therefore be interpreted with care. In addition, the Evans blue technique has been questioned for estimating RCV after hypoxic exposure because of possible albumin leakage after exposure to altitude that would result in false high RCV values [2].

24.3.2 Effects of Sea Level Training on RCV and Hb_{mass}

Higher Hb_{mass} and BV in endurance athletes have been frequently assumed to be due to erythropoietic adaptation to the training process. Sawka et al. [74] concluded that exercise training, less than 11 days, leads to no change in RCV and that exercise training of more than 21 days leads to an increase in RCV of about 8 %. The conclusion that RCV did not change within 11 days seems to be clear. Several studies showed no increase after 10–12 days endurance training [13, 33, 34]. However, in our opinion, it is not clear that RCV increased after 21 days: with one exception, all other studies, which used radioactive isotope methodologies, showed no increase in RCV. In the study of Green (RCV measured with ^{51}Cr method), RCV did not increase after 4 weeks endurance training [34]. This is supported by the results of Ray et al. [63], where the subjects either trained for 8 weeks in a supine or an upright position and RCV did not increase (RCV measured with the ^{99}mTc method). Also Shoemaker failed to provoke changes in RCV with the ^{51}Cr method [79]; RCV was unchanged after 3, 6 and 11 weeks of endurance training. Only Remes et al. [64] (^{51}Cr method) reported that a group of 30 subjects increased RCV 4.1 % after 6 months of military training. Further evidence that 3–4 weeks endurance training does not increase RCV or Hb_{mass}, comes from a series of recent experiments where the Hb_{mass} has been measured with the "new" CO-rebreathing method. Gore et al. [28] reported, that neither endurance training for 4 weeks in a cold nor hot environment increased Hb_{mass} in male and female endurance athletes. Also 12 weeks of rowing in elite endurance athletes did not increase Hb_{mass} [28]. These findings are in line with our recent study, where national team cross-country skiers did not increase Hb_{mass} with 5 month of endurance training (Wehrlin et al. [86]), 1 year of endurance training in adolescents [17] and even 3 years of endurance training in national team cross-country skiers (see Fig. 24.4). Interestingly, the studies that measured RCV with the Evans blue-dye technique (T-1824) increased RCV after endurance training: Schmidt et al. [75] reported an 8 % increase in RCV after 3 weeks and Wartburton et al. [85] showed an increase of +12.5 % in an interval training group and +11.5 % in a continuous training group, but subjects increased RCV already in the first training week by about 8 %. This means in absolute values, that RCV in the interval group increased 24 ml/kg from the pretest, to 26 ml/kg in week 1 and 27 ml/kg in week 12, while the continuous training group started with 26 ml/kg and increased to 28 ml/kg after 1 week and 29 ml/kg after 12 weeks; this seems to be questionable. However, Schmidt and Prommer [76] reported recently an increased (6.4 %) Hb_{mass} in relatively untrained subjects after training 9 months for a marathon.

In summary, when measurement technique is taken into account, it seems it is very difficult to increase Hb_{mass} and RCV with normoxic endurance training, especially for already endurance trained athletes.

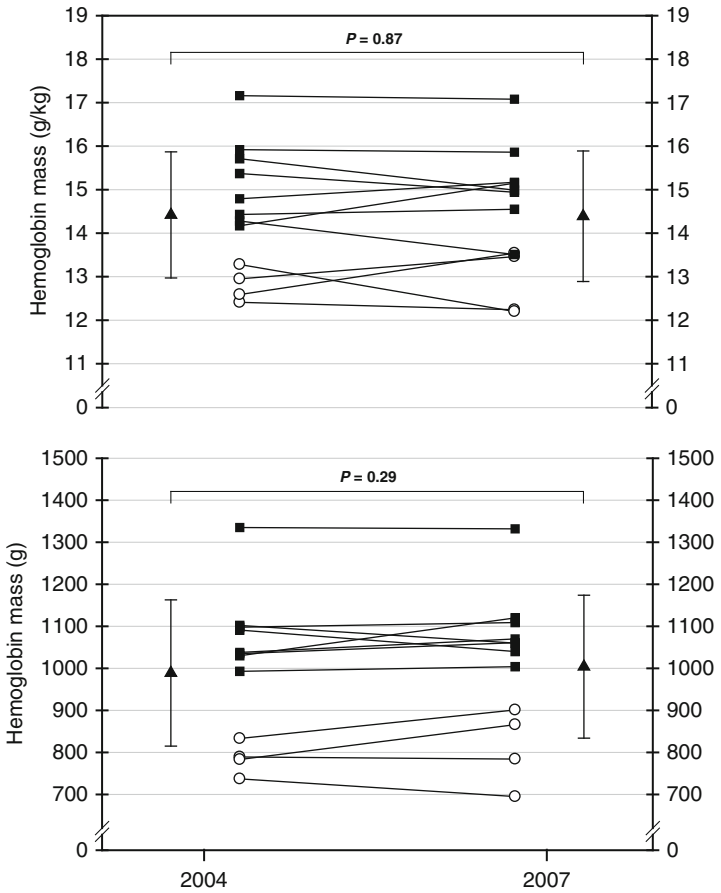

Fig. 24.4 Effect of 3 years endurance training between 2004 and 2007 on total (g) and relative (g/kg) hemoglobin mass in 12 Swiss national team cross-country skiers. ○ represent female, ■ male athletes and ▲ represent mean values ± SD. *P* indicates the *P*-value

24.3.3 Hemoglobin Mass: Effects of Moderate Altitude Exposure in Elite Endurance Athletes

When we started our experiments in 2002, it was quite unclear if a normal LHTL camp increases RCV and Hb_{mass} because the different studies showed controversial results: in the classic, carefully controlled, LHTL study conducted by Levine and Stray-Gundersen [49], RCV increased by ~5 % in the LHTL group after living for 4 weeks at 2500 m and training at 1250 m. These results were questioned [2] however, because they measured RCV indirectly with the Evans blue dye method and there have been doubts about the adequacy of this method for estimating RCV after hypoxic exposure [2, 35], and they reported similar increases in RCV in the 4-week

sea-level training phase and even a decrease in RCV in the control group [49]. However, at first glance, the effect of LHTL on RCV and Hb_{mass} was confusing. In some studies, Hb_{mass} and/or RCV was increased after real [49] and artificial LHTL altitude training camps [46, 67] while other studies reported no change after LHTL with real [15], artificial [2, 3] as well as LHTH at real altitude [24, 27, 28, 81, 82]. Ashenden pointed out, that with one exception [27] all studies using the Evans blue dye method showed an increase in RCV whereas all studies using the CO-rebreathing method to directly determine Hb_{mass} showed no increase in Hb_{mass}. However, when looking at the "hypoxic dose" (living altitude combined with the duration of altitude exposure) it was obvious, that most studies that showed no increase in Hb_{mass} and RCV used a lower hypoxic dose than the studies that reported increases in Hb_{mass} and RCV. We therefore started a controlled study with elite endurance athletes (national team orienteers and cross-country skiers) to measure the changes in Hb_{mass} and RCV with the CO-rebreathing method and an estimated adequate hypoxic dose similar to that used by Levine and Stray-Gundersen [49]. The orienteers (altitude group) completed a 24-day LHTL phase living 18 h per day at 2456 m and training at 1800 and 1000 m above sea level in the Swiss Alps. The cross-country skiers (control group) completed a normal training phase, which consisted of living and training between 500 and 1600 m for 24 days. Indeed, Hb_{mass} and RCV were increased by 5.3 and 5 % ($p < 0.01$) in the orienteers whereas there was no change in Hb_{mass} and RCV of the cross-country skiers. The changes in Hb_{mass} and RCV were different between the groups ($p < 0.01$) [89]. Because another theory [27] to explain the failure to increase Hb_{mass} with LHTL was that the athletes in the studies with increased Hb_{mass} were not "world class," our two best world class runners (we did not have more) also performed a LHTL training camp. They lived for 26 days at the same place (Muottas Muragl, Engading valley, Switzerland) at 2456 m and trained at 1800 m. Hb_{mass} (+3.9 and +7.6 %) and RCV (+5.8 and +6.3 %) were increased [87] indicating that it is possible to increase Hb_{mass} and RCV in world class athletes.

In Fig. 24.5, we include the results of all studies in which endurance-trained athletes participated in either a LHTH or LHTL altitude training camp and measured Hb_{mass} and/or RCV [2, 3, 8, 15, 23, 27, 36, 46, 49, 54, 60, 65–67, 72, 73, 81, 86, 87, 89]. At first glance, the effect of LHTL and LHTH on Hb_{mass} and RCV is confusing reaching from no effect to increases in Hb_{mass} and RCV of about 10 %.

We grouped the studies according to the hypoxic doses they used (hours spent at altitude) (Fig. 24.5). Group A [2, 3, 15, 54, 65, 72] includes the studies where the athletes spent about 100–300 h at altitude and reported no change in Hb_{mass} or RCV. The studies in group B [23, 37, 46, 49, 67, 73, 86, 87, 89] include athletes who spent between 350 and 550 h at altitude and whose Hb_{mass} or RCV increased about 4–7 %. Group C is the LHTH group from the classic Levine and Stray-Gundersen study [49] in which athletes spent about 700 h at altitude and RCV increased by 10 %. In group D, athletes spent between about 500 and 750 h at altitude, but Hb_{mass} remained unchanged [27, 60, 81]. In group E, the athletes spent only about 200–250 h at altitude, but Hb_{mass} was increased by 8–10 % [8, 66]. Based on the fact that Hb_{mass} in lifelong residents [37] of moderate altitude

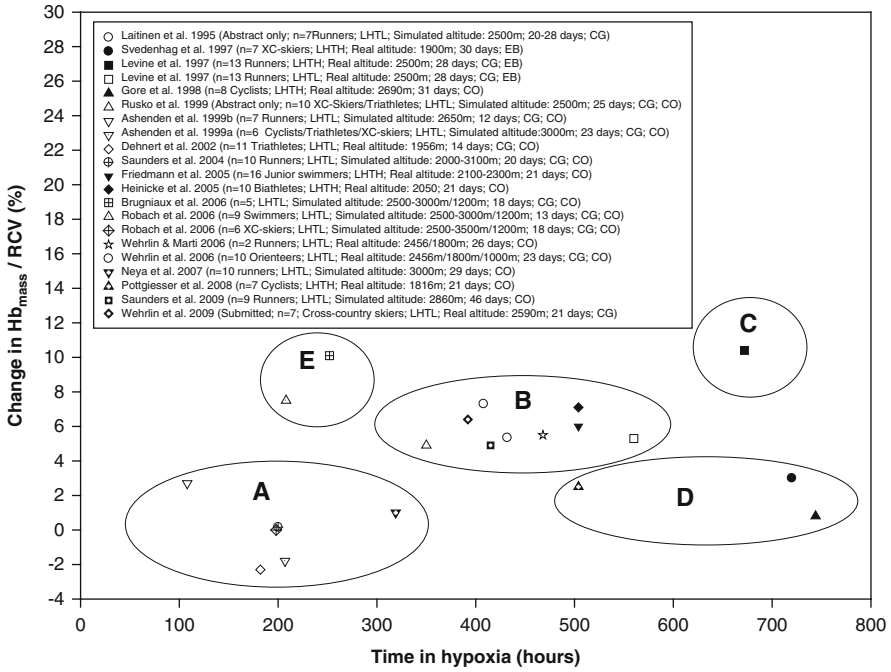

Fig. 24.5 Change in hemoglobin mass (Hb_{mass}) or red cell volume (RCV) in relation to time spent at altitude in studies with endurance-trained athletes RCV [2, 3, 8, 15, 23, 27, 36, 46, 49, 54, 60, 65–67, 72, 73, 81, 86, 87, 89]. Reported are: number of subjects (*n*), the sport, the type of altitude training (LHTH = live high–train high; LHTL = live high–train low), the nature of the altitude (real or simulated), the living altitude, days spent at altitude, use of a control group (CG) and technique used for measurement of Hb_{mass} or RCV (EB = Evans blue dye; CO = carbon monoxide rebreathing). A, B, C, D and E refer to the text

(2600–3550 m), including athletes [7, 76], is elevated, it has been suggested [48, 68, 69] that moderate altitude increases Hb_{mass} and RCV and that the "hypoxic dose" (living altitude combined with the time spent at altitude) used in altitude training plays a major role in whether or not Hb_{mass} and RCV are increased. Rusko et al. [68] concluded, that the minimum dose necessary to attain a hematological acclimatization is >12 h per day for at least 3 weeks (about 250 h) at an altitude of 2100–2500 m. Wilber, Stray-Gundersen, and Levine recommend to live for 4 weeks, >22 h per day at an altitude between 2000 and 2500 m [91]. As expected, there is a clear dose-response relationship between the groups A, B, and C in Fig. 24.5. In group A, the hypoxic dose was probably too low, whereas the hypoxic dose (350–550 h at 2100–2600 m) in group B was high enough to increase Hb_{mass} or RCV by about 5 %. The results of Group C results indicate that Hb_{mass} and RCV can be increased further with a higher hypoxic exposure, as shown by Heinicke et al. [37], where Hb_{mass} was increased by 11 % after a 6-month exposure to 3550 m in soldiers.

In group D, Hb_{mass} was unchanged despite the fact that the athletes in the two studies [27, 81] spent more than 700 h at altitude. In the first study [81], the athletes spent 30 days LHTH at an altitude of 1900 m, an altitude which might be too low to cause an increase Hb_{mass} and RCV. In the second study, Gore et al. [27] reported no increase in absolute Hb_{mass} after 31 days LHTH at 2690 m, though the authors pointed out that all athletes succumbed to illness during the period, which can have depressive effects on erythropoiesis [25]. Finally, group E showed a Hb_{mass} increase of 8–10 % with a relatively low hypoxic dose. The nine AG athletes in the study by Robach et al. [66] lived at simulated altitudes between 2500 and 3000 m for only 13 nights (16 h per day). However, the reproducibility of the method used to determine Hb_{mass} was not investigated and one athlete increased Hb_{mass} by 31 %, which seems to be an unnaturally high increase in after only 13 days at altitude. The mean increase would have been reduced to about 4.7 % when excluding the result of this athlete. In the study by Brugniaux et al. [8] five athletes from the AG lived for 18 days at simulated altitudes between 2500 and 3000 m. Hb_{mass} increased by 10.1 % and RCV was elevated by 9.2 % though the latter result was not statistically significant. Visual analysis of the individual RCV data showed that two of five athletes increased RCV by 20–30 %, which also seems to be unnaturally high. In both studies, a low amount of CO (44 and 49 ml) was used. In endurance athletes with high absolute Hb_{mass} and RCV this will lead to a very low ΔCOHb and low reproducibility of measurement [9].

In summary, we conclude that one altitude training period (LHTL) with a hypoxic dose of living more than 400 h at an altitude of about 2300–2500 m can increase Hb_{mass} and RCV. A lower hypoxic dose may have little or no effect on erythropoiesis. Hb_{mass} and RCV can even be increased in world class athletes with already high Hb_{mass} and RCV levels.

24.3.4 Hemoglobin Mass: Effects of Long-Term Living at Moderate Altitude in Elite Athletes

There is no doubt that moderate altitude residents posses higher Hb_{mass} than comparable habitants from lowland. Schmidt and Prommer [76] recently performed a Meta-Analysis (with their own data) where they with a cross-sectional design compared Hb_{mass} in sea-level and altitude (2600 m) resident subjects, subdivided in four groups characterized by different VO_{2max}. In all male groups, Hb_{mass} was between 9 and 14 % higher in the altitude than in the sea level groups, and a similar picture was found for the females with slight differences [76]. The higher RCV was compensated by a lower plasma volume that resulted in similar blood volumes in altitude and sea level resident subjects. It remains to be investigated whether endurance athletes reach these values with a series of LHTL camps.

24.4 Conclusions

In acute hypoxia, VO_{2max} is reduced linearly by about 6–8 % per 1000 m increasing altitude in elite athletes from sea level to 3000 m, with corresponding higher relative training intensities for the same absolute work load. With 2 weeks of acclimatization, this initial deficit can be reduced by about one half. In elite endurance athletes, Hb_{mass} is not increased with years of normal sea-level endurance training. However, when exposed for more than 400 h to altitudes between 2300 and 2500 m, Hb_{mass} increases temporarily by 5–6 %. This effect size is smaller than the reported 10–14 % higher Hb_{mass} values of endurance athletes living permanently at 2600 m.

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Chapter 25
Does the Sympathetic Nervous System Adapt to Chronic Altitude Exposure?

Mikael Sander

Abstract During continued exposure to hypobaric hypoxia in acclimatizing lowlanders increasing norepinephrine levels indirectly indicate sympathoexcitation, and in a few subjects serial measurements have suggested some adaptation over time. A few studies have provided direct microneurographic evidence for markedly increased muscle sympathetic nervous activity (MSNA) after 1–50 days of exposure of lowlanders to altitudes of 4100–5260 m above sea level. Only one study has provided two MSNA-measurements over time (10 and 50 days) in altitude (4100 m above sea level) and continued robust sympathoexcitation without adaptation was found in acclimatizing lowlanders. In this study, norepinephrine levels during rest and exercise also remained highly elevated over time. In comparison, acute exposure to hypoxic breathing (FiO_2 0.126) at sea level caused no change in sympathetic nervous activity, although the same oxygen saturation in arterial blood (around 90 %) was present during acute (FiO_2 0.126) and chronic hypoxic exposure (4100 m above sea level). These findings strongly suggest that the chemoreflex-mechanisms underlying acute hypoxia-induced increases in MSNA are sensitized over time. Collectively, the MSNA data suggests that sensitization of the sympathoexcitatory chemoreflex is evident but not complete within the first 24 h, but is complete after 10 days of altitude exposure. After return from high altitude to sea level the MSNA remains significantly elevated for at least 5 days but completely normalized after 3 months. The few MSNA measurements in high altitude natives have documented high sympathetic activity in all subjects studied. Because serial measurements of MSNA in high altitude natives during sea level exposure are lacking, it is unclear whether the sympathetic nervous system have somehow adapted to lifelong altitude exposure.

Keywords Muscle sympathetic nerve activity • MSNA • Sympathoexcitation • Chemoreflex

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25.1 Introduction

Humans encounter hypoxemia due primarily to decreased pulmonary oxygen uptake by virtue of geography (high altitude) or illness (e.g., heart and lung disease). During hypoxemia many of the important counterregulatory mechanisms which minimize development of tissue hypoxia and organ dysfunction revolve around local tissue responses to hypoxemia and chemoreflex engagement, which causes respiratory and autonomic nervous adjustments. The present topical review has the following objectives: First, to present a review of the current knowledge about the pathways and mechanisms controlling autonomic nervous function during hypoxemic challenges; Second, to present and discuss previously published data on the autonomic adjustments to acute and sustained hypoxic breathing in man.

25.2 Pathways in Autonomic Nervous Control During Hypoxemia

The identification and characterization of the oxygen sensors, brainstem centers and efferent neuronal pathways which are thought to be involved in the cardiopulmonary responses to hypoxemia have primarily been accomplished by the use of reductionistic anesthetized animal and ex vivo preparations, and to a minor extent by studying the effects of pharmacological manipulation or surgery in humans. The reflex adjustments to hypoxemia are characterized by changes in the nervous control of ventilation, the parasympathetic and sympathetic output to the heart, and the sympathetic vasoconstrictor drive. The hypoxia-induced reflex adjustments are modulated by increased ventilation both via activation of pulmonary stretch receptors and via resultant decreases in arterial PCO_2. However, a discussion of these modulatory effects has been omitted because of length restraints.

25.2.1 Oxygen Sensors

The sensing of low oxygen in arterial blood or in tissue is a prerequisite for reflex adjustments to hypoxemia. If the hypoxia is severe enough many if not all neuronal pools respond with increased activity. However, the levels of hypoxemia which can be endured by conscious mammals, for example by high altitude sojourn, activates well defined neural oxygen sensors located both peripherally and centrally.

The primary peripheral oxygen sensors are cells of neuronal origin, a subpopulation of the glomus cells or type I cells, located within the carotid and aortic arch bodies [46, 73, 76, 104]. In the hierarchy of the peripheral chemoreceptors, the carotid bodies are considered most important, as denervation of the aortic receptors has minor consequences for the autonomic adjustments to hypoxemia [46, 61]. The

glomus cells directly sense and are progressively activated (depolarized) by decreasing arterial oxygen tension below a threshold of about PiO_2 70–80 mmHg. This activation is initiated by hypoxia-induced inhibition of K-channels and subsequently leads to neurotransmitter-release within synapses formed with the apposed afferent nerve fibers [73, 76, 104]. The precise mechanism(s) of oxygen sensing and the excitatory neurotransmitter(s) are incompletely understood and may vary between species [53, 76]. Recent studies in gene knock-out mice strongly suggest that, at least in mice, ATP-signaling via P2X-receptors are a prerequisite for carotid body hypoxemia-sensing [15, 28, 29]. Hypoxemia leads to increased activity in the afferent fibers from the carotid and aortic arch bodies. Together the glomus cells and the afferent fibers constitute peripheral chemoreceptors. The chemoreceptor afferents directly excite neurons in the nucleus tractus solitarius (NTS) by glutamatergic and purinergic neurotransmission [10, 17, 48, 67, 103] (Fig. 25.1). The adaptive response of the carotid body afferent activity to chronic hypoxia had been studied in animal models. Within the first hour of ongoing hypoxic exposure in anesthetized rabbits direct recordings of chemoreceptor afferent activity during hypocapnic hypoxemia showed some adaptation (the activity at 60 min was 70 % of the activity at 5 min) [56]. However, in general prolonged hypoxic exposure (5 h to 28 days) caused sensitization of the carotid bodies to an acute hypoxic exposure [4, 14, 72, 98]. Overall, the carotid body becomes twice as sensitive to hypoxia within hours of hypoxic exposure and this level of sensitization is retained even after weeks of exposure.

The central oxygen sensors involved in chemoreflexes are located in at least two neuronal pools. First, subgroups of the neurons involved in the brainstem circuitry controlling ventilation are thought to be oxygen sensors. Most notably, the ventral respiratory group (vRG), more specifically the pre-Bötzinger complex within the vRG, is thought to have specific sensitivity for hypoxia [88, 89]. The pre-Bötzinger region in humans has very recently been identified [86]. Second, brain stem neurons located within the nucleus reticularis rostroventrolateralis, also known as rostral ventrolateral medulla (RVLM), have been shown to respond to hypoxia [89, 92, 93]. The RVLM, unlike most surrounding brain stem centers, is served by a well-developed capillary network, providing these cells with the opportunity to at least indirectly sense blood oxygen tension, and in brain stem slice preparations these cells are progressively activated by decreasing oxygen tension below a threshold of about 50 mmHg [92, 93].

If applying the described neural oxygen sensor thresholds decreasing arterial oxygen tension would first activate peripheral (below 80 mmHg) and subsequently (below 50 mmHg) the peripheral and central oxygen sensors would become activated in concert. During moderate systemic hypoxemia, where both peripheral and central chemoreceptors are normally activated, it has been demonstrated in animal and human models that engaging the central oxygen sensing neurons alone are able to produce substantial reflex adjustments to hypoxemia. However, some differentiation between these reflex responses seems to exist and this differentiation may also be species specific. Thus, peripheral (carotid body) deafferentation only slightly limits cardiac and vascular responses, although it may limit or even prevent

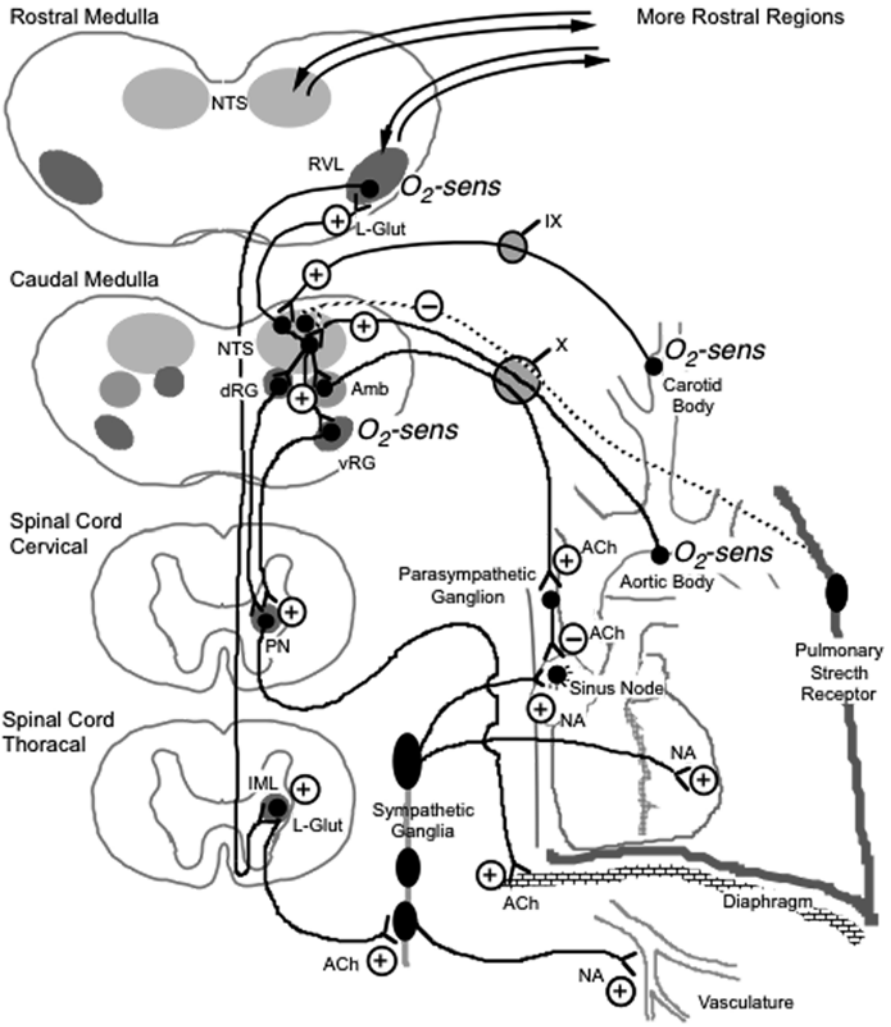

Fig. 25.1 Model of the primary neuronal circuitry involved in the autonomic nervous adjustments to hypoxemia. "+" denotes excitatory and "–" inhibitory neurotransmission. On the *left* are sketched brainstem and spinal cord slices at different levels, on the *right* the heart, lungs, and vasculature. For clarity, the afferent input to the NTS is depicted only in caudal medulla, whereas in reality the input is divided to also innervate NTS in the rostral medulla. O_2-sens, neural oxygen sensor; IX, glossopharyngeal nerve; X, vagal nerve; PN, phrenic nuclei and nerve; Amb, nucleus ambiguus; dRG and vRG, dorsal and ventral respiratory group; NTS, nucleus tractus solitarius; RVL, rostral ventrolateral reticular nucleus; IML, intermediolateral cell column; L-Glut, L-glutamate; Ach, acetylcholine; NA, noradrenaline

hypoxemia-induced ventilatory responses [19, 33, 34, 60]. In a study in conscious dogs, peripheral "deafferentation" was accomplished by extracorporeal normoxemic carotid body perfusion [19], and central nervous hypoxia still caused a significant hyperventilatory response. In contrast, in humans experimental "deafferentation" by bilateral local anesthetic blockade of the carotid body afferents prevented hypoxemia-induced hyperventilation [33, 34]. In animals, the dependence on the peripheral chemoreceptors seems to diminish over time after severing the peripheral afferents. Thus, in conscious carotid body denervated rats, the overall hypoxia-induced ventilatory response was initially only 35 % of sham-controls (after 2 days), but fully recovered over time (45 days) [61]. In contrast, patients having undergone bilateral glomectomy (carotid body removal) as a (now abandoned) experimental treatment for asthma or chronic pulmonary disease or to remove tumors have been tested both acutely and years after surgery and apparently do not regain hypoxia-induced ventilatory responses [57, 94, 100]. However, bilateral carotid body tumorectomy apparently does not prevent hypoxemia-induced increases in muscle sympathetic nervous activity [95].

25.2.2 Central Neuronal Pools

Ventilation is complex, as it is both under conscious and autonomic control. The autonomic control of ventilatory rhythm and depth is thought to be accomplished by either pacemaker or neural network activity in neurons located in the nucleus ambiguus (nA), dorsal vagal nucleus (DVN), hypoglossal nucleus (HN), and the ventral (vRG) and dorsal respiratory groups (dRG) [77, 84, 85]. Specific preganglionic vagal and hypoglossal neurons exhibit ventilatory rhythms and coordinate upper airway and bronchial relaxation at the onset of inspiration [26]. The respiratory group neurons activate anterior horn motoneurons in the phrenic nuclei (PN) at the level of C3–C5, and the phrenic nerves innervate the diaphragm, which in mammals is the main muscle group creating inspiratory work [27]. Autonomic control is also exerted on thoracic nerves that innervate intercostal muscles and abdominal muscles, that help stabilize the thorax and aid expiration, respectively, exert further autonomic control. The hypoxia-induced activation of these neuronal pools is mediated through several pathways [7, 13]. During hypoxemia, the complexity is further emphasized, since decerebration studies have indicated that mesencephalic and suprapontine centers become involved in the control of respiratory frequency and the coordination between frequency and volume [71].

Parasympathetic neural activity to the heart in larger mammals is primarily controlled by preganglionic vagal neurons located within nA, with minor input from the DVN. Neurons in the NTS receiving afferent input from the peripheral oxygen sensors, project to the nA and thereby provide excitatory afferent input controlling parasympathetic output to the heart during hypoxemia [3]. The DVN and nA neurons are not specifically sensitive to hypoxemia compared to other neuronal pools [21].

Central sympathetic outflow to both the heart and the vasculature is derived from a small population of neurons within the RVLM (in rats about 200 neurons) [78]. These neurons have pacemaker properties, and they receive a barrage of input, including excitatory input from a majority of the chemoreceptor-activated NTS neurons [48], and inhibitory input from the caudal ventrolateral medulla relaying baroreflex activation [66]. As mentioned above, the RVLM neurons, at the same time, are the primary central oxygen sensors. These neurons are thought to integrate relayed peripheral afferent and central nervous input as well as the surrounding tissue oxygen tension and the resultant level of activation determine central sympathetic outflow.

Evidently the chemoreceptor-afferent excitatory input to NTS neurons is a unifying characteristic for the hypoxemia-induced increases in parasympathetic and sympathetic drive. It is likely, that the primary excitatory neurotransmitter in the NTS is L-glutamate, and that both NMDA and AMPA-receptors are involved. In support of this concept, microinjection of L-glutamate into the NTS of conscious rats induces hemodynamic effects consistent with parallel vagal and sympathetic activation [17].

25.2.3 Efferent Neuronal Pathways

In anesthetized rats, the onset of vagal and hypoglossal inspiratory activities always precedes the abrupt start of the phrenic nerve discharge, and the frequency of all three inspiratory nerves increases during hypoxia [26]. In another study, hypoxia (FiO_2 0.10) induced increases in minute ventilation, which were unaffected by atropine [8]. In anesthetized rabbits hypoxia also causes an increase in both respiratory frequency and tidal volume, and the latter was fully dependent on carotid body function [47]. In conscious ponies, hypoxia (FiO_2 0.12) caused an increase in ventilation that was largely dependent on intact carotid body function [11].

The preganglionic parasympathetic neurons of the nA (and DVN) project via the vagus nerve to the peripherally located parasympathetic ganglia of the heart. In these ganglia release of acetylcholine activates nicotinic receptors on the postganglionic parasympathetic neurons. The vagal input to the heart is directed to the sinus node and other parts of the conduction system but not to the vasculature or myocytes. In the heart, vagal activation causes release of acetylcholine which acts on muscarinic receptors and decreases frequency of depolarisation in the cardiac pacemaker cells. The vagal activity and heart rate responses to hypoxia, before and after muscarinic blockade, have been studied in animals of different sizes. In rats, hypocapnic hypoxia (FiO_2 0.08) elicited decreases in heart rate which were abolished by the muscarinic receptor antagonist, atropine [5]. In fetal lambs, hypoxia (FiO_2 0.10 for the ewe) causes bradycardia, which can be prevented by atropine [16]. It is likely that the bradycardia seen in response to hypoxia in small animals and in utero in larger animals are mediated by increased parasympathetic activity and may correspond to the depression of heart rate induced by stress in human

fetuses [60]. In larger animals and humans hypoxia leads to increases in heart rate [60]. These differential responses may be related to the different balance between sympathetic and parasympathetic control of heart rate at baseline in smaller (more sympathetically controlled) and larger mammals (more vagally controlled). In support of this interpretation, the lowering of maximal heart rate during exercise after sojourn in altitude seems to be related to an increased parasympathetic tone (in a situation where the heart normally would be under sympathetic control) [9].

The sympathetic outflow from the brainstem originates in the RVLM. Each of the few hundred RVLM neurons project to several neurons within the intermediolateral cell column (IML) of the spinal cord, where the preganglionic sympathetic neurons are activated by glutamatergic neurotransmission. In the sympathetic ganglia, the preganglionic fibers release acetylcholine which via nicotinic receptors activates postganglionic sympathetic fibers supplying the heart and vasculature. Peripherally postganglionic sympathetic nerves primarily release norepinephrine, which cause beta-adrenoceptor-mediated excitation of the sinus node and conduction system of the heart, and alpha-adrenoceptor-mediated increases in cardiac contractility and vasomotor tone.

Co-activation of the antagonistic sympathetic and parasympathetic fibers innervating the heart has been demonstrated during hypoxemia by direct nerve recordings in the anesthetized rat [27] and by pharmacological and surgical inhibition in the cat and dog [32, 74], and in all these studies sympathetic efferent nerve discharges tended to predominate causing an increased heart rate. In conscious rats and dogs, systemic hypocapnic hypoxemia also caused an increase in heart rate [40, 49]. One may speculate that co-activation of cardiac sympathetic and parasympathetic fibers might be needed to optimize the cardiac work–metabolism relationship during hypoxemia. The sympathetic activation increase contractility, but the parasympathetic activation keeps heart rate low and provide the time for cardiac muscle oxygenation during diastole and for optimal diastolic filling [50].

Direct recordings of postganglionic sympathetic activity has documented hypoxia-induced increases in sympathetic nerve activity of the renal nerve in anesthetized as well as conscious rats and pigs [27, 40, 102], and directed to the hindlimb muscle vasculature in cats [30]. In conscious rats, acute hypoxemia caused a slight increase [40], and 5 days at a simulated altitude of 4350 m caused a tripling in plasma noradrenaline levels [59].

25.3 Autonomic Adjustments to Hypoxic Breathing in Man

The effects of hypoxic breathing in volunteers have been studied in three different ways. First, in low-altitude laboratories, a frequently used approach is mask or mouthpiece breathing of gas-mixtures with different fractions of oxygen (FiO_2) for minutes to hours (laboratory studies). Second, in hypobaric chambers, subjects breathe ambient "chamber"-air in which the tension of oxygen (PIO_2) is determined by the adjustable levels of hypobaric pressure (chamber studies).

Third, in high altitude laboratories, low-altitude residents sojourning for days to weeks or high-altitude residents residing for months to life breathe ambient air, in which PIO_2 is predetermined by the level of hypobaric pressure (altitude-studies). The autonomic adjustments to acute and chronic exposure to hypoxic breathing in humans are many and include changed neural control of hormone production and metabolism. The remainder of this review focuses on the hypoxia-induced ventilatory and sympathetic vasoconstrictor responses in humans, leaving out heart rate responses because of manuscript length restraints. The chemoreflex control of ventilation and sympathetic vasoconstriction share several communalities with regards to the anatomical organization as outlined above. However, within the last few years data are emerging to suggest that hypoxia-induced increases in ventilation are dependent upon an intact carotid body, whereas hypoxia-induced increases in sympathetic vasoconstrictor drive is not dependent on the carotid body. It is evident from the presented data below, that chronic exposure to hypoxia is accompanied by a robust augmentation of ventilation and sympathetic drive compared to acute hypoxic exposure. In the latter part of the review some of the potential mechanisms responsible for this sensitization will be presented.

25.3.1 Ventilatory Responses

In humans the ventilatory response to acute hypoxic breathing in laboratory studies is characterized by increased respiration which is primarily related to an increased tidal volume with only a minor increase in respiratory frequency [22]. In the classic dose–response study by Dripps and Comroe [22], minute ventilation was demonstrated to increase with severity of hypoxemia. In another classic study, FiO_2 of 0.06, 0.05 and 0.04 (An FiO_2 of 0.06 is equivalent to the top of Mount Everest) caused even further increases in respiration, now accomplished by both increased tidal volume and frequency [41]. No later studies have described the magnitude of the hypoxemia-induced hyperventilation in man better than the combination of these studies. However, microneurographic studies are inherently good for evaluating other cardiovascular responses to hypoxic breathing at rest, because the method demands relaxation in all larger muscle groups in order not to loose the nerve-recording. In recent microneurographic studies [20, 23, 31, 35, 37, 43, 55, 69, 70, 75, 81–84, 90, 91, 96], FiO_2 around 0.10 caused minute ventilation to increase by a range of 7–115 % above baseline (Fig. 25.2b). In these studies there were no clear correlation to whether or not the hypoxic challenge was accompanied by isocapnia (by titrating the $FICO_2$) or poikilocapnia (by hypoxia-induced hyperventilation). Besides an increase in minute ventilation, it has also been described in laboratory studies that the upper airways dilate in response to isocapnic hypoxemia (Sat 70–85 %), such that both the inspiratory and expiratory airway resistance are decreased [44].

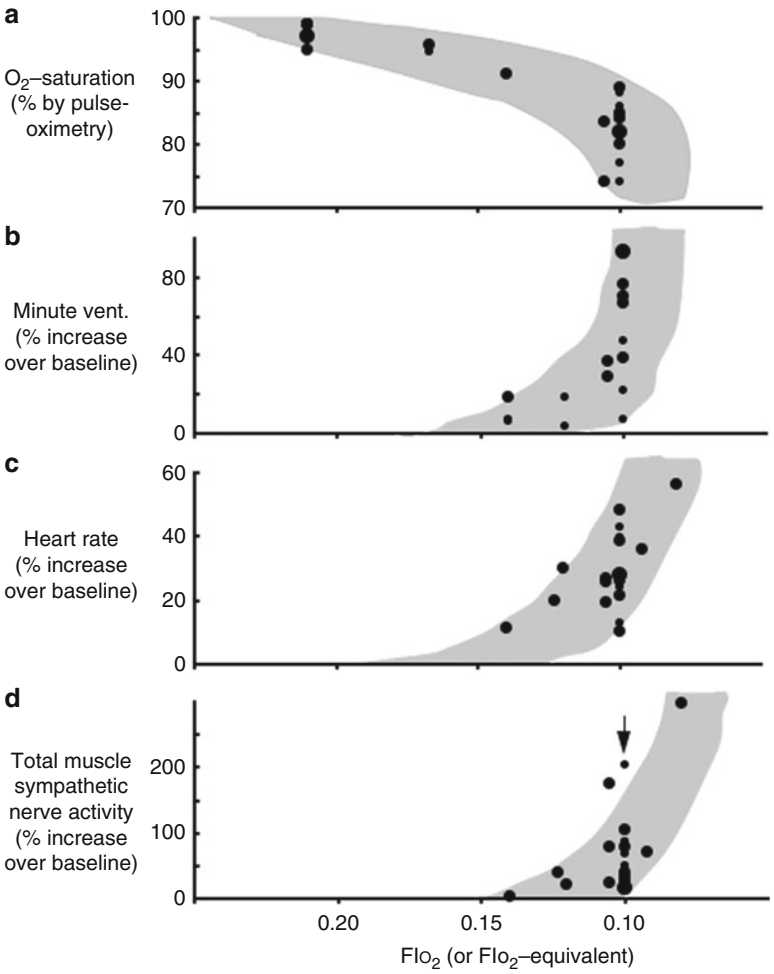

Fig. 25.2 Summary of previously published microneurographic studies of pulmonary and cardiovascular responses to acute hypoxemia in humans. All five panels depict values as a function of FiO_2, some studies investigating more than two levels. Each study is represented for each FiO_2 by a solid circle, the size of which is proportional to the number of subjects included ($n = 6$–17). (**a**) arterial oxygen saturation as measured by pulse oximetry or arterial blood gasses; (**b**) ventilatory response as determined by the increase in minute ventilation (in % above baseline); (**c**) heart rate response (in % above baseline); (**d**) Mean arterial pressure (% change from baseline); (**e**) total muscle sympathetic nerve activity response as measured by microneurography (in % above baseline), there were significant increases in each of the studies at least at the lowest FiO_2 for changes in saturation, ventilation, and mean arterial pressure

In a chamber study, the hyperventilation induced by stepwise increases in simulated altitude to 6000 m was unaffected by the combination of atropine and propranolol [51].

In altitude studies, the ventilation also increases during the first hours of exposure; a small decrease may then occur during the first 24 h, but subsequently the ventilation seems to increase further as judged by continued lowering of the arterial PCO_2-levels until quite low levels PCO_2 are reached. It should be mentioned that it has been difficult to demonstrate a continued increase in ventilation by direct measurements of ventilation in the supine resting human (which is in contrast to the ease of measuring an increased ventilation in acute hypoxia studies). In a large study, high altitude residents (3800–4065 m) of the Andes and the Himalayas exhibited significantly higher resting ventilation than sea-level controls [6].

25.3.2 Adjustments of Sympathetic Vasoconstrictor Drive

Sympathetic vasomotor tone during hypoxemia has been determined by two different approaches. First, sympathetic nervous activity can be directly recorded by nerve puncture and intraneural microneurographic recordings of postganglionic activity. Second, whole body sympathetic nervous activity can be indirectly estimated by measurement of norepinephrine concentration in plasma or urine, or changes in whole body as well as local neurotransmitter uptake/release can be determined by noradrenaline spill-over measurements in the body, a limb, or an organ. Both approaches have inherent strengths and weaknesses. Microneurography offers reproducible, specific and sensitive measurements of regional sympathetic nerve activity with excellent time-resolution for responses to acute interventions. However, the technique is technically demanding making field studies troublesome, and specifically studies during exercise of larger muscle groups are very difficult. As a safety precaution the same nerve should not be punctured twice inside 4 weeks, which provides important restraints on repeated measurements in the same subjects. Norepinephrine spill-over also gives reliable determinations of whole body or regional sympathetic activity. Norepinephrine spill-over in the forearm or leg often correlates well with direct recordings of muscle sympathetic activity [39, 55]. However, this method requires an intra-arterial line, which also provides important restraints on repeated measurements in the same subjects. Noradrenaline concentration in both plasma and urine has been used for decades and has the advantage that the data is easily collected daily. However, the method is not very specific or sensitive for detection of acutely released noradrenaline, since the concentration also depends on the rates of plasma clearance due to excretion, metabolism and specific reuptake. These factors result in high intra- and interindividual variability of both plasma and urine concentrations. The use of noradrenaline measurements is further subject to the specific problem that hypoxemia has been reported to increase noradrenaline clearance [54].

In laboratory studies and a few chamber studies, acute hypoxemia causes sympathetic excitation as measured by microneurographic recordings from the peroneal nerve during supine rest [20, 23, 31, 35, 37, 43, 55, 69, 70, 75, 81, 84, 90, 91, 96] (or from the tibial nerve during sitting rest [82, 83]) (Fig. 25.2e). Microneurographic data may be acquired from a mean voltage integrated neurogram by counting the number of bursts per minute or determining the sum of the burst amplitude per minute (termed total activity, and often expressed as %-change from baseline). The latter is considered the most sensitive measure of minute-to-minute changes in sympathetic activity. It is evident from Fig. 25.2d, that all studies find increases in total sympathetic activity, but equally evident that this sympathetic response is variable. The sympathetic response to a FiO_2 of 0.10 ranges from 15 to 204 % above baseline. The studies are relatively uniform in protocol regarding microneurography-method and protocol time-sequence, since all studies have used 5–30 min of hypoxic exposure on each level of FiO_2. The explanation for the variability may be partly related to subject differences with regard to gender (only Jones et al. studied a group of women) [43], age (Davy et al. studied a group of elderly men) [20], or residential altitude (Rowell et al., Davy et al. and Jones et al. performed their baseline studies at 700–1700 m above sea level) [20, 43, 81]. In these studies, hypoxemia-induced hyperventilation resulted in hypocapnia (poikilocapnic approach), unless isocapnia was accomplished by $FICO_2$-titration. No apparent differences in muscle sympathetic nerve responses are noted for poikilocapnia vs. isocapnia between studies. The issue has been addressed directly by comparing the same subjects during successive hypoxemia/isocapnia and hypoxemia/hypocapnia [90]. The authors concluded that hypocapnia does not significantly alter the hypoxemia-induced changes in sympathetic tone.

One microneurographic study has failed to find a significant increase in muscle sympathetic nerve activity during hypoxic breathing of FiO_2 of 0.10 with concurrent isocapnia in two groups of healthy young subjects ($n=7$ and $n=8$) (not included in Fig. 25.3 since the actual data were not reported in the original article) [87]. In this study, the period of hypoxemia was brief, and in other studies by the same group it has been convincingly described that there is a delay from the start of hypoxic breathing to measurable changes in muscle sympathetic nerve activity. This time-delay is undoubtedly related in part to the time required for arterial and tissue hypoxemia to develop.

In laboratory studies and chamber studies the hypoxemia-induced effects on resting norepinephrine levels have been determined in several studies (e.g., [25]). The majority of these studies have been reviewed recently [79]. Out of 14 studies of acute hypoxemia, only one study has reported significantly elevated norepinephrine levels during acute hypoxemia, and that study also showed hypoxemia-induced increases in forearm norepinephrine spill-over and peroneal nerve sympathetic activity [55].

High altitude field studies of muscle sympathetic nervous activity are rare. I am aware of four studies, but so far only two publications [23, 36]. In the first published report microneurography was performed in a high altitude laboratory (Monte Rosa 4559 m) 18–24 h after subject arrival to altitude [23]. This study had specifically

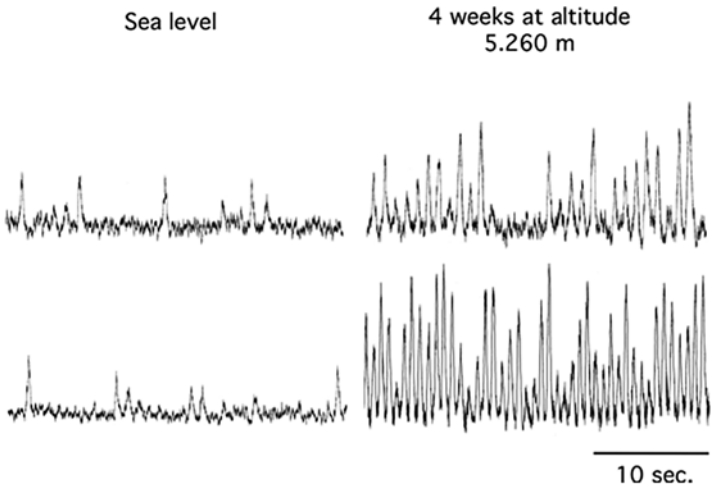

Fig. 25.3 Exemplary microneurographic recordings showing muscle sympathetic nerve activity from the peroneal nerve in two subjects during normoxemia (Copenhagen, Denmark, sea level) and during prolonged hypoxemia induced by high altitude exposure (Chacaltaya, Bolivian Andes, 5260 m above sea level; original records from Hansen and Sanders Chacaltaya studies)

selected two groups on the basis of susceptibility ($n=6$) or resistance ($n=5$) to high altitude pulmonary edema (HAPE). Sympathoexcitation on the mountain was evident as determined by an increased burst-frequency in both groups, however, the change in HAPE-prone subjects was significantly higher than in the HAPE-resistant subjects, raising the possibility that HAPE could be caused in part by sympathetic vasomotor drive to the lung. Only one other field study performed in high altitude has been published [37]. In that study, eight lowlanders (living in Copenhagen, Denmark, <50 m above sea level) acclimatized to 5260 m above sea level (Chacaltaya, Bolivian Andes) for about 4 weeks. While the subjects acclimatized well as judged by their ability to do physical exercise, every subject was demonstrated to have extremely high sympathetic traffic (an average of about 300 % of sea level values) (Fig. 25.3). More recently, a few abstract presentations have described that even moderate altitude (4100 m of El Alto, La Paz, Bolivia) causes robust increases in sympathetic activity without any apparent adaptation during the first 8 weeks of sojourn, and in that same study high sympathetic nervous activity was found in seven out of seven high altitude native males of La Paz, Bolivia (studied in El Alto) (Sander, unpublished results).

In high altitude studies, several expeditions have measured norepinephrine plasma or urine levels [2, 12, 18, 45, 62–65, 77, 79] (Fig. 25.4). These studies have primarily been carried out at altitudes between 4200 and 4559 m. During the first days at high altitude norepinephrine levels are not easy to predict, as they may increase or decrease. However, after the first days there is a relatively uniform increase in norepinephrine, which at altitudes below 5000 m perhaps wear of after the first 1–2 weeks (although data on the long-term effects are scarce). Two studies

Fig. 25.4 Summary of previously published data on resting noradrenaline plasma levels (as %-change from sea level values) as a function of time in high altitude. The majority of the studies (*solid line* and *circles*) have been performed at altitude between 4200 and 4559 m. One study was performed at 6542 m (*arrow*)

have examined norepinephrine levels at higher altitudes. During 3 weeks at 6542 m, norepinephrine plasma concentrations were higher and with a seemingly more sustained increase compared to lower altitudes [2]; and after 8 weeks at 5260 m, norepinephrine levels were three-times higher than sea level values [12] (Fig. 25.4). Similarly, in a study of Indian soldiers stationed for 12 weeks at 6000 m, norepinephrine plasma concentration was three times above normal after 10 weeks [1]. A few studies have determined norepinephrine spill-over [12, 63] and report clear increases in norepinephrine spill-over after 3 weeks at 4300 m and an even larger increase after 8 weeks at 5260 m.

25.3.3 Relations Between Sympathetic Activity, Blood Pressure, and Flow During Hypoxemia

It is somewhat controversial to which degree properties of the arterial baroreflex are changed by hypoxemia. In animals, hypoxia-exposure induced simultaneous increases in blood pressure and directly recorded cardiac and renal sympathetic nerve activities in anesthetized rabbits which provides evidence for hypoxia-induced resetting of the baroreflex [42]. Conversely, arterial baroreflex activation inhibited the chemoreflex-mediated vascular responses in anesthetized dogs [58].

In patients with angina pectoris resistant to medical therapy, bilateral carotid sinus nerve stimulation through surgically implanted electrodes undoubtedly activates both baroreceptor and chemoreceptor afferents. In four such patients afferent stimulation caused a marked decrease in heart rate, muscle sympathetic nerve activity and blood pressure indicating that the baroreflex prevails over the chemoreflex during acute parallel engagement [99]. In healthy volunteers, the arterial baroreflex control of heart rate has been studied by use of neck-suction and pressure before and during an acute hypoxic challenge and was reportedly unaffected by mild to moderate hypoxia [24]. However, in another study arterial baroreflex deactivation inhibited the hypoxemia-induced sympathoexcitation, without inhibition of the hypercapnia-induced or cold pressor-induced sympathoexcitation [91].

The documented increased sympathetic nervous activity during acute and sustained hypoxemia in humans is generally not confounded by baroreflex activation, since blood pressures in most acute studies are unchanged or slightly elevated [20], and in high altitude often significantly elevated [101]. Indeed, it has been argued that the blood pressure elevation by (24 h-monitoring) occurring over time at 4300 m is sympathetically mediated [101]. However, it should be mentioned that one chamber and two laboratory studies have reported relatively large decreases in mean arterial blood pressures once subjects were submitted to severe hypoxia (FiO_2 in the range of 0.07–0.09) [80–82]. This phenomenon is unlikely to be caused by sudden sympathetic withdrawal, since in two of the studies sympathetic traffic was still very high [81, 82], but more likely related to exaggerated hypoxia-induced cardiac dysfunction and peripheral vasodilation including uncoupling of sympathetic vasoconstrictor signal transduction. It is beyond the scope of this paper to discuss the local hypoxia-induced mechanisms leading to bradycardia and decreased cardiac contractility [68, 97] and peripheral vasodilation [38, 60]. However, it is important to understand (1) that the functional significance of sympathoexcitation in response to hypoxemia is counteracted by these local mechanisms; (2) peripheral vasodilation seen during hypoxemia does not exclude simultaneously increased sympathetic vasomotor activity.

In a chamber study, stepwise increases in simulated altitude to 6000 m was accompanied by decreases in leg vascular resistance which was unaffected by the beta-adrenoceptor blocker, propranolol and the muscarinic receptor antagonist, atropine [51]. The authors suggest that the decreased peripheral resistance is related partly to acutely and specifically decreased sympathetic vasomotor activity is due to central nervous hypoxia [51]. However, their data does not support this concept, but merely indicates that the vasodilation is not caused by circulating adrenalin, which causes peripheral vasodilation by binding to beta-adrenoceptors.

In more chronic hypoxemia of disease, the peripheral chemoreceptors have been found to undergo hypertrophy [52]. In conscious rats, 5 days at simulated altitude of 4350 m caused a 30–40 % decrease in plasma-membrane beta-adrenoceptor density in the heart, as measured with a hydrophilic ligand, and a similar reduction in beta-receptor-agonist-induced increase in cAMP in cardiac homogenates [59]. The authors suggest that this decrease primarily is related to internalization of beta-receptors as a more lipophilic ligand indicated no difference in receptor density between hypoxia and control. In high altitude pigs, a lower density of beta-adrenergic and a higher density of muscarinic receptors have been described [54].

25.4 Summary

Autonomic adjustments to hypoxemia in man differ over time. Acutely, the cardiovascular responses seem dominated by increases in ventilation, heart rate, and sympathetic vasoconstrictor tone, without any large changes in blood pressure, cardiac output or peripheral blood flow. During more sustained hypoxemia, such as

acclimatization to high altitude, the adjustments include a continued increase in ventilation and sympathetic vasomotor tone, while heart rate decreases towards baseline values. Blood pressure becomes slightly but significantly elevated, and this increase is likely caused by an altered balance between the sympathetic vasoconstrictor drive and the direct hypoxia-related vasodilation.

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Chapter 26
Integrative Conductance of Oxygen During Exercise at Altitude

José A.L. Calbet, Carsten Lundby, and Robert Boushel

Abstract In the oxygen (O_2) cascade downstream steps can never achieve higher flows of O_2 than the preceding ones. At the lung the transfer of O_2 is determined by the O_2 gradient between the alveolar space and the lung capillaries and the O_2 diffusing capacity (DLO_2). While DLO_2 may be increased several times during exercise by recruiting more lung capillaries and by increasing the oxygen carrying capacity of blood due to higher peripheral extraction of O_2, the capacity to enhance the alveolocapillary PO_2 gradient is more limited. The transfer of oxygen from the alveolar space to the hemoglobin (Hb) must overcome first the resistance offered by the alveolocapillary membrane (1/DM) and the capillary blood ($1/\theta$Vc). The fractional contribution of each of these two components to DLO_2 remains unknown. During exercise these resistances are reduced by the recruitment of lung capillaries. The factors that reduce the slope of the oxygen dissociation curve of the Hb (ODC) (i.e., lactic acidosis and hyperthermia) increase $1/\theta$Vc contributing to limit DLO_2. These effects are accentuated in hypoxia. Reducing the size of the active muscle mass improves pulmonary gas exchange during exercise and reduces the rightward shift of the ODC. The flow of oxygen from the muscle capillaries to the mitochondria is pressumably limited by muscle O_2 conductance ($DmcO_2$) (an estimation of muscle oxygen diffusing capacity). However, during maximal whole body exercise in normoxia, a higher flow of O_2 is achieved at the same pressure gradients after increasing blood [Hb], implying that in healthy humans exercising in normoxia there is a functional reserve in $DmcO_2$. This conclusion is supported by the fact that during small muscle exercise in chronic hypoxia, peak exercise $DmcO_2$ is similar to that observed during exercise in normoxia despite a markedly lower O_2 pressure gradient driving diffusion.

Keywords VO_2max • Oxygen transport • Fatigue • Lung • Diffusing capacity

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26.1 Introduction

In the oxygen (O_2) cascade downstream steps can never achieve higher flows of O_2 than the preceding ones. This process is driven by of O_2 pressure (PO_2) gradients, which should overcome the resistances to O_2 flow from the atmosphere to the mitochondria. Consequently, the end PO_2 after the passage of O_2 across each resistance is the starting PO_2 for the next step in the transfer of O_2. Oxygen conductance may be calculated for each step of the oxygen cascade by dividing O_2 flow through each resistance by the corresponding O_2 pressure gradient. Oxygen flow may be increased by enhancing O_2 diffusing capacity and the O_2 pressure gradient. At any point step of the O_2 cascade, O_2 flow may be increased even when there is a reduction in PO_2 gradients if diffusing capacity is not the factor limiting O_2 diffusion, i.e., if a functional reserve exists in O_2 diffusing capacity. On the other hand, O_2 flow may be also sustained in presence of a limited O_2 diffusing capacity if it is possible to increase the PO_2 gradient.

During whole body exercise the O_2 demand increases with exercise intensity stressing the O_2 transport system to its limits at maximal exercise. When the flow of O_2 arriving to the muscles is insufficient to satisfy the O_2 demand, the oxygen deficit is compensated for by augmenting the anaerobic energy production accelerating the rate of fatigue development [1, 2, 24]. Thus, during exercise, homeostatic responses are designed to secure enough flow of O_2 so as to avoid a mismatch between O_2 delivery and demand. In hypoxia, the O_2 transport system is challenged because the PO_2 gradients driving the flow of O_2 are reduced. At low levels of hypoxia, the homeostatic responses may succeed at maintaining O_2 flow preserving VO_2 and exercise capacity. However, at moderate and high altitude exercise VO_2max and exercise capacity are reduced, even in the acclimatized human. With acclimatization to altitude arterial O_2 content (CaO_2) is increased to values similar or even higher than those observed at rest at sea level [8, 46]. During maximal exercise in chronic hypoxia CaO_2 is similar or just a little lower than that observed at sea level before acclimatization [10]. However, maximal oxygen uptake (VO_2max) improves little with altitude acclimatization [6]. In this article we examine the effects of acute and chronic hypoxia on the oxygen conductance focusing on two regions: the lung and the muscle. We will use the term *severe acute hypoxia* to refer to a level of hypoxia equivalent to altitudes above ~4500 m and the term *chronic hypoxia* for the situation created by permanent residence for at least 2 weeks at altitudes above 3000 m.

26.1.1 Lung Oxygen Conductance

The most critical step in the transfer of oxygen from the atmosphere to the mitochondria is blood oxygenation at the lungs. Pulmonary O_2 exchange depends on the pressure gradient driving O_2 diffusion (P_AO_2-$P_{mean}O_2$, where $P_{mean}cO_2$ is the mean PO_2 in the

lung capillaries) and the O_2 diffusing capacity of the lung (DLO_2), such that O_2 flow (VO_2) is equal to the product of $DLO_2 \times (P_AO_2\text{-}P_{mean}cO_2)$. Since DLO_2 expresses the amount of O_2 that can diffuse in 1 min per mmHg of O_2 pressure gradient this implies that the process of O_2 gas exchange is not instantaneous and may be limited if the contact time between the alveolar air and the capillary blood is too short [52]. With enough time for gas equilibration, in the absence of alveolar ventilation/perfusion (V_A/Q) heterogeneity and shunt, P_AO_2 and PaO_2 would reach the same value. For this reason, the difference between P_AO_2 and PaO_2 ($AaDO_2$) is used to assess the efficiency of pulmonary gas exchange.

26.1.1.1 The Alveolar-to-Mean Capillary PO_2 Gradient ($P_AO_2\text{-}P_{mean}cO_2$, Where $P_{mean}cO_2$)

During exercise in hypoxia, the main contributing factor limiting the O_2 diffusion in the lung is the reduction in P_IO_2 caused by the lower atmospheric pressure. As a consequence P_AO_2 is also reduced and hence, so too is the PO_2 gradient driving O_2 diffusion. When the PO_2 gradient driving diffusion is small, as occurs during exercise in hypoxia, then transfer of O_2 from the atmosphere to the lung capillaries becomes more sensitive to changes in DLO_2 [7]. Although DLO_2 increases linearly with intensity during exercise in normoxia and hypoxia [49], in most humans this increase is insufficient to account for the reduction in $P_AO_2\text{-}P_{mean}cO_2$ gradient and consequently the flow of oxygen is reduced below that observed at sea level. To compensate for the reduced inspiratory O_2 pressure (P_IO_2) at altitude the organism initiates a series of physiological adjustments to maintain the flow of O_2 by attenuating the reduction of the $P_AO_2\text{-}P_{mean}cO_2$ gradient and by increasing the whole solubility of O_2 in blood to increase DLO_2.

In theory the $P_AO_2\text{-}P_{mean}cO_2$ can be defended by increasing P_AO_2 and also by reducing $P_{mean}cO_2$. This is accomplished in part by increasing pulmonary ventilation (V_E), which contributes to mitigate the reduction of P_AO_2 caused by low P_IO_2. In fact, at a given absolute workload exercise-induced hyperventilation is exaggerated in hypoxia, and reflected in a higher V_E/VO_2. For example, during submaximal exercise (~120 W) in severe acute hypoxia equivalent to 5300 m ($F_IO_2 = 0.105$) V_E was 72 % higher in hypoxia than in normoxia [10]. Submaximal exercise hyperventilation (same absolute workload) is not enhanced by acclimatization while peak exercise V_E is increased at moderate to high altitudes [10, 38] and reduced at 4000 m [34]. However, peak exercise V_E in altitude acclimatized lowlanders may be similar [10], higher [38] or slightly lower than observed in normoxia [34].

However, the improvement in P_AO_2 that can be achieved through hyperventilation is limited by physiological dead space and the P_IO_2. At rest in normoxia the $P_IO_2\text{-}P_AO_2$ difference lies close to 50 mmHg and to 30 mmHg at peak exercise [9, 50]. During

maximal exercise in severe acute hypoxia ($F_IO_2 = 0.105$) the P_IO_2-P_AO_2 difference is reduced to 19 mmHg [9] and does not change after 9–10 weeks of acclimatization to this level of hypoxia (5260 m) [10]. The minimum value reported for this gradient was observed during exercise at the barometric pressure equivalent to the summit of Mt. Everest, over the course of Operation Everest II, when it was 12 mmHg at rest, and remained close to this value during maximal exercise [50].

Another contributing factor to attenuate the impact of low P_IO_2 on P_AO_2-$P_{mean}cO_2$ is the increase in systemic oxygen extraction that accompanies incremental exercise intensity. However, during maximal whole body exercise in hypoxia mixed venous O_2 content is similar or only slightly lower than during maximal exercise in normoxia [9, 10], implying that this compensatory mechanism can contribute very little to blunt the reduction in the P_AO_2-$P_{mean}cO_2$ gradient. Compared to normoxia, $P_{mean}cO_2$ is reduced in acute and chronic hypoxia due to the lower P_AO_2. Using data from the Chacaltaya Expedition and assuming minimal or no V_A/Q mismatch or shunt (both assumptions are reasonable at peak exercise in severe hypoxia [54]), we have estimated that the values of $P_{mean}cO_2$ at maximal exercise on the cycle ergometer were 28 mmHg and 28.5 mmHg in acute and chronic hypoxia, respectively; and the corresponding P_AO_2-$P_{mean}cO_2$ gradients were 28 mmHg and 29 mmHg, and the corresponding DLO_2 were 78 ml $O_2 min^{-1}$ $mmHg^{-1}$ and 86 ml $O_2 min^{-1}$ $mmHg^{-1}$, respectively. In normoxia, $P_{mean}cO_2$ was 60 mmHg, P_AO_2-$P_{mean}cO_2$ 60 mmHg and DLO_2 68 ml $O_2 min^{-1}$ $mmHg^{-1}$ (Fig. 26.1). Thus, in acute hypoxia equivalent to an altitude of 5260 m above sea level and after 9–10 weeks of residence at 5260 m DLO_2 at maximal exercise is rather similar, which is in agreement with the findings reported at lower altitudes [5]. In acute hypoxia and in chronic hypoxia DLO_2 was slightly higher than in normoxia. This increase in maximal exercise DLO_2 could partly compensate for the increase diffusional limitation in hypoxia [5].

According to the alveolar gas equation ($P_AO_2 = P_IO_2$-($PaCO_2/R$, where "R" is the respiratory quotient)), it is implied that hyperventilation also augments P_AO_2 not only by renewing the alveolar gas at a faster rate but also by eliminating CO_2. For a given level of V_E, compared to acute hypoxia, both resting and exercising $PaCO_2$ values are lower after altitude acclimatization, due to the increased bicarbonate excretion by the kidneys [29]. Compared to acute hypoxia, after 9–10 weeks at 5260 m $PaCO_2$ was reduced by 6 mmHg, and without this reduction in $PaCO_2$ the P_AO_2 would have been about 5 mmHg lower than in acute hypoxia.

26.1.1.2 The Components of Lung O_2 Conductance (DLO_2)

The resistance to the diffusion of O_2 ($1/DLO_2$) can be divided into the resistances at the capillary membrane ($1/DM$) and the reaction rate of O_2 with Hb in the red blood cells ($1/\theta Vc$) such that $1/DLO_2 = 1/DM + 1/\theta Vc$, where DLO_2 is the overall diffusing capacity of the lung, DM is the diffusing capacity of the membrane separating the alveolar air from the blood, Vc is the total volume blood in the lung capillaries exposed to alveolar air, and θ is the diffusing capacity of the red blood cells.

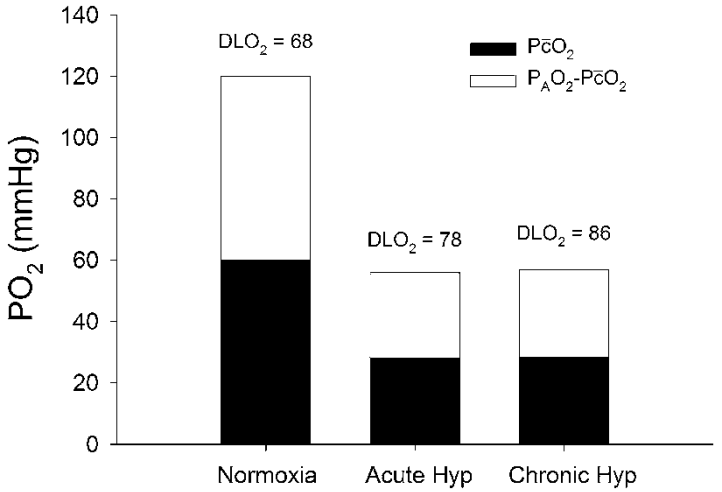

Fig. 26.1 Alveolar PO_2, mean capillary PO_2 and PO_2 gradients driving O_2 diffusion in the lung at maximal exercise in normoxia, acute hypoxia, and chronic hypoxia. Measurements were performed at maximal exercise on the cycle ergometer breathing room air at sea level, acute hypoxia ($F_IO_2 = 0.105$; $P_IO_2 = 75$ mmHg) and after 9–10 weeks of residence in Mount Chacaltaya (Bolivia) at 5260 m above sea level. Mean capillary PO_2 was obtained by first calculating the mixed venous PO_2 using the oxygen dissociation curve of the hemoglobin adjusted for femoral vein blood temperature, arterial pH, and arterial PCO_2. For sea level calculations a concentration of 4.65 mM of 2,3-DPG was assumed. For chronic hypoxia the amount of 2,3-DPG (2,3 diphosphoglycerate) needed to right shift the ODC to achieve the standard P_{50} measured in these subjects in chronic hypoxia was iteratively calculated to be 8 mM, and this value was used instead. With mixed venous PO_2 the mean capillary PO_2 was calculated by Bohr numerical integration, as reported elsewhere [28]. DLO_2:lung O_2 diffusing capacity (ml O_2 min^{-1} $mmHg^{-1}$)

During exercise more capillaries are recruited, increasing the O_2 conductance of the membrane (DM); however, this increase could be blunted in acute hypoxia if some degree of interstitial edema develops, due to higher pulmonary artery pressures in hypoxia than in normoxia [20]. Nevertheless, a mild interstitial edema does not seem to affect O_2 transport in men [27].

The conductance of the lung capillary blood (θVc) is also increased during exercise by recruiting more capillaries [18, 30]. Some controversy exists regarding whether the major fraction of the resistance to O_2 diffusion resides in the membrane or in the lung capillary blood. Is all the available DLO_2 utilized during exercise in normoxia? In humans VO_2max may be enhanced by increasing [Hb] via blood autotransfusion [22] or erythropoietin treatment [36]. Both manipulations essentially increase the O_2 conductance of the capillary blood, implying that during exercise in normoxia not all the available O_2 conductance of the membrane is utilized. Therefore, humans have a functional reserve in membrane O_2 conductance. In chronic hypoxia [Hb] increases, and this should enhance the O_2 conductance of the

capillary blood. However, the improvement in θVc with altitude acclimatization is higher than the enhancement of DLO_2, as observed after 9–10 weeks of residence at 5260 m. Why is this occurring? One possibility is that most of the resistance for O_2 diffusion resides in the membrane after altitude acclimatization. If this is true, then reducing [Hb] should not affect DLO_2 in the altitude-acclimatized human. During the Chacaltaya Expedition the subjects were also studied 1 h after isovolemic hemodilution with Dextran 70 (1 l of blood was replaced by 1 l of 6 % Dextran). This reduced [Hb] by 24 %, i.e., to same value they had before altitude acclimatization [16]. At maximal exercise on the cycle ergometer after hemodilution DLO_2 was 82 ml $O_2\,min^{-1}\,mmHg^{-1}$, i.e., similar to that observed in acute hypoxia. The fact that a substantial reduction of the O_2 carrying capacity of blood had no negative effect on DLO_2 may have two main explanations. One possibility is that the DLO_2-limiting factor resides at the membrane, in which case an improvement in the erythrocytic component would only exert a small, if any, effect on maximal exercise DLO_2. Another possibility is that the main limiting factor acts on the mechanisms determining mixed venous O_2 content. Mixed venous O_2 content at VO_2max depends on muscle O_2 extraction capacity and the distribution of blood flow. In addition, as the main mechanisms causing task failure during incremental exercise to exhaustion in hypoxia is more central (central nervous system) than peripheral [3, 12, 32, 39], an improvement of θVc may not result in increased exercise capacity if brain interstitial PO_2 is not enhanced [4, 26].

Altogether these findings support the concept that humans are not limited by the membrane component of the O_2 conductance during maximal exercise in normoxia and that not all of the available membrane O_2 conductance is utilized during maximal exercise in normoxia. It seems that some of this functional reserve in membrane O_2 diffusing capacity can be recruited during exercise in acute and chronic hypoxia. This conclusion is further supported by the fact pneumectomy in humans reduces DLO_2 less than half [31, 57]. Thus, DLO_2 does not seem to limit VO_2max at altitude in the healthy human, despite the fact that DLO_2 (measured at rest) is positively correlated with VO_2max [7]. Moreover, Sherpas and Quechuas (altitude natives) have much higher (>40–50 %) lung O_2 diffusing capacities than acclimatized lowlanders; however, maximal exercise capacity (Wmax) and VO_2max values are similar when measured at 5000–5300 m above sea level in the three populations [23, 54].

Using the model of Piiper and Scheid [40], which establishes that $VO_2max = Q\beta \cdot \{1 - \exp[-(D/Q\beta)]\} \cdot (P_AO_2 - PvO_2)$, where Q is the cardiac output, β the capacitance coefficient, D is DLO_2 and PvO_2 is the PO_2 at the beginning of the alveolar capillaries (i.e., the mixed venous PO_2), we have obtained the β values for normoxia (2.4), severe acute hypoxia (3.9), chronic hypoxia (7.7), and chronic hypoxia after isovolemic hemodilution to the [Hb] observed before altitude acclimatization (4.7). Using these values we have determined the influence of β on VO_2max, using the maximal values of DLO_2 obtained in these experiments (Fig. 26.2). Theoretically, increasing β should have a positive effect on $VO2_2max$, leaving the other factors of the Piiper and Scheid equation unmodified. In fact, this is depicted in Fig. 26.2, where the VO_2max observed in Chronic hypoxia after

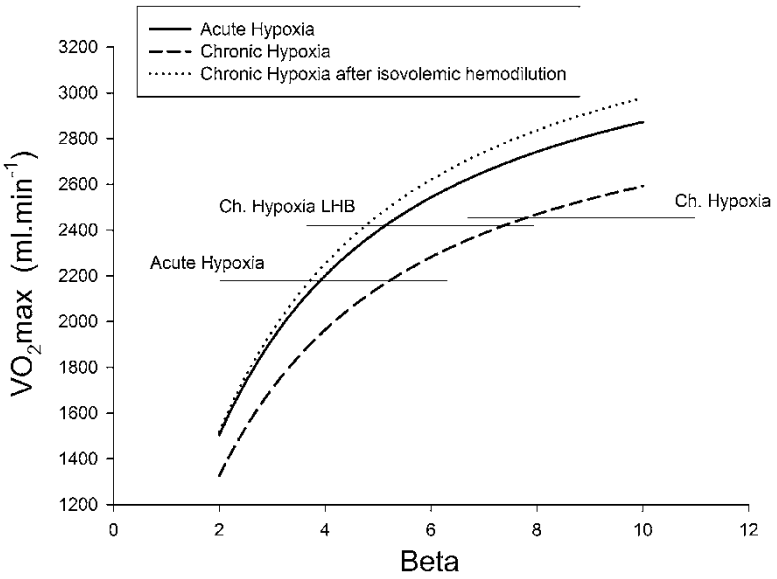

Fig. 26.2 Theoretical analysis of the influence of the coefficient β on VO_2max in acute and chronic hypoxia using the model of pulmonary gas exchange proposed by Piiper and Scheid. VO_2max during exercise on cycle ergometer is predicted to increase with β (the capacitance coefficient) during exercise in acute hypoxia ($F_IO_2 = 0.105$; $P_IO_2 = 75$ mmHg) and after 9–10 weeks of residence in Mount Chacaltaya (Bolivia) at 5260 m above sea level. However, the impact of β is attenuated in chronic hypoxia. Reducing isovolemically [Hb] (LHB) to achieve the same [Hb] observed in acute hypoxia returns the relationship between VO_2max and β to almost the same position as predicted by the model for acute hypoxia. Calculations were performed by using all the parameters of the Piiper and Scheid equation [40] obtained at maximal exercise in each specific condition and changing only the value of β

hemodilution to the same [Hb] seen before exposure to altitude can be predicted from the variables obtained in acute hypoxia just by replacing β by the value observed after hemodilution. About 75 % of the increase of β with chronic hypoxia can be explained by the increased [Hb]. However, the benefit from increasing β at altitude cannot be fully exploited because the resistance of the capillary blood is not the main limiting factor of DLO_2 at altitude. In fact, low [Hb] in Tibetan males is associated with greater high-altitude exercise capacity [48].

In severe acute hypoxia the increase of β is due to the leftward shift of ODC elicited by hyperventilation [13]. For example during maximal exercise in severe acute hypoxia ($F_IO_2 = 0.105$) the left shift of the ODC allowed for SaO_2 to increase by 8 % units compared to the SaO_2 expected from the ODC corresponding to normoxia [9]. Moreover, during maximal exercise at 5260 m the improvement in SaO_2 after acclimatization would have been 10 % higher if the P_{50} had remained in the left-shifted position observed at the same level of hypoxia but under maximal exercise in acute conditions [9]. However, the position of the ODC in the lung at VO_2max in chronic hypoxia is similar to that observed in normoxia at sea level [9, 10]. The importance

of this factor has been overlooked in the past. Examination of pulmonary gas exchange with a small and large muscle mass revealed that the rightward shift of the ODC elicited by exercise is lower during small mass exercise allowing for a 5–6 unit higher SaO_2 during exercise in severe and chronic hypoxia [15]. Therefore, an attenuation of exercise-induced rightward shift of the ODC by, for example, reducing the level of exercise-induced hyperthermia or lactic acidosis has a positive effect on exercise capacity by allowing a higher convective O_2 transport, particularly when the exercise is performed in hypoxia. It remains to be shown if a leftward shift of the ODC will increase DLO_2 and VO_2max during whole body exercise in humans acclimatized to altitude. DLO_2 will not increase if most of the resistance for O_2 flow resides in the membrane and not in the capillary blood, or if task failure is due to central mechanisms.

26.1.1.3 Diffusion Limitation in the Lung

The main mechanism limiting pulmonary gas exchange during exercise at altitudes above 3000 m is diffusion limitation [51, 56] due to incomplete gas equilibration between the alveolar gas and the capillary blood [17, 52]. During exercise in moderate hypoxia and normoxia the $AaDO_2$ increases linearly with Q with a slope of approximately 1.5–2 mmHg l^{-1} min^{-1} [17, 51]. According to the model of Piiper and Scheid [40] and to the data published by Wagner's group [51, 55, 56] pulmonary gas exchange should be more sensitive to an elevation of Q in acute hypoxia than in normoxia.

Pulmonary gas exchange during exercise is improved with altitude acclimatization likely due to a smaller diffusional limitation [5, 53], linked to the reduction in submaximal and maximal Q [53] or increased recruitment of pulmonary capillaries. However, at altitudes above 4500 m, for a given Q the $AaDO_2$ is higher in acute than in chronic hypoxia, implying that the improvement in $AaDO_2$ with acclimatization should also depend on other mechanisms [15, 17].

In Fig. 26.3 we have applied the model of Piiper and Scheid to generate a series of values of $AaDO_2$ for increasing values of Q for the conditions observed at maximal exercise in acute and chronic hypoxia during the Chacaltaya Expedition (Fig. 26.3). With this model it is predicted that increasing cardiac output in acute hypoxia by 6 l min^{-1} would have increased the $AaDO_2$ by 5 mmHg, due to a reduction of PaO_2 by 5 mmHg, if the other parameters of the equation remained unaltered. In chronic hypoxia, however, the impact of a similar increase in peak Q on $AaDO_2$ will be a little less (~4 mmHg). This slightly attenuated effect of cardiac output in chronic hypoxia is due to the higher values of β after acclimatization. Thus, although a reduction in peak Q with chronic hypoxia could contribute to a lower $AaDO_2$ after acclimatization, this effect is small. In agreement, isovolemic hemodilution during submaximal exercise in chronic hypoxia was compensated for by increasing mean Q from 14 to 16 l min^{-1} without any significant effect on pulmonary gas exchange [16].

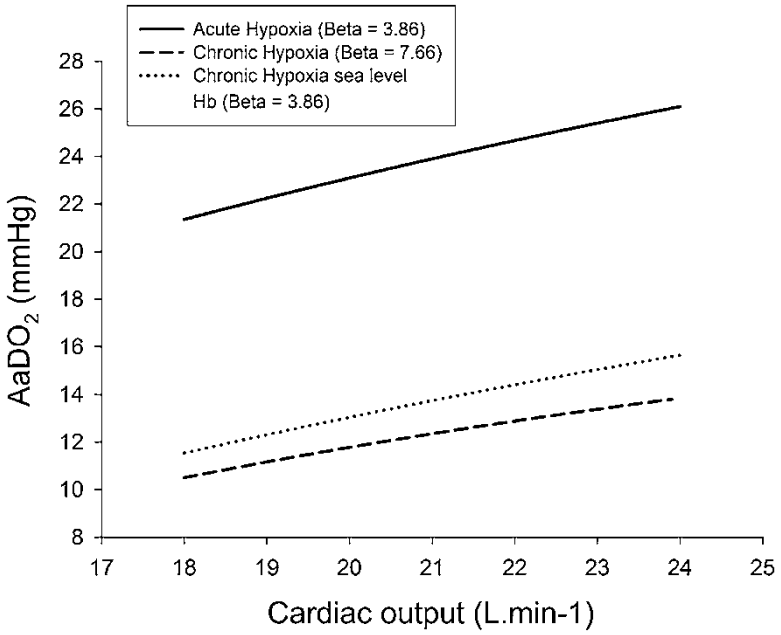

Fig. 26.3 Theoretical model of the impact of cardiac output on the alveoloarterial PO_2 difference ($AaDO_2$) in acute hypoxia and chronic hypoxia according to the model of Piiper and Scheid. Maximal whole body exercise $AaDO_2$ increases with cardiac output with a higher slope in acute than chronic hypoxia. However, when the impact of cardiac output in chronic hypoxia is modeled using the β coefficient experimentally obtained at VO_2max in acute hypoxia ($\beta = 3.86$) $AaDO_2$ is increased in the same proportion as in acute hypoxia, implying that the increase of β attenuates the impact of cardiac output on $AaDO_2$ in chronic hypoxia. Measurements were performed at maximal exercise on the cycle ergometer breathing room air at sea level, acute hypoxia ($F_IO_2 = 0.105$; $P_IO_2 = 75$ mmHg) and after 9–10 weeks of residence in Mount Chacaltaya (Bolivia) at 5260 m above sea level

By combining the experimental conditions studied during the Chacaltaya Expedition, it can be shown that the impact of cardiac output on pulmonary gas exchange depends on the size of the active muscle mass [15]. In subjects acclimatized to 5260 m during 9–10 weeks, peak leg extension exercise elicited a Q of 12 l min^{-1} while the accompanying $AaDO_2$ was only 2 mmHg; this condition can be compared with the values obtained during submaximal exercise on the cycle ergometer after acclimatization (~120 W) which elicited a Q of 14 l min^{-1} and a $AaDO_2$ of 15 mmHg. The 13 mmHg difference in $AaDO_2$ between one leg knee extension and cycle ergometer exercise is too large to be explained by the small difference in Q between these two conditions alone [15, 17]. The smaller $AaDO_2$ with the small muscle mass is linked to greater hyperventilation, diminished rightward shift of the ODC, higher distribution of flow to the contracting muscle and a higher SvO_2 and mixed venous PO_2, which also lowers the P_AO_2-$P_{mean}cO_2$ gradient.

26.1.2 Muscle O_2 Conductance (DmO_2)

The flow of O_2 from the muscle vascular bed to the mitochondria cannot be higher that the magnitude of oxygen delivered. Determination of maximal DmO_2 at the muscle level ideally requires measurements with varying blood O_2 content (i.e., reduced levels of F_IO_2) to ascertain if a plateau appears in the relationship between $DMO_2/PmcO_2$ (where $PmcO_2$ is the mean capillary PO_2 in the muscle vascular bed).

Since at maximal whole body exercise, demand for blood flow exceeds the pumping capacity of the heart in humans the whole body vascular conductance is higher that the pumping capacity of the heart [11, 47], and thus, the available flow must be distributed with priority to the most active muscle fibers so that the respiratory muscles, the myocardium and the brain receive an adequate the supply of O_2. This competition may lead to premature fatigue if the brain or the respiratory muscles do not receive enough O_2 as to maintain their metabolic rate [1, 21]. During whole body exercise in severe acute hypoxia the relative distribution of blood flow is rather similar to normoxia [9]. In contrast, in chronic hypoxia a lower proportion of the available Q is diverted to irrigate the active leg muscles and consequently leg O_2 delivery increases less than it could (theoretically), limiting leg and whole body VO_2max [10]. However, during exercise with a small muscle (one-legged knee extension exercise) peak LBF is not reduced.

Inside the muscle, the blood flow must be distributed to the most active muscle fibers, and this is likely achieved by the combined action of an increased sympathetic activation [14, 33] with selective sympatholysis nearby the muscle fibers consuming more O_2 [41]. In close proximity to the active muscle fibers the O_2 must diffuse from the Hb to the mitochondria. This process does not seem to limit the flow of O_2 from the vascular bed to the mitochondria, since $DmcO_2$ is not reduced when the in vivo P_{50} is lowered as occurs in severe acute hypoxia [12]. The diffusion of O_2 in the active muscles depends on the pressure gradient between the capillaries (mean muscle capillary PO_2) and the mitochondrial PO_2, which lies close to 0 mmHg at maximal exercise [19, 25]. In hypoxia the PO_2 gradient driving this diffusion is reduced, simply because PaO_2 is lower. Theoretically there may be a diffusion limitation in the active muscles caused by too short a mean transit time, low O_2 pressure gradient, and insufficient muscle O_2 diffusing capacity, which in part depends on the capillary density [13, 42].

Using proton nuclear magnetic resonance spectroscopy (^{1}H NMR) it has been shown that even at low exercise intensities the PO_2 inside the active muscle fibers is reduced to 3 and 2 mmHg [43, 44], implying that there is a gradient between the mean capillary PO_2 and intracellular PO_2 [44]. The presence of this gradient has been interpreted as an indication of diffusion limitation from red cell to the sarcoplasma in human skeletal muscle [44]. Nevertheless, the presence of the gradient does not imply necessarily that muscle VO_2max is limited by

a diffusional limitation. In fact, we have shown that muscle VO_2max may be increased without increasing the PO_2 gradient driving diffusion [12, 37].

The gradient driving the diffusion of O_2 ($P_{mean}cO_2$-$P_{mitochondrial}O_2$) is just a little greater during one leg kicking than during whole body exercise [15]. However, the blood flow in ml/kg of active muscle mass is also higher during small mass exercise implying a shorter transit time. Despite the shorter transit time, leg VO_2max is reduced much less during small muscle than during whole body exercise implying that proportionally a remarkably greater amount of O_2 can diffuse from the capillaries to the muscle fiber during small muscle exercise [15]. The latter indicates that the main mechanism limiting muscle VO_2max during exercise in hypoxia is O_2 delivery while muscle O_2 diffusing capacity may have a secondary role [12, 15]. Moreover, during one leg knee extension exercise in chronic hypoxia the PO_2 gradients driving diffusion are much lower than during the same exercise performed in normoxia; however, leg VO_2max was similar [15], further indicating that $DmcO_2$ does not limit muscle VO_2max during exercise at altitudes up to 5260 m, but it may do at higher altitudes.

Increasing [Hb] facilitates the diffusion of O_2 from the alveolar space to the vascular space [45]. However, muscle O_2 conductance (an estimation of muscle diffusing capacity) was not influenced by [Hb] during maximal exercise in chronic hypoxia [16]. In addition, increasing [Hb] with erythropoietin treatment allowed a higher diffusion of O_2 in the active muscle, despite no effect on capillarization or muscle oxidative enzymatic activity [35, 36], indicating that not all the available muscle O_2 diffusing capacity is used during exercise in normoxia. This has been confirmed recently by studying muscle O_2 diffusion during sprint exercise in normoxia and severe hypoxia [12]. Thus, most experimental evidence shows that a functional reserve exists in muscle O_2 diffusing capacity during whole-body exercise, that is exploited during maximal exercise in hypoxia to compensate for the reduction in the O_2 pressure gradient.

In summary, during exercise at altitude the transfer of O_2 from the atmosphere to the mitochondria of the active muscle fibers is mostly limited by the flow of O_2 in the lungs, which is reduced due to the lower alveolocapillary PO_2 gradient driving O_2 diffusion in the lung. Increasing blood hemoglobin concentration with altitude acclimatization increases the O_2 carrying capacity of blood and the fraction of the lung DLO_2 due to the capillary blood oxygen conductance. However, this adaption does not increase lung maximal exercise DLO_2. We provide some evidence indicating that the impact of cardiac output on pulmonary gas exchange is attenuated in chronic hypoxia. Although the PO_2 gradient driving O_2 diffusion is reduced in hypoxia, similar levels of muscle O_2 diffusion are observed during small mass exercise in chronic hypoxia and in normoxia, suggesting than humans have a functional reserve in muscle O_2 diffusing capacity, likely to be recruited during exercise in hypoxia.

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Chapter 27
Modeling Variable Phanerozoic Oxygen Effects on Physiology and Evolution

Jeffrey B. Graham, Corey J. Jew, and Nicholas C. Wegner

Abstract Geochemical approximation of Earth's atmospheric O_2 level over geologic time prompts hypotheses linking hyper- and hypoxic atmospheres to transformative events in the evolutionary history of the biosphere. Such correlations, however, remain problematic due to the relative imprecision of the timing and scope of oxygen change and the looseness of its overlay on the chronology of key biotic events such as radiations, evolutionary innovation, and extinctions. There are nevertheless general attributions of atmospheric oxygen concentration to key evolutionary changes among groups having a primary dependence upon oxygen diffusion for respiration. These include the occurrence of Devonian hypoxia and the accentuation of air-breathing dependence leading to the origin of vertebrate terrestriality, the occurrence of Carboniferous-Permian hyperoxia and the major radiation of early tetrapods and the origins of insect flight and gigantism, and the Mid-Late Permian oxygen decline accompanying the Permian extinction. However, because of variability between and error within different atmospheric models, there is little basis for postulating correlations outside the Late Paleozoic. Other problems arising in the correlation of paleo-oxygen with significant biological events include

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tendencies to ignore the role of blood pigment affinity modulation in maintaining homeostasis, the slow rates of O_2 change that would have allowed for adaptation, and significant respiratory and circulatory modifications that can and do occur without changes in atmospheric oxygen. The purpose of this paper is thus to refocus thinking about basic questions central to the biological and physiological implications of O_2 change over geological time.

Keywords Hypoxia • Hyperoxia • Evolution • Tetrapod • Paleozoic • Paleoatmosphere • Oxygen

27.1 Introduction

Texts on historical geology chronicle how the structure of the Earth's surface has been modified over time by forces of climate and tectonics. They also date the progression in the diversity, abundance, and complexity of life on Earth as well as its ebb and flow as a result of climatic and tectonic influences and events such as asteroid impacts. The record of biosphere evolution was first revealed by the fossil record which documented the continuity between extinct and extant organisms throughout the Phanerozoic Eon, the time span "of visible life" extending from the Cambrian (550 million years ago, MYA) to the present. Nearly the entire history of the appearance, proliferation, and diversification of plants and animals and their integration into complex ecological communities defining Earth's biosphere plays out over Phanerozoic time.

Although a somewhat later addition to the documentation of Earth's history, studies of the paleoatmosphere, including the origin and growth of atmospheric O_2, verify this molecule's gradual accumulation and significant changes over time as well as its critical role in biosphere evolution [3, 13]. The primitive atmosphere's O_2 content (about 0.001 present atmospheric level, PAL = 20.95 %) came from the photochemical dissociation of water vapor and was largely consumed in the oxidation of lithosphere sediments. Further O_2 increases awaited the evolution of photosynthesis about 2800 MYA. When atmospheric O_2 reached 0.01 PAL, it was sufficient to block ultraviolet light penetration into water, which expanded opportunities for life in the ocean. Additional radiation of life awaited the rise to 0.1 PAL, which opened land habitats [2, 3, 6].

The biological significance of atmospheric O_2 for organism and biosphere evolution is thus tied to step functions associated with its gradual accumulation in sufficient amounts to form the ultraviolet-ozone shield, enable the appearance of the diffusion-limited Ediacaran fauna, support the Cambrian explosion, and possibly even drive the radiation and metabolic physiology of various taxa and contribute to the occurrence of global extinctions [26]. Indeed, biologists have long attributed the occurrence of some phenomena documented in the fossil record as being the result of O_2 changes, and some have further considered atmospheric O_2 to be an ecological resource that affected biodiversity [15, 23, 30]. In the case of the giant dragonflies of the Carboniferous Period (354–290 MYA), Schidlowski [49]

observed that their tracheal respiratory system would not have been adequate for sustaining flight metabolism at PAL and thus predicted a hyperoxic Carboniferous atmosphere.

Whereas early models of atmospheric evolution indicated a gradual increase in O_2, through the first half of the Phanerozoic, more advanced, geochemically based studies show a more complex pattern. The model published in 1989 by Berner and Canfield [5] was one of the first to present data in a manner that drew additional interest from biologists in examining the role of O_2 in evolution and the enrichment of biodiversity [30]. This model (Figs. 27.1 and 27.2) showed a rise of O_2 to above 30 % in the Carboniferous and Permian followed by a drop to below 15 % in the Late Permian and Triassic. In the same way that a new fossil stimulates a paleontologist to compare its size, structure, and ecological setting with other fossils and extant species, the Berner and Canfield O_2 curve provided a window into deep time for comparative biologists and physiologists. This paper refocuses thinking about

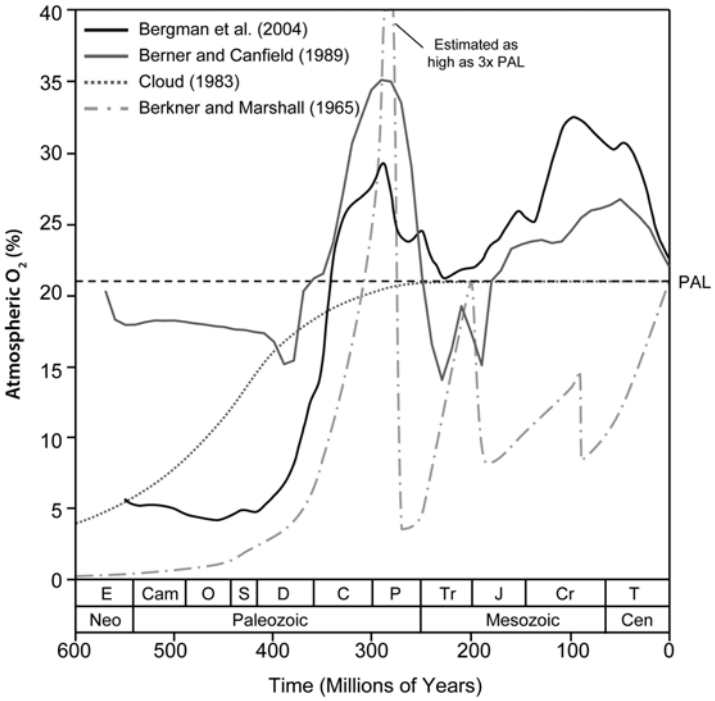

Fig. 27.1 Estimated paleoatmospheric O_2 levels from four different models. Estimates are shown in relation to the present atmospheric level (PAL; *dashed line* = 20.95 %). Geologic period abbreviations: *C* Carboniferous, *Cam* Cambrian, *Cr* Cretaceous, *D* Devonian, *E* Ediacaran, *J* Jurassic, *O* Ordovician, *P* Permian, *S* Silurian, *T* Tertiary, *Tr* Triassic. Geologic era abbreviations: *Cen* Cenozoic, *Neo* Neoproterozoic

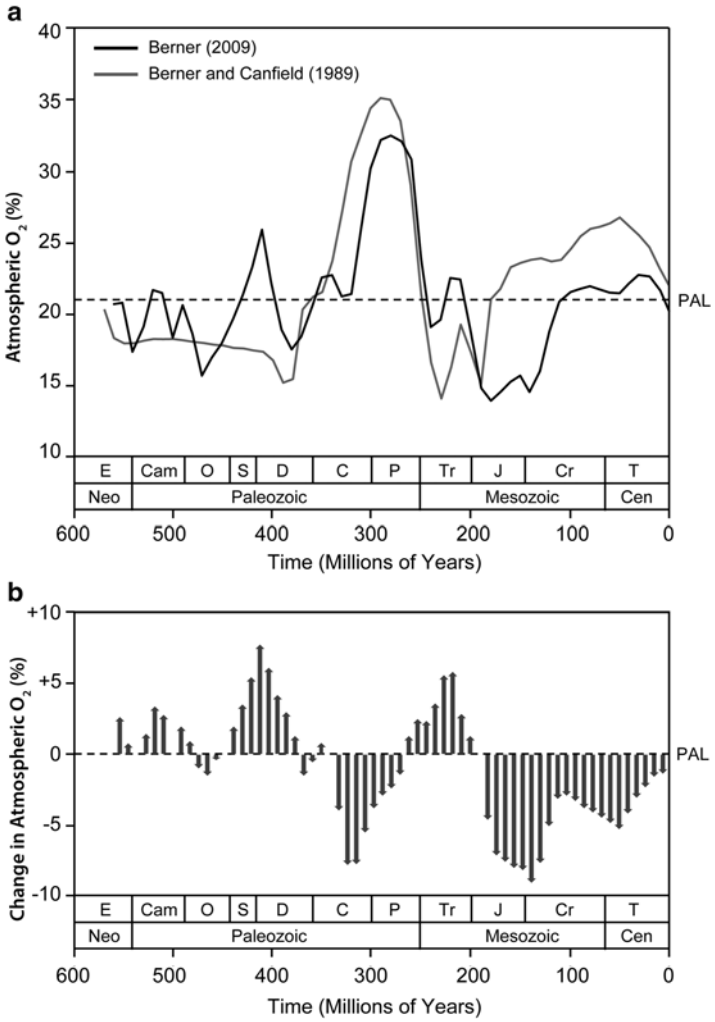

Fig. 27.2 (**a**) Comparison of the estimated paleoatmospheric O_2 levels reported by Berner and Canfield [5] and Berner [4]. *Dashed line* is the present atmospheric level (PAL) of O_2 (20.95 %). (**b**) Percentage differences in the 2009 and 1989 paleoatmospheric curves. Geologic period and era abbreviations are given in Fig. 27.1

the basic questions that are central to the biological and physiological implications of O_2 change over geological time. These questions concern the extent to which the resolution of these models can actually be used in support of physiological inference about O_2 effects, given both the millions of years involved in the completion of these O_2 transitions and the rate and extent to which physiological systems could, through mechanisms of phenotypic plasticity, homeostasis, and natural selection, become adjusted to them.

27.2 Models of Paleoatmospheric Oxygen

Most of the geochemical models of paleoatmospheric O_2 and CO_2 have their basis in estimates of fixed carbon and exchange processes with the inorganic, carbon-silicate cycle. They attempt to quantify sedimentary records reflecting the formation rate of oxidized and reduced forms of iron, carbon, sulfur, and other elements. Their basis is in estimates of the degree of O_2 shuttling between Earth's large carbon and sulfur reservoirs. When these reservoirs are oxidized, atmospheric O_2 is lowered; when these reservoirs are buried, O_2 increases. Figure 27.1 compares the paleoatmospheric O_2 models of Cloud [13], Berkner and Marshall [3], Bergman et al. [2], and Berner and Canfield [5]. Cloud's model is not based on geochemical relationships and assumes a gradual but steady rise in O_2 with time. It shows an increase from 0.25 to nearly 1 PAL over the 300 MY (million years) of the Paleozoic Era. By contrast, the geochemical models of Berkner and Marshall [3] and Bergman et al. [2] show a lower O_2 in the Early Paleozoic but then a rise to levels exceeding those indicated by Cloud [13]. This differs greatly from the Berner and Canfield [5] construction which shows Early Paleozoic levels close to PAL. The three geochemical models do agree in terms of a marked O_2 rise in the Carboniferous followed by an abrupt decline in the Permian (Fig. 27.1). However, there is little agreement among them for O_2 over the 250 MY span from the beginning of the Triassic to the present.

The differences in the O_2 curves generated by these different studies reflect the parameters of the models as well as operating assumptions. Berner and Canfield [5], for example, developed separate models for O_2 and CO_2, whereas Bergman et al. [2] combined these in the same model. Also, a fire-feedback effect on O_2 added to the Bergman et al. [2] model reduced the Devonian-Carboniferous-Permian excursion of their O_2 curve. Thus, while the three geochemical models shown in Fig. 27.1 vary greatly in their resultant O_2 curves, there is some agreement between them insofar as the rise and then fall in O_2 during the Carboniferous and Permian. This said, the timing of these events shown in the different models still differs by as much as 20 MY or more and there are also considerable differences in the magnitude of the shifts.

Berner and colleagues have consistently reported sampling error estimates with all of their O_2 curves [4–6]. However, model refinements and successive iterations are a normal course of events in this type of research and subsequent models usually differ from earlier ones. The summary of 20 years of model refinement by Berner and colleagues is captured in Fig. 27.2 which compares the curve of Berner and Canfield in 1989 [5] with that of Berner in 2009 [4] and quantifies the percentage differences between them (Fig. 27.2b). Relative to the 1989 curve, the 2009 version indicates a generally higher O_2 for the early part of the Phanerozoic and for the Triassic, but shows much lower O_2 levels than previously estimated in the Late Paleozoic and from the Jurassic to the present. Although the general structure of the 1989 and 2009 curves remains similar, the newer model identifies a new O_2 spike in the early Devonian (also supported by ocean O_2 models, Ref. [14]) and indicates less extreme changes in the upward and downward O_2 oscillations occurring across the Carboniferous, Permian, and Triassic.

27.3 The General Premise for Phanerozoic O_2 Effects

The initial hypothetical exploration of the biological significance of the Berner and Canfield [5] paleoatmospheric O_2 model sought to identify signature respiratory and metabolic transformations having the potential to impact the physiology and life history of key groups in a way that influenced biosphere evolution [30]. The premise was that while hyperoxia held the potential for enhanced respiratory and metabolic function and increased biosphere complexity, atmospheric hypoxia had the potential not only to refine respiratory function but also to limit it. The latter could have impacted fitness leading to the winnowing of certain groups, thus setting the stage for extinction and the subsequent reconstitution of biosphere complexity. The focus of Graham et al. [29, 30] was on the biological processes that unfolded over the Devonian, Carboniferous, and Permian Periods in the Late Paleozoic. This segment of time was chosen because of the general agreement among the geochemical models for the rise and fall of atmospheric O_2 and because there were numerous "biological firsts" and other significant events documented by the fossil record. Also, central to the initial theory was the effect of paleoatmosphere O_2 shifts on organisms (insects, amphibious tetrapods, and others) having respiratory systems entirely or somewhat dependent upon the diffusive flux of O_2 for respiration.

27.4 The Problem: Fitting Paleoatmospheric O_2 Models to Fossils and Physiology and Vice Versa

In view of the variation in the modeled curves for paleoatmospheric O_2, the question of fitting or correlating these with evolutionary events that were possibly linked to a change in O_2 is problematic, especially for times outside of the Late Paleozoic. However, even within this narrow sector of geologic time, there are still differences among the models in both the magnitude of the O_2 changes and their timing.

Another timing problem is that the O_2 changes of interest in the Late Paleozoic span millions of years. Physiologists interested in studying the effects of variable O_2 on metabolism or other processes do so most often by evaluating responses over time spans ranging from fractions of a second to a few months [24, 27, 32, 44, 46, 47]. For *Drosophila*, *Caenorhabditis*, and other small model organisms, experiments with O_2 exposure effects can extend over generations [1, 33, 42, 54, 55]. However, to put this into perspective, the 2009 Berner curve (Fig. 27.2a) shows about a 12 % O_2 rise over the approximately 45 MY between the Late Carboniferous to Middle Permian, a rate of O_2 increase of 2 Torr MY^{-1}. Referring again to the 2009 Berner curve [4], the decline in O_2 between the Late Permian and the Early Triassic is about 14 % over 35 MY, 3 Torr MY^{-1}. Given that the estimated time of existence for most biological species is less than 2 MY, these slow changes in atmospheric O_2 need to be considered not for their effect on a species but rather on the fates of an assemblage of clades derived from species living at the time O_2 began to change and prevailing through its duration.

In terms of vertebrate adaptive mechanisms for the gradual Late Permian-Triassic O_2 decline, this may have occurred slowly enough to be compensated by functional plasticity in Hb-O_2 affinity through, for example, intracellular shifts in allosteric modulators. Two natural occurrences of gradual O_2 decline are the uplifting events in the Andes and Himalaya mountains, both of which appear to have had effect on the respiratory physiology of resident vertebrates.

Each autumn bar-headed geese (*Anser indicus*), living on the plains of Siberia, migrate over the Himalaya to winter feeding grounds in India, Pakistan, and Myanmar. Over the span of time that this migration has occurred (40–50 MY), the Himalaya has slowly ascended at a rate that lowers migratory route PO_2 by about 2 Torr MY^{-1} or about 100 Torr total [47]. A higher Hb-O_2 affinity (lower P_{50}), which enhances O_2 uptake in the lungs, is a general adaptive feature of endotherms living at high altitude, and the bar-headed goose has a higher Hb-O_2 affinity than other *Anser* species that do not fly as high. This affinity difference is due to one amino acid substitution in the α Hb chain [39]. Assuming a general rate of amino acid substitution of 1 per 5 MY (determined for β globin by Ref. [22]), this affinity increase is well within the time frame for selection to have acted on the ancestral clade of the bar-headed goose and to thereby fix a random mutation into a high-altitude adaptation [47], even though exposure to hypoxia on this high altitude migration is abrupt and temporary (bar-headed geese cross the Himalaya in 1 day).

A similar pattern is seen for two altitude dwelling Andean camelids, the vicuña (*Vicugna vicugna*) and the guanaco (*Lama guanicoe*), both of which have a left-shifted Hb-O_2 dissociation curve relative to other mammals [47]. Ghosh et al. [25] estimated the rate of ascent of the Bolivian Altiplano 7–11 MYA to be about 1 mm per year, which, over a span of about 5 MY, is 5000 m. This altitude shift reduced atmospheric PO_2 from 160 to 87 Torr or about 15 Torr MY^{-1}, which is much greater than the estimated rate for the Permian-Triassic O_2 reduction. Assuming that the ancestral groups of the vicuña and guanaco rode along with this geologic transit to higher elevation, there would have been a slowly paced natural selection for life at high altitude which, in addition to a lowered PO_2, would have also involved colder temperature, less precipitation, increased ultraviolet light, less productivity leading to fewer ecological resources, and a reduced habitat complexity [35, 52].

Comparative studies of human and other high-altitude dwellers provide insight into the roles of phenotypic plasticity, physiological acclimatization, and genetic change associated with high-altitude colonization [52]. Recent work documents how intense selection for survival at high altitude among human populations in Tibet and in the Andes has induced the increased expression of genes enhancing respiration in low O_2 and blunting erythropoietic and pulmonary vasoconstriction responses to hypoxia demonstrated for low land populations placed at altitude. However, the extrapolation of the migration of extant species into hypoxic zones is not the same as the gradual atmospheric shifts experienced by Late Paleozoic organisms [28, 30, 33, 35]. In this regard, one of the key omissions in much of the thinking about the effect of O_2 fluctuations on vertebrates has been a lack of distinction between the diffusive and convective aspects of O_2 transport. Changes in ambient PO_2 affect diffusion and thus ventilation; however, as long as lung PO_2 remains above P_{50}, a greater

or lower environmental PO_2 will not impact convective transport [30, 33, 47]. Thus, attribution of a hyperoxic atmosphere to changes in body size or specialized physiology in vertebrates with fully functional circulatory systems [18] largely ignores the important regulation of O_2 delivery by hemoglobin and other factors [29, 30].

27.5 Examining the Premise: The Case for and Against Atmospheric O_2 in Landmark Respiratory Transformations

This section surveys the occurrence of landmark transformations possibly related to respiration and metabolism as suggested by the fossil record and recent investigations. It sets the stage for evaluating the merits of attributing such changes to shifts in atmospheric O_2 as opposed to other factors. These considerations are informed by Fig. 27.3, which shows the 1989 Berner and Canfield [5] and 2009 Berner [4] O_2 curves in relation to the timing of several significant biological events (numbered), including transformations in the respiratory function of specific groups.

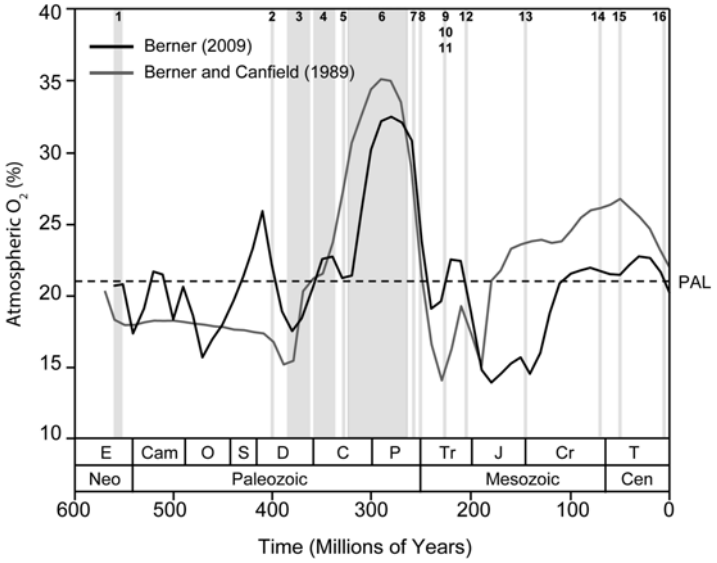

Fig. 27.3 Comparison of the estimated paleoatmospheric O_2 levels reported by Berner and Canfield [5] and Berner [4] in relation to the present atmospheric level (PAL). *Vertical grey fields* indicate the timing of certain events that may have been influenced by changes in atmospheric O_2 and specific groups for which "transformative" modifications in respiration, physiology, functionality, or ecological radiation could potentially relate to paleo-oxygen level: (1) Ediacara radiation; (2) Radiation of large predatory fishes; (3) Vertebrate land invasion; (4) Early tetrapod radiation; (5) First synapsids; (6) Insect gigantism and the origin of flight; (7) Mammal-like reptiles; (8) Permian Extinction; (9) Probable origin of synapsid endothermy; (10) Early crocodilians; (11) Dinosaurs (Ornithischia and Saurischia); (12) Early mammals; (13) First birds; (14) *Majungasaurus*; (15) First lungless salamanders; (16) Icefish. Geologic period and era abbreviations are given in Fig. 27.1

27.5.1 O_2, Ediacara, and the Age of Fishes (Events 1, 2)

Dahl et al. [14] reported analyses of the isotopic composition and the concentration of molybdenum in sedimentary rocks that identify two periods of ocean oxygenation. The first of these corresponds to the Pre-Cambrian (550–560 MYA) appearance of the Ediacaran fauna, which were large, thin, motile, bilaterally symmetrical, and diffusion-limited animals. The second oxygenation event occurred in the Devonian and correlates with both the radiation of vascular plants (which by fixing carbon and emitting O_2 contributed to atmosphere oxygenation) and the radiation and increase in body size of large predatory fishes (the Devonian Period is referred to as the "Age of Fishes"). While the Pre-Cambrian ocean oxygenation is not reflected in paleoatmosphere models, the Devonian episode described by Dahl et al. [14] appears to line up with the O_2 rise shown in Berner's [4] latest curve (Figs. 27.2 and 27.3).

Assumptions regarding the benefit of increased O_2 to diffusion-limited animals like the Ediacaran fauna are consistent with the basic hypothesis linking O_2 to transformative evolutionary events. On the other hand, the extension of the idea of O_2 enhancement contributing to an increase in Devonian fish size misses a fundamental point: For a Devonian fish (or any vertebrate) having a functional circulation and effective Hb-O_2 binding and transport mechanisms complete with the capacity for HIF-1α modulation of respiratory adaptation, gradual changes in O_2 over millions of years would arguably contribute less to increases in body size and respiration than would the myriad of ecological changes afforded by increased productivity (more O_2), such as opening new niches in which there would be selection for increased swimming speed and body size.

27.5.2 Vertebrate Land Invasion and the Early Tetrapods (Events 3, 4)

Vertebrates evolved in water, and the first air-breathing vertebrates were fishes living in lowland environments subject to drought and hypoxia [27]. A critical lowering of aquatic oxygenation in shallow water habitats is believed to have been the main driving force for the independent origin of air breathing in many Devonian fishes [12]. The occupation of land by vertebrates was initiated by a lineage of the lobe-finned (Sarcopterygii) osteichthyan fishes, the tetrapodomorphs, which came ashore during the Middle Devonian (370 MYA) and became the ancestral group of all tetrapods [10, 28, 50]. With respect to the question of how atmospheric O_2 may have related to the land invasion, both atmospheric hypoxia (a Middle Devonian nadir) and an O_2 rise throughout the Middle to Late Devonian (Figs. 27.1, 27.2, and 27.3) have been invoked to explain it [10, 12, 30].

Paralleling these events, the 250 MY history of fish evolution since the Paleozoic has seen the persistence of other air-breathing fish taxa as well as the independent

origin of air breathing among many fish groups, novel air-breathing organs distinct from the lung, and amphibious behavior [27, 28]. The extant air-breathing fishes present proxy models for the Paleozoic evolution of vertebrate aerial respiration and terrestriality [31, 40, 41, 45]. Particularly relevant are the mudskippers (family Gobiidae, subfamily Oxudercinae), a modern teleost group that appeared in the early to mid Tertiary (about 40–50 MYA), in which the characters of air breathing and terrestriality are well developed. Mudskippers live on mudflats and can respire aerially and aquatically, although species in the genus *Periophthalmodon* are obligate air breathers and will drown if denied air access [38, 53]. They engage in complex air management behaviors in order to brood their eggs in an air phase in hypoxic mud burrows [36, 37]. Recent work has shown that mudskipper aerial respiration and exercise capacity are limited by hypoxia and enhanced in hyperoxia [40]. Their capacity to repay an exercise-induced O_2 debt is also enhanced in a hyperoxic atmosphere [40].

Based on these findings, global atmospheric hyperoxia likely aided the vertebrate invasion of land in several ways. Breathing hyperoxic air reduced the ratio of evaporative water loss to O_2 uptake thus lowering desiccation. Hyperoxia also increased lung efficiency. (Early tetrapods had both gills and lungs, but gills are not effective for aerial gas exchange, particularly CO_2 discharge, which, because of its implications for acid–base balance, is a more acute constraint for prolonged aerial respiration.) Early tetrapods were heavily dependent upon the lungs because their large size and thick body armor limited their capacity for aerial cutaneous respiration. The rise in atmospheric O_2 coupled with a reduction in CO_2 would have elevated primitive lung effectiveness. Subsequent changes in these gas ratios could have influenced the transition from ventilation by buccal force pumping to aspiration and also led to a greater role of CO_2 in the control of breathing. A reduced ratio of water loss to O_2 uptake possible in hyperoxic air may have also been an important factor in the origin of the cleidoic egg. Finally, hyperoxia would have also enhanced tetrapod metabolic capacity and thus contributed to the sustained power production required to overcome gravity. As a result, increased metabolic performance expanded ecological options within the rapidly expanding terrestrial biosphere.

27.5.3 Gigantism and the Origin of Insect Flight (Event 6)

The Carboniferous and Permian saw the rise of gigantism in protodont dragonflies, such as *Meganeura* (wing span up to 71 cm, thorax diameter 2.8 cm), and other insect groups including Ephemeroptera (mayflies), Palaeodictyoptera, Diplura (bristletails), and Thysanura (silverfish) [11, 33]. Gigantism also occurred in other Carboniferous arthropods (millipedes and scorpions), the extant relatives of which are diffusion-dependent for respiration [30]. Tests to model Carboniferous and Permian O_2 atmosphere effects on insect body size have been done by rearing

Drosophila in hypoxia and normoxia over several generations [42]. Such studies (reviewed by Ref. [33]) demonstrate that *Drosophila* and most other insects are smaller when reared in hypoxia, while in hyperoxia some develop and evolve larger sizes. In addition, insects reared in higher PO_2 developmentally and evolutionarily reduce their proportional investment in the tracheal system, suggesting that there are significant costs associated with its structure and function. Thus, the hyperoxic atmosphere of the Late Paleozoic was likely beneficial in both overcoming diffusion limitation and relaxing investment in the energetically expensive and elaborate tracheal system.

Flight is an essential mechanism for insect dispersal and diversification. The earliest proto-wings had a possible respiratory function and were used for locomotion and predator escape. Some insects evolved flight in the Devonian, but it was likely a hyperoxic and a more dense Carboniferous atmosphere that aided the origin of flight in many groups by increasing the lift generated by small winglets [15, 16, 30]. The need to power flight required sustained high levels of oxidative metabolism which would have been facilitated by a hyperoxic atmosphere. Recent work [11] has shown that fossil insect wing length parallels estimates of atmospheric O_2, concentration throughout the Carboniferous and Permian when O_2 levels were at their highest and then through the Triassic which saw a decrease in both atmospheric O_2 and insect wing length (large flying insects did not survive beyond the Permian when 27–30 % of the known insect orders went extinct). However, following the Jurassic, the correlation of insect wing length and atmospheric O_2 decoupled as biotic factors (e.g., the rise of insect-eating birds and the advent of vertebrate flight) are hypothesized to have applied selective pressures that reduced flying insect size, even in the wake of rising O_2 concentrations during the Cretaceous [11].

27.5.4 The Permian Extinction (Event 8)

About 80 % of marine and terrestrial species were lost in the Permian Extinction. Included in this loss were 75 % of the tetrapods (amphibians and amniotes). Causal factors suggested for the Permian Extinction include the formation of the Pangea supercontinent and the corresponding changes in habitat surface area, primary production and glaciation, as well as extreme volcanism associated with formation of the Siberian Steps [17]. Also occurring was a sharp drop in global atmospheric O_2, which is registered in each of the geochemical models except that of Bergman et al. [2] (Figs. 27.1, 27.2, and 27.3). Model-indicated values for hypoxia at this time range between 0.2 and 0.9 PAL. This fall in global O_2 would have been restrictive and may have modified or eliminated some taxa that had radiated in the preceding period of hyperoxia; however, given the slow rate of change in O_2, these levels of hypoxia cannot be regarded as the primary cause of the Permian Extinction [30].

27.5.5 Synapsids, Mammal-Like Reptiles, Endothermy, and Early Mammals (Events 5, 7, 9, 12)

Tetrapod diversification began in the Devonian and accelerated in the Carboniferous when at least 11 of 16 known basal lineages, including the three primary groups of amniotes, the synapsids (ancestral group of mammals), diapsids (ancestral group of reptiles and birds), and anapsids, all appeared. While tetrapod diversity decreased in the late Permian, the synapsids underwent a pronounced diversification from the pelycosaurs to the therapsids, a diverse assemblage of herbivores and carnivores known as the mammal-like reptiles [8]. Synapsid success has been partially attributed to the effects of hyperoxia and to a denser atmosphere, which enhanced specializations such as metabolic heat retention, an important precursor for endothermy that was thought to be present in certain groups [29, 30]. Although the mammal-like reptiles flourished in the Late Permian and Early Triassic, they were later outcompeted by the diapsids.

27.5.6 Diapsids: Crocodiles, Dinosaurs, and Birds (Events 10, 11, 13, 14)

The Middle Triassic saw the diapsids become the dominant terrestrial vertebrates. Appearing at this time were the archosaurian reptiles, a group that includes crocodiles, dinosaurs, and birds. This group holds the interest of comparative biologists for the possible influence of paleoatmospheric O_2 in the development of respiratory and metabolic physiology [44, 57]. It is now known that the highly specialized respiratory system of birds, which features fixed-volume lungs that have a unidirectional airflow driven by bellow-like air sacs, is a shared feature with dinosaurs such as the late Cretaceous *Majungasaurus* (= *Majungatholus*) [43], and that unidirectional pulmonary airflow is also found in crocodilians [19]. Another structural similarity for dinosaurs and birds is that the air sacs extend into the skeleton where they form air pockets that are presumed to lessen body mass [58].

The bird pulmonary system is essential for the maintenance of stable flight and is thought to be closely tied to this group's high metabolism and endothermy. Birds did not appear until the Jurassic; however, the presence of a structurally similar lung in dinosaurs and unidirectional pulmonary airflow in crocodilians [19] suggest that the metabolic physiology of birds may be synapomorphic within the archosaur lineage. Although the crocodiles differ from birds by lacking air sacs and ventilating using a hepatic piston [19], these differences may reflect the secondary requirements for an aquatic existence such as apnea and buoyancy regulation. Fossils suggest that many of the early archosaurs including ancestral crocodilians were highly active and some were bipedal [7, 8]. Moreover, the presence of a four chamber heart in crocodiles and birds implies that this too was a synapomorphy for the group, and, by extension, higher metabolic rates, and possibly endothermy,

were within the repertoire of the early archosaurs. Finally, the unidirectional cross flow of blood in the bird lung is highly effective for respiration in hypoxia, and this suggests that a similar lung in the early archosaurs could have enabled their survival, radiation, and competitive dominance over other groups in the relatively low O_2 regime of the Mesozoic (Fig. 27.3). However, the recent discoveries of unidirectional pulmonary airflow in both monitor lizards and iguanas (lepidosaurian reptiles) calls into question such hypotheses and suggests this ventilatory mechanism may have evolved in early diapsids before the archosaur and lepidosaur lineages split [9, 48].

27.5.7 Lungless Amphibians (Event 15)

For amphibians, but no other tetrapods, the lungs appear to be an expendable commodity that can be traded for other life-history advantages. Loss of this organ has occurred independently at least three times in amphibians. The majority of salamanders are lungless, including all of the nearly 380 species (9 genera) in the family Plethodontidae. Lunglessness also occurs in two other salamanders (both in the genus *Onychodactylus*, family Hynobiidae), a South American caecilian (*Atretochoana*, Typhlonectidae), and in an Indonesian frog (*Barbourula*, Bombinatoridae). Although *Barbourula* is small and has a favorable surface area to volume ratio for cutaneous gas exchange, *Atretochoana* is one of the largest caecilians and is the largest lungless amphibian.

In some groups, lunglessness is accompanied by the complete loss of the pulmonary circulation and closure of the nasal choanae. Lunglessness is possible for amphibians because of their low metabolic rates and their well-developed capacity for cutaneous respiration. Because respiratory capacity defines metabolic scope and the potential for life-history specialization, lunglessness implies the presence of a complex of selective trade-offs between buoyancy and metabolic performance and feeding mechanisms [20, 21]. The lungless frog and caecilian, as well as many plethodontids, occur in flowing highland streams where cool oxygenated water optimizes cutaneous O_2 uptake, while the absence of a lung lessens buoyancy, which optimizes locomotion and reduces the risk of being swept downstream by strong flow. Plethodontids, however, are highly diverse and some are terrestrial. This means that even if initial selection for the loss of lungs was rooted in buoyancy compensation and cutaneous respiration, this limitation for aerobic metabolism has not precluded this group's radiation into ecological niches where life without a lung remains manageable. In all lungless amphibians, elimination of the buccal chamber's involvement in breathing has led to altered feeding mechanisms and largely precluded vocalization. The origin of the Plethodontidae in the Early Tertiary (some estimates put this in the Late Cretaceous, Ref. [56]) and this group's radiation in the Early to Middle Tertiary probably encompasses the timing of lung loss in other lungless amphibians, implying that this feature evolved in O_2 levels at or near PAL (Fig. 27.3). This argues that biological forcing functions, and not a relaxation of respiratory drive attributable to elevated O_2, were the agents for amphibian lung loss.

27.5.8 Antarctic Icefish (Event 16)

Icefish (Channichthyidae) live in extremely cold (+1.5 to −1.9 °C) waters of the Antarctic continental shelf and are the only adult vertebrates without Hb or red cells [51]. Channichthyids are one of eight families in the perciform suborder Notothenioidei, which is the dominant fish group in Antarctic waters. The loss of Hb in icefish occurred at the time (2–5.5 MYA) of the group's separation from other Antarctic notothenioids, which have hematocrits and mean corpuscular Hb concentrations that are comparable to those of other fish species inhabiting cold water [51]. All of the 11 genera in the Channichthyidae lack the β globin gene and only have remnants of the gene for α globin. Of the 16 species in this family, none express myoglobin (Mb) in their striated muscle, and only ten have Mb in their cardiac myocytes [51].

Icefish demonstrate that the loss of seemingly vital components of the respiratory system can occur in the absence of pronounced changes in atmospheric O_2 (Fig. 27.3). The absence of these proteins in icefish is not, as initially hypothesized, an adaptation to cold, oxygenated water. Rather, the loss of Hb and Mb are mutations that would not support physiological function in warmer temperatures or in a habitat having much greater levels of predatory pressure and interspecific competition. Sidell and O'Brien [51] describe Hb loss in this group as a "naturally occurring genetic knockout," noting that the subsequent loss of cardiac Mb within some species is the result of four independent mutational events.

Blood lacking Hb transports less than 10 % of the O_2 carried in blood having red cells with Hb. It was initially thought that the higher solubility of O_2 in extremely cold plasma compensated for the loss of red cells and provided physiological advantage by reducing blood viscosity. However, the work of Hemmingsen [34] and colleagues and others demonstrates that the heart function of icefish has undergone adaptations that compensate for the lack of Hb. Relative to other notothenioids, channichthyid hearts are larger, have a larger mass-specific cardiac output, and the cost of their cardiac work for circulation is much greater (22 % of total VO_2). Icefish also have a fourfold greater blood volume and larger capillary diameters which allows a larger, faster, and lower-pressure blood flow through tissues. The absence of Hb and Mb in striated and cardiac muscle also limits the nitric oxide (NO)-oxygenase activities which may have accelerated the acquisition of morphological changes such as increased tissue vascularization, capillary diameters, and the mitochondrial densities needed to compensate for hypoxia.

27.6 Conclusions

In the time since the publication of the Berner and Canfield [5] paleoatmospheric model and the first synthesis of its implications for life in the Phanerozoic [30], over 50 contributions have provided new ideas and suggested specific ways that changes in atmospheric O_2 could have exercised signature effects on respiration,

metabolism, and biodiversity. The main focus of the Graham et al. [30] hypothesis was the correlation between changes in Late Paleozoic (Devonian, Carboniferous, Permian) O_2 levels and transformative changes in the pattern of life that took place at that time, particularly in organisms having respiratory systems dependent upon the diffusive flux of O_2 for respiration. However, many more recent formulations of the "O_2 correlation" have extended into regions of the Phanerozoic where the paleoatmosphere O_2 is less well known by modelers, and to a range of biological processes, from the formation of shells, segments, and body shapes and sizes in various phyla, to behavioral and ecological changes. While all of these can be loosely correlated to O_2, implying a cause-effect relationship is simplistic. Critically important in many of these newer contributions is an apparent lack of understanding of how the presence of a circulatory system, replete with an effective O_2 binding respiratory pigment, would obviate slight changes in atmospheric O_2 over geologic time. Simply stated, the O_2 affinity of a respiratory pigment is set at a level that maximizes O_2 loading at partial pressures far less than atmospheric, and modulation of this affinity in response to changes in atmospheric O_2 over geologic timescales is a reasonable expectation. This paper verifies the "O_2 correlation" by stressing the importance and clear relevance of O_2 access to the radiation of certain groups such as the diffusion-dependent Ediacaran fauna and the giant Carboniferous arthropods, and the development of innovative functional changes such as insect flight, vertebrate terrestrial locomotion, and the cleidoic egg. However, it also describes cases in which there have been large-scale changes in vertebrate respiratory and circulatory systems (lungless amphibians and Hb-lacking icefish) in the absence of marked changes in atmospheric O_2, thus demonstrating that the composite effects of habitat and life history can both select for and sustain major changes in organismal physiology. Thus, when attempting to understand the causation of transformative evolutionary events, it is essential to consider the interplay of potentially numerous abiotic and biotic factors.

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Chapter 28
Caudwell Xtreme Everest: An Overview

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Abstract The Caudwell Xtreme Everest (CXE) expedition in the spring of 2007 systematically studied 222 healthy volunteers as they ascended from sea level to Everest Base Camp (5300 m). A subgroup of climbing investigators ascended higher on Everest and obtained physiological measurements up to an altitude of 8400 m. The aim of the study was to explore inter-individual variation in response to environmental hypobaric hypoxia in order to understand better the pathophysiology of critically ill patients and other patients in whom hypoxaemia and cellular hypoxia are prevalent. This paper describes the aims, study characteristics, organization and management of the CXE expedition.

Keywords Acclimatization • Extreme altitude • Arterial blood gases • Climbing

28.1 Introduction

In the spring of 2007, the Caudwell Xtreme Everest (CXE) medical research expedition studied 222 healthy subjects on the trek to Everest Base Camp (EBC, 5300 m) [1]. One group of 198 volunteer subjects trekked to EBC and back, whilst a second group of 24 investigator subjects ascended the same route but remained at the altitude of EBC for a prolonged period, or in some cases ascended higher on the mountain (Fig. 28.1: Ascent profiles of CXE subjects). Four fixed laboratories and two temporary mountain laboratories staffed by a team of more than 60 investigators were required to achieve the planned measurements.

The scale of the CXE study required both a substantial logistical and organizational infrastructure and detailed preparatory work to explore the feasibility, reliability and validity of a variety of study techniques. Furthermore, comprehensive risk management and extensive medical support were essential to achieve these goals in a high-risk remote environment.

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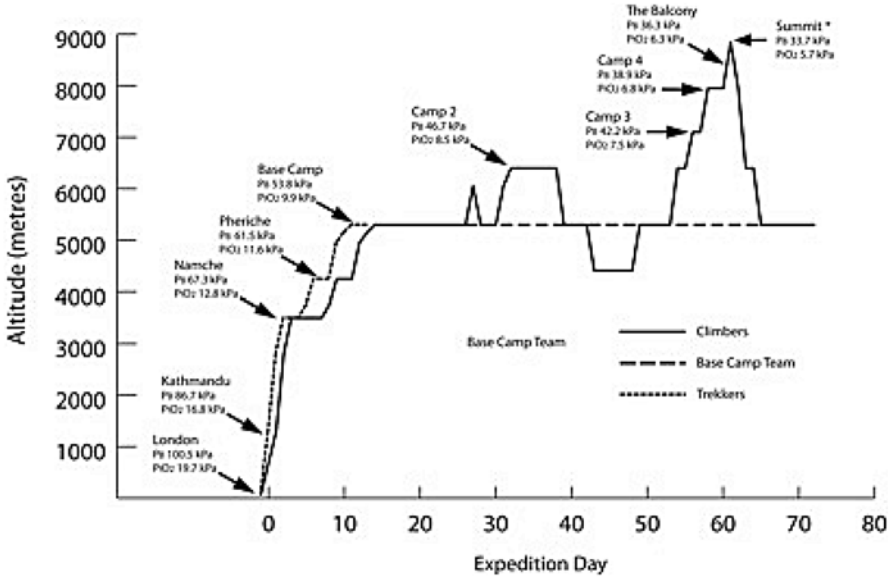

Fig. 28.1 Ascent profiles of CXE subjects

28.2 Aims and Priorities

CXE was based on the premise that the physiological responses of healthy subjects to environmental hypoxia at high altitude are a valid model for exploring pathophysiological responses to hypoxia in critical illness and other illnesses where hypoxaemia or cellular hypoxia are common. Our overall aim was to use this model to improve understanding of critical illness and to develop novel management strategies for critically ill patients. Cellular hypoxia, globally or localized to specific tissues, is a universal feature of critical illness, and occurs frequently in a variety of other illnesses. We have discussed elsewhere the concept that physiological and pathophysiological responses to extreme environmental challenges may be similar to responses seen in critical illness [2, 3]. Furthermore the study of critically ill patients is hindered by the heterogeneity of presenting complaints, patient premorbid state, administered treatment and responses to that treatment. In addition, baseline "control" data are rarely available in critically ill patients adding to the difficulty of interpreting observed phenomena. Consequently establishing a causal relationship or robust association between an isolated physiological variable (e.g. level of hypoxaemia) and specific response is limited by the high noise to signal ratio. Taken together, these concepts led us to adopt this model, whilst keeping in mind its limitations.

Specifically, we proposed that exercise at altitude would be a useful model for exploring the balance between oxygen delivery and utilization in critical illness. Our two central hypotheses were that functional changes occurring with increasing altitude (more profound hypoxia) would be explained by factors other than "oxygen

delivery" (the product of oxygen content and cardiac output) and that variation between individuals in their responses to hypoxia would be explained by genetic differences. The first of these hypotheses arose from two observations. First, that changes in physiological variables related to oxygen delivery do not account for changes in observed performance at altitude [4, 5]. Second, that function (e.g. maximum exercise capacity) is significantly limited following adaptation to high altitude (real or simulated), in the face of normal or supra-normal blood oxygen content and systemic oxygen delivery [6–8]. In order to explore alternative explanations for adaptive changes in function, we measured indices of microcirculatory function (using Sidestream Dark-field Microscopy) and tissue oxygenation (using Near Infrared Spectroscopy), and explored the relationship between oxygen utilization and work output (using steady-state measurement of oxygen consumption at different work rates) under a variety of conditions. In order to investigate the genetic determinants of these adaptive responses, we designed and conducted a large (>100 subjects) study such that there was adequate statistical power to identify associations between observed phenotypic characteristics and specific alleles [9].

Overall CXE aims were prioritized as safety first, science second, and climbing third (and then only in support of the scientific goals). The mantra of "safety-science-summit" provided an effective framework for guiding decisions when unexpected circumstances arose during the expedition. For example, the use of supplemental oxygen for climbing above 7100 m was based on the recognition that this would reduce risk of adverse events to the climbing investigators [10]. Ensuring that subjects breathed ambient air for at least 20 min prior to all measurements minimized the effect of this approach on measured variables. In general, we sought to avoid decision making during the expedition through extensive contingency planning prior to departure.

Among the research, individual studies were prioritized based on their relationship with the core hypotheses and the number of other studies with which they interacted. For example, the exercise protocol incorporating a work-rate ramp to volitional exhaustion was given high priority, as it was both a central measure of adaptive response and incorporated measurement of a variety of additional physiological variables under conditions of dynamic physiological stress.

Detailed preparatory validation studies and a continuous program of device calibration throughout the duration of the expedition assured measurement validity. For example, one individual travelled continuously between fixed laboratories conducting a program of cycle ergometer and breath-by-breath device calibration. In addition, each type of device had been demonstrated as accurate at an equivalent pressure to 9000 m altitude in a hypobaric chamber prior to the start of the expedition.

28.3 Study Design and Subjects

The study design is described in detail elsewhere [11] and has several important elements: longitudinal observational (no intervention) design, baseline (sea-level) control data, large study population, tightly matched participant ascent profiles, two

distinct groups of participants addressing different sets of questions, reliable valid measurement and multiple recorded variables at each altitude.

Previous studies at altitude have tended to have relatively small groups (between 10 and 30 subjects) [12–14] or had participants with un-matched ascent profiles, therefore limiting the validity of between-participant comparisons [15]. By ensuring that our participant groups followed identical ascent profiles while trekking to EBC, we are able to attribute differences between participants to true physiological differences (and their genetic determinants) with greater confidence.

While data from the large trekker group is being used to explore inter-participant variability (heterogeneity) of responses to the imposed hypoxic stimulus and associate these responses to specific allelic variants, the smaller investigator group is providing information about prolonged exposure to altitude as well as unique measurements at extreme altitude. Although previous expeditions have conducted studies at or close to the summit of Everest [16, 17], we were able to use a variety of devices and techniques that had not previously been used at such extreme altitudes to obtain unique measurements in this group [18–20] REFS. A companion pilot study evaluated the physiological responses of a small group of children (Smith Young Everest Study) ascending to altitudes of up to 3500 m (Namche Bazaar) [21, 22].

28.4 Study Setting

Pre-expedition sea-level control data were obtained in the investigators laboratory in London during the 3 months prior to the expedition [11]. This phase of the study was also used to train the field investigators new to the group in the study techniques. Four semi-permanent and two temporary laboratories were established in Nepal. Permanent laboratories (for the duration of the expedition) were established in Kathmandu (1300 m), Namche Bazaar (3500 m), Pheriche (4200 m) and at Everest Base Camp (EBC) (5300 m) [11]. Temporary mountain laboratories were established at Camp 2 in the Western Cwm (6400 m) and on the South Col (7950 m) [11]. A small shelter (Ultralight Shelter, Terra Nova Equipment Ltd, Alfreton, UK; www.terra-nova.co.uk/) was briefly erected at 8400 m during the summit attempt in order to provide a protected environment for the highest blood gas measurements [18].

Each laboratory had a named laboratory manager and a medical officer who was a qualified and appropriately experienced doctor. The Kathmandu laboratory was situated in a large hotel room and was staffed by eight investigators and two hospitality staff. The Namche laboratory was situated in several small rooms within a trekking lodge and was staffed by eight investigators. The Pheriche laboratory was situated within two small rooms within a trekking lodge and was staffed by three investigators. The EBC laboratory was situated in three Deployable Rapid Assembly Shelters (DRASH) (MilSys (UK) Ltd, Hereford, UK; www.milsys.co.uk/) and was staffed by 13 investigators. An additional large DRASH was divided

and used as a medical centre and logistics power unit. The laboratory at Camp 2 was situated within a small DRASH and was staffed by the climbing investigators, as was the laboratory on the South Col which was housed within an 8-person high altitude tent (Cosmos, Terra Nova Equipment Ltd, Alfreton, UK; www.terra-nova.co.uk/).

Mains type electricity (240 V, alternating current) was provided at all laboratories up to and including Camp 2 using a combination of petrol generators and photovoltaic cells linked to storage batteries by combined inverter/charger and uninterruptable power supply units (Victron Energy B.V., Almere Haven, The Netherlands; www.victronenergy.com/). The South Col laboratory utilized a 24-V direct current electricity supply. Although mains electricity was available in Kathmandu, we used an uninterruptable power supply system with backup battery and generator system in addition to assure reliability of supply.

28.5 Preparation

The aim of pre-expedition preparation was to minimize the risk that planned studies would fail or produce erroneous results. In order to ensure that all measurements were feasible, reliable and valid we tested all equipment, protocols and people in conditions as similar to those that we would find on Everest as possible. Sea-level studies in cold and hypobaric chambers were used to evaluate the functional and measurement characteristics of individual devices. Field preparation allowed integrated testing of study protocols and team effectiveness in environments and conditions similar to those we would encounter on the main expedition. Two expeditions to the summit of Cho Oyu (8201 m) were conducted in 2005 and 2006 as well as shorter visits to the European Alps. These allowed us to test risk management, organization, and individual and team performance prior to the main expedition and thereby have a high level of confidence that our central aims were achievable.

28.6 Logistics

A full-time expedition manager (AM) was responsible for expedition logistics and procurement of non-research, non-medical equipment. A store-man and assistant assisted him immediately before and during the expedition and this team was responsible for maintenance and repair. During the expedition setup phase (early-mid March 2007), the logistics team was supplemented by two of the investigators who were not study participants. This set-up team was responsible for building all non-permanent lab structures (e.g. base camp DRASH shelters) as well as electrical power systems. In collaboration with the Sherpa team and helicopter contractors, they also coordinated transport of all equipment (expedition and personal) not carried in with individual teams. In total, 26 tonnes of equipment were shipped from

the UK to Nepal and dispatched to the appropriate laboratory location in >400 blue plastic barrels (55 gal, 208 l), >100 toughened plastic cases (Trifibre Ltd, Leicester, UK; www.trifibre.co.uk/), >100 compressed gas cylinders (calibration, investigational and medical) and numerous custom containers and kit bags. All equipment was delivered to the appropriate location, on or ahead of schedule and no equipment was lost.

The expedition manager also took principal responsibility for the management of routine sponsor liaison for all but the largest of sponsors. Finally, the expedition manager coordinated training of expedition members in the use of equipment (e.g. generators, electrical systems). Decommissioning of the expedition logistics and feedback to sponsors required 5 months to complete after the end of the main expedition. The expedition manager also took principal responsibility for the management of routine sponsor liaison for all but the largest of sponsors. Finally, the expedition manager coordinated training of expedition members in the use of equipment (e.g. generators, electrical systems). Decommissioning of the expedition logistics and feedback to sponsors required 5 months to complete after the end of the main expedition.

28.7 Organization and Management

CXE involved more than 60 investigators along with a dedicated logistics team of three in the field during spring 2007. In addition, more than 30 Sherpa's and numerous porters and yak drivers took part in the expedition. The successful completion of the expedition was only achieved because of the quality and commitment of all these individuals and their ability to work together, often under adverse conditions, to achieve shared goals.

Central elements of a successful conclusion were clearly prioritized goals (see above), a defined organization structure with clearly devolved responsibility and reporting lines, regular scheduled communication at all levels, and a clearly understood contract of involvement.

The person "in charge" of each element of the expedition was clearly identified (Fig. 28.2: Organizational structure) and this structure was maintained throughout the expedition. Where specific individuals were both mission critical and functioning in a high-risk environment, a named deputy was identified to replace them in the event of incapacity or communication breakdown. For example, the expedition leader's (MD) role would have been taken by the Climbing Leader (SD) above EBC and the Project Manager (KM) at or below EBC. Within the overall structure, laboratory managers (MGM) provided an essential local leadership role in such a dispersed team. The expedition project manager (KM) organized all trekker arrangements (in collaboration with the commercial company providing logistics for their treks), managed external communications and the website for the duration of the expedition, and deputized for the expedition leader in the day-to-day running of the expedition when they were above base camp.

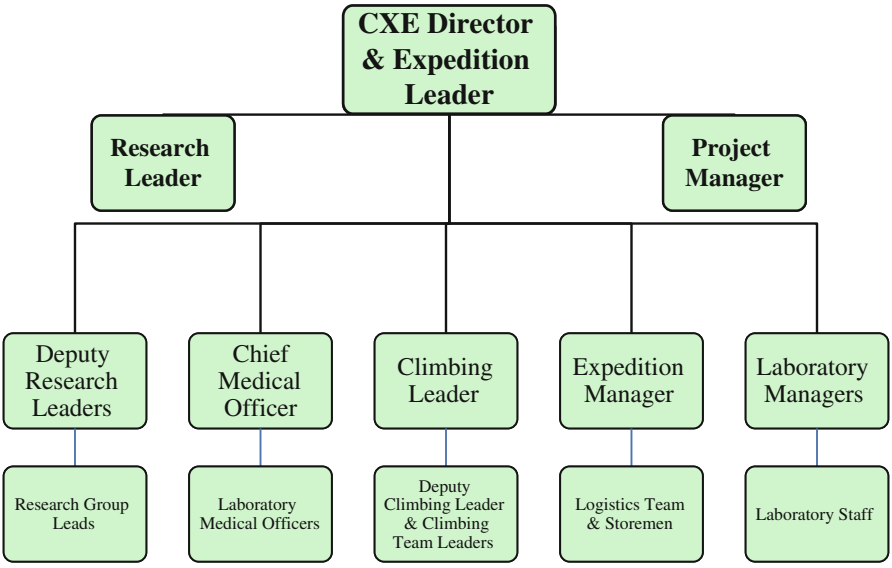

Fig. 28.2 CXE organizational structure

All communication was coordinated from EBC. Above EBC communication was principally by VHF radio with satellite phone backup. Scheduled calls occurred twice daily to all teams above EBC and combined research, logistics and medical communication. VHF radio was monitored continuously from EBC by the expedition manager (or named deputy) and by the Sherpa team. Daily scheduled management and medical calls took place between the fixed laboratories and EBC. Close liaison between the expedition leader and the expedition sirdar and members of his team on a daily basis was central to the effective conduct of the expedition.

A clear understanding of the shared goals and priorities was achieved in part by clear discussion of these issues at interview stage prior to the expedition. In addition, all investigators and other team members agreed verbally and in writing to a simple expedition contract covering issues of responsibility, accountability, ownership of intellectual property rights and attribution of publications resulting from the expedition.

28.8 Risk Management

A detailed risk management plan was prepared in advance of the expedition. This incorporated the medical plan, evacuation plan, communication protocol and climbing plan. Further details of the risk management plan (including evacuation, communication and climbing plans) will be reported separately.

28.9 Medical

The expedition medical team comprised the laboratory medical officers reporting to the chief expedition medical officer (DZHL) and was responsible for all medical decisions. All trekkers were medically screened by the CXE chief medical officer and by doctors working for the trekking logistics company. A comprehensive medical plan covering altitude and non-altitude-related illness was produced prior to the expedition and was also submitted as a companion document to the application for ethical approval. All conditions were treated according to evidence-based guidelines drawn up by the expedition medical officer as part of the medical plan.

All participants were requested not to self-medicate for altitude or other illnesses. However, it was emphasized that in the event that any subject did take medicine not prescribed (and therefore documented) by the expedition medical team, then it was most important that they reported this to the investigators in order to avoid confounding by undocumented treatments.

The laboratory medical officers held twice daily medical clinics. Medical decisions between laboratories were devolved to trek leaders who were asked to follow the expedition guidelines and document all consultations and interventions. Trekkers and investigators who were medically qualified were asked not to advise fellow expedition members, but to refer requests for medical attention to one of the expedition medical officers. Details of the expedition medical screening will be reported separately.

28.10 Research Management and Governance

The CXE director/expedition leader (MG) was principal investigator for all studies. Ethical approval for all studies was obtained from the UCL Research Ethics Committee. The CXE director (MG) and research leader (HEM) maintained an overview of the research portfolio. The two deputy research leaders (DSM, DZHL) managed equipment procurement, laboratory design, study conduct, and data management before and during the expedition. Each study was allotted to one of four research groups led by a named individual who was responsible for detailed issues relating to the studies within their group. Day-to-day conduct of the studies was devolved to laboratory managers leading local staff.

28.11 Data Management and Analysis

The volume of data generated by the longitudinal study of >200 participants was large and presented significant management challenges. For example, more than 4500 diary days (>12 person-years) of data was obtained from the daily collection

of simple physiological variables on all participants, more than 1800 exercise tests were completed and analyzed and there are more than 10,000 separate stored plasma samples. In order to minimize the risk of data loss, all electronic data were backed up locally at each laboratory onto solid-state storage. In addition, each trek leader carried a solid-state storage device containing all electronic files for each subject in their trek. These were backed up at each laboratory on arrival and on arrival back at Kathmandu. All blood samples were collected in duplicate and stored separately to minimize data loss in the event of damage to sample storage containers.

Each participant was responsible for the paper diary recording simple physiological variables and symptoms for the duration of the study. No diary data was mislaid. All data stored on paper was subsequently transcribed onto a database where it was combined with electronically stored data. Where paper records were collected these were retained for source verification of all data. Transcription, verification and quality control of the main expedition database took more than 12 months to complete.

Data analyses involving a subjective component (e.g. lactate thresholds on exercise data, microcirculation images) were undertaken in duplicate (separately by two independent investigators) to ensure reliability of interpretation. Analysis of the exercise data by this method took 18 months to complete and verify.

The trekker and investigator cohorts were treated separately for all data analyses because their ascent profiles differed (investigators ascended more slowly) and because the investigator cohort were a group selected in part because of previous problem free exposure to high/extreme altitude.

In general, exploratory analyses were performed on the investigator group prior to a limited number of a priori defined hypothesis-driven analyses on the trekker data. A detailed publication plan was drawn up prior to commencement of analysis and comprises a three-stage approach. Stage 1 papers will be descriptive for discrete types of phenomena (e.g. exercise, microcirculation, NIRS data). Stage 2 papers will combine different types of data from the CXE BioResource in correlative and model-building studies to explore the determinants of change in certain key variables (e.g. peak exercise capacity [VO_2 peak]) seeking to identify novel biomarkers of susceptibility to and tolerance of hypoxia as well as candidate mechanisms. Stage 3 papers will comprise a small group of overarching thematic papers drawing these strands together along with integrative analyses (computational biology/systems medicine) of the combined BioResource. Finally, we will explore the rich CXE BioResource in a hypothesis generating exercise for future studies. Comparison between CXE data and other comparable data resources will become important at this stage in order to validate findings derived from the CXE data and to further develop the BioResource [23, 24].

Early publications from the study have focused on unique high altitude measurements [18–20, 25–27], manuscripts exploring metabolic adaptation to hypoxia [28–33], novel breathing circuit performance [34, 35] and pilot data from the preparatory studies [36–38]. Manuscripts reporting data from the large trekker cohort are currently under review.

28.12 Conclusions

Large-scale, tightly controlled applied human physiology studies with high quality measurement can be safely conducted in the remote and austere environment of the trek up to EBC and the terrain higher on Everest. CXE achieved this goal through a combination of clearly prioritized aims, careful preparation, and a team of exceptional individuals working together within a defined and accepted organizational structure. The success of this approach should be judged on the quality of the scientific work resulting and achievement of the primary stated aim: to improve understanding of critical illness and to develop novel management strategies for critically ill patients.

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Chapter 29
Energy Flux, Lactate Shuttling, Mitochondrial Dynamics, and Hypoxia

George A. Brooks

Abstract Our understanding of what happens in working muscle and at the whole-body level at sea level and at high altitude is different from that a few years ago. If dietary CHO and nutrition are adequate, at sea level metabolism shifts from a mix of lipid and CHO-derived fuels toward carbohydrate (glycogen, glucose, and lactate) oxidation at moderate and greater exercise intensities. As given by the Crossover Concept, a percentage to total energy expenditure, lipid oxidation is greatest at exercise power outputs eliciting 45–50 % of VO_2max with greater intensities requiring relatively more CHO and lesser lipid oxidation. At altitude, a given exercise power output is achieved at a greater relative intensity expressed as % VO_2max. Hence, exercise under conditions of hypoxia requires greater glycolytic flux, and lactate production than does the same effort at sea level, normoxic conditions. Glycolytic flux is further augmented at altitude by the effect of hypoxemia on sympathetic nervous system activity. Hence, augmented lactate production during exercise is adaptive. Over the short term, accelerated lactate flux provides ATP supporting muscle contraction and balances cytosolic redox. As well, lactate provides and energy substrate and gluconeogenic precursor. Over a longer term, via redox and ROS-generating mechanisms, lactate may affect adaptations in mitochondrial biogenesis and solute (glucose and lactate) transport. While important, the energy substrate, gluconeogenic, and signaling qualities of lactate production and disposal at altitude need to be considered within the context of overall dietary energy intake and expenditure during exercise at sea level and high altitude.

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29.1 Background

29.1.1 Historical Perspective

Questions related to the balance of oxidative and glycolytic energy flux are traceable to the beginnings of modern biology. Working from the perspective of nineteenth century fermentation technologists who observed acid byproducts to accumulate in cultured yeast and bacteria cultured in the absence of oxygen, in the early twentieth century investigators working with non-perfused and deoxygenated frog muscle preparations readily concluded that lactic acidosis was attributable to oxygen lack [1]. However, it is now apparent that the Warburg Effect of aerobic glycolysis is not specific to tumor cells, but that glycolysis leading to lactate production is characteristic of mammalian cells whether studied in vitro or in vivo. Since the 1920s, interest in understanding the control of lactate production has been common to such disparate fields as exercise physiology, neuroscience, and tumor biology. More recently, focus has been on the Lactate Shuttle Mechanism to understand the pathways, controls, and physiological roles of lactate production and disposal. Based on simultaneous determinations of glucose and lactate flux rates in laboratory rodents [2, 3], as originally conceived, the Lactate Shuttle was hypothesized to be a mechanism to support glycolytic and aerobic energy production via oxidation and glucose homeostasis via gluconeogenesis [4]. However, via redox, ROS, and other mechanisms, lactate is being investigated for its role as a signaling molecule, i.e., as a "Lactormone." In the contemporary literature in the field of neurobiology contains many investigations on Lactate Shuttling for neuron metabolism, glutaminergic signaling, peripheral energy store sensing, and appetite regulation [5] (Fig. 29.1). Not surprisingly, Larsen et al. [6] have shown that when arterial lactate rises during exercise, lactate is taken up and oxidized such that it substitutes, in part, for glucose. This very same effect has been demonstrated by Miller et al. [7] using lactate-clamp technology. Broad acceptance of the Lactate Shuttle Concept was demonstrated by investigators who have sought to disrupt the Lactate Shuttle as a mechanism for killing tumor cells [8]. In sum, while the field has been, and remains, controversial, there is widespread interest in lactate as is it obvious to many that critically important stress and strain mechanisms involve lactate formation and disposal.

29.1.2 Early Studies at Altitude

Not to be inattentive or otherwise neglectful of then contemporary science, as described by Reeves [9], pioneer workers such as Dill et al. [10] and Edwards et al. [11] took an early interest in the lactate response at altitude. In the 1929 expeditions to Leadville, CO [10] and the Andes in Chile [11] observed in unacclimatized sojourners that during rest and exercise, blood lactate concentrations were evaluated at altitude above those seen at sea level. The conclusion at that time regarding

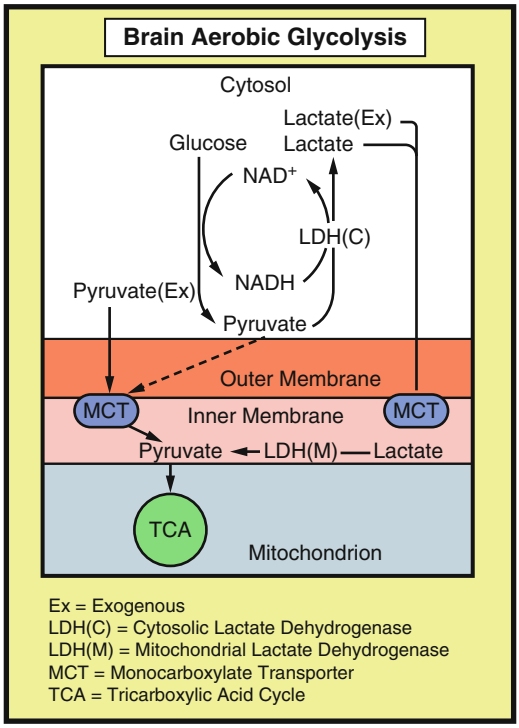

Fig. 29.1 A schematic illustration of the brain glycolytic pathway under aerobic conditions. A schematic illustration of the brain glycolytic pathway under aerobic conditions in which glucose is broken down to the glycolytic end-product lactate. Lactate, in turn, becomes the mitochondrial substrate, via its conversion to pyruvate, for the tricarboxylic acid cycle. At maximal work load, as measured in the study of Larsen et al. [6], most of the lactate that is utilized by brain mitochondria is muscular in origin and thus exogenous. Under resting conditions, most of the lactate used by brain mitochondria originates from glucose that is metabolized glycolytically in neurons and glia. From Schurr [5]

the elevation in blood lactate was classic and based on the Pasteur Effect, glycolytic flux was increased because oxidative metabolism was limited. However, the pioneer investigators observed that after acclimatization to altitude, resting, and exercise, blood lactate levels declined despite the persistence of hypoxemia. And, moreover, other paradoxical observations, such as an inverse relationship between altitude and blood lactate levels, at the end of maximal exertion while blood lactate is elevated during submaximal exertion have been of interest [12]. Like the pioneers, we and others (e.g., [14]) have continued to be perplexed by the original and subsequent observations. In all of this could invocation of the term "paradox" simply mean that our understanding is inadequate?

Certainly there are many complications in studying metabolic responses to stress, but prominent in the field of carbohydrate metabolism at altitude are factors of ambient hypoxia, cachexia and malnutrition, changes in body mass and composition, changes in temperature, humidity, and photoperiod. Superimposed on those, some-

times also are factors associated with gender and racial differences, the stresses of travel, change of season, and hemisphere. And to complicate things more has to be recognition that acclimatization occurs and that experience at altitude and exposure to hypoxia affect outcomes. Hence, it is the position of this writer that the seemingly incongruent results obtained by diverse researchers are all appropriate for the conditions studied. However, with my colleagues at the USARIEM and inspired by J.T. Reeves and colleagues at the UCHSC, we have tried to dissect out and describe the effects of acute and chronic exposure hypoxia on energy substrate partitioning. Over the course of almost a decade, most of it before and subsequent to my participation in the effort, many contributions have been made in describing the metabolic responses of men and women to high altitude. I will try to do justice to this work, but at the outset I need to identify the key and essential contribution of Gail Butterfield to our efforts. Describing her seminal contribution provides insight into what we did, how we interpret the results, and how we read and interpret the literature.

From working with my colleagues on Pikes Peak, and now after almost four decades of studying intermediary metabolism, I have come to ask beginning students the following question: "What are the boundaries between nutrition and biochemistry, and between biochemistry and physiology?" Then, after hearing that the boundaries are hard to identify, I sometimes ask as second question: "What is the difference between stress and strain." Ultimately, we end up in a continuing discussion about the interrelatedness of biological processes and conclude with increasing admiration for the complicated beauty of regulatory processes.

29.1.3 Dietary Controls

Because Gail Butterfield anticipated that the stress of altitude could affect metabolic rate, but that hypoxemia would cause a cachexia such that together the two effects of acute altitude exposure would cause an energy deficit that would affect metabolism, body mass, and body composition. She also knew that changes in the latter two (body mass and composition) would further affect metabolism. Hence, Gail insisted on tight dietary controls at lea level and at altitude. The following figures for the 1988 Pikes Peak Study illustrate what happens. Indeed, she and colleagues showed that BMR is elevated at 4300 m altitude and the increase persists over 3-weeks acclimatization [16] (Fig. 29.2). As well, because of the stress of altitude and the resulting energy deficit nitrogen balance is negative. The problem with nitrogen balance was corrected by the addition of ≈500 kcal/day depending on the subject's response, so weight loss at altitude was minimized, though not prevented in two of seven subjects. And finally, in the same study it was shown that catecholamines are elevated on arrival at altitude, and while the epinephrine response diminishes over time, if anything circulating norepinephrine rises showing adrenal medullary activation at altitude [17]. Consequently, in subsequent investigations, investigators revisited issues related to sympathetic influences in the acute and chronic responses to altitude exposure.

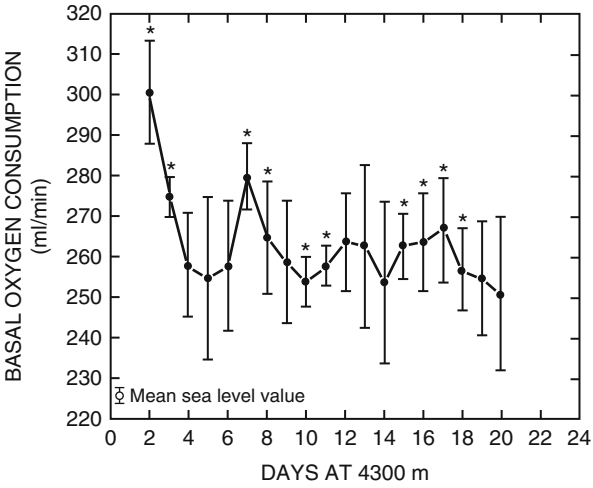

Fig. 29.2 Basal oxygen consumption at sea level and at 4300 m. Values are mean ± SEM for seven males. * Significant difference from sea level throughout. The elevation at day 6 was associated with the addition of ≈500 kcal/day to cover in increase in basal energy expenditure. From Butterfield et al. [16]

With adequate controls of macronutrient and energy nutrition in two studies on Pikes Peak, we [18, 19] saw increased glucose utilization during rest and a given absolute intensity of exercise (e.g., 100 W); effects on glucose flux and oxidation are greatest in men on arrival, but persists for 3 weeks at least even with best attempts at insuring dietary energy balance. With regard to hypoxia increasing glucose use, this observation is typical of tissues and cells in vitro [20], and nonhuman mammals [21]. However, what appears to be clear for the effect of hypoxia on metabolism is, in fact, not clear at all because according to the Crossover Concept [2], substrate flux and energy substrate partitioning are geared to relative exercise intensity. This effect of a given task being relatively more difficult at altitude than sea level was addressed by Braun et al. [23] who studied women at sea level at a power output eliciting 50 % VO_2peak as was done previously for men [18, 19], but they determined also the glucose flux at 65 % VO_2peak at SL in order to make a relative comparison to what happens at 4300 m. What Braun et al. found was that women responded differently from men, and when normalized to total energy expenditure (kcal/min), glucose flux was normalized over altitude and exercise intensity in women and 10 days after exposure to 4300 m.

29.1.4 The Fatty Acid Paradox

In the past, weight loss has been part of the tradition of altitude exposure, but the ritualistic hypophagia and dehydration dietary practices are not conducive to determining the effects of hypoxia on metabolism. And, the data obtained in the 1991 Pikes Peak study in which half the subjects were β-blocked reveals yet another, and relatively uninvestigated phenomenon; this is the "Fatty Acid Paradox." Whether rest or exercise, β-blocked or not, arterial [FFA] rises during exercise [24]

(Fig. 29.3). However, in contrast to what is typical at sea level, a rise in [FFA] during exercise at altitude reflects not increased, but decreased use as the net FFA uptake [limb blood flow (a-v)] for working limb muscle declines to zero as the RQ approaches unity [24]. Clearly, within the context of energy balance and appropriate CHO nutrition working muscle uses little lipid as a substrate. In fact, we [25, 26] have recently shown that little lipid is used in working limb muscle at sea level. In

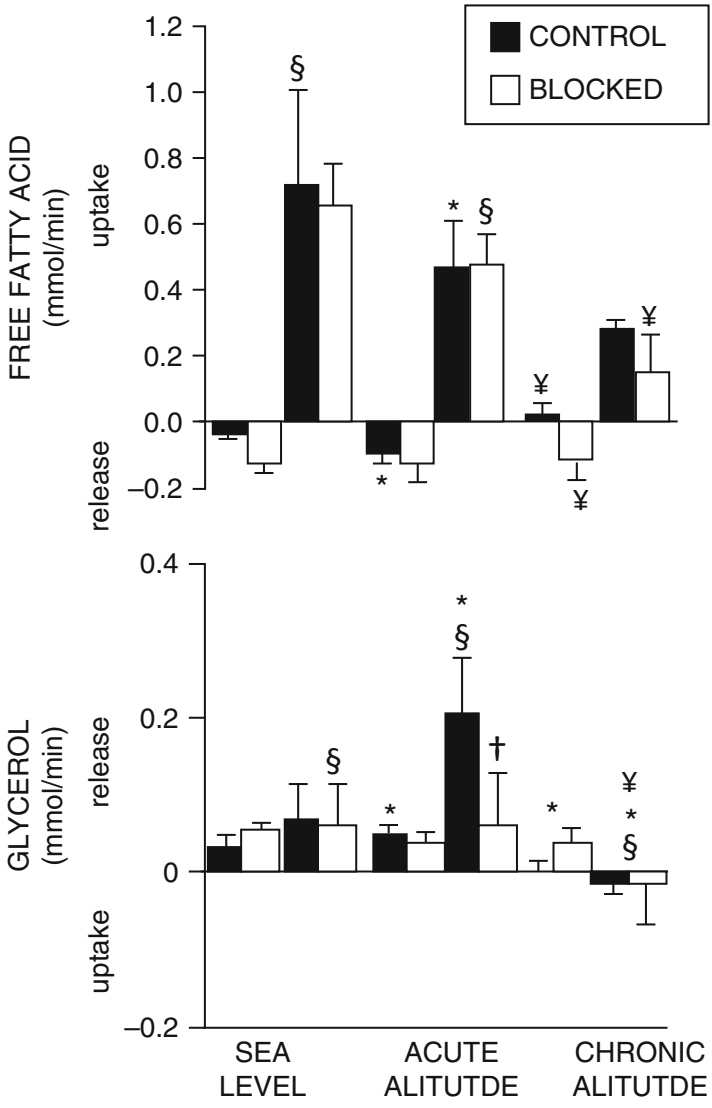

Fig. 29.3 Mean free fatty acid and glycerol exchanges across the lets (mean ± SEM) in five control and six β-blocked men at rest and during exercise at sea level, upon acute exposure to 4300 m altitude, and after 3 weeks at 4300 m. Data from Roberts et al. [24]

the light of these data showing the dominance of CHO fuel energy use in working muscle, if it occurs inadequate total and CHO nutrition at altitude deprives muscle of it's preferred substrate, forces use of less preferred fatty- and amino acids and results in the loss of body mass. That mountaineers reach great heights while malnourished and dehydrated is sheer testament to their inner and physical strength.

29.2 What People Do Determines What They See

29.2.1 The Lactate Faux Pas

It is historic that much of the discussion of metabolism at high altitude has centered on lactate. In a broader sense, this focus on but one aspect of CHO metabolism has been to ignore effects of hypoxia and environment on the possibilities of fatty acid and amino acid paradoxes. With regard to the historic interest and discussion of a "Lactate Paradox," the dialogue is useful to the extent that it furthers our understanding of what happens at altitude and during exercise at altitude. Hence, while the pioneers and more recent investigators have observed phenomena descriptive of a "paradox," others have not. To reiterate, because I believe all the data to be valid, variability in the results means, to me, that different studies were done on different persons, at different times in different places. Time and space are limited so the discussion is compressed to CHO nutrition, gender, and mechanism.

29.2.2 Macronutrient Nutrition and Exercise Metabolism

In the early 1980s, we [27] and Heigenhauser et al. [28] independently studied the effects of glycogen depletion on ventilatory and blood lactate responses to graded exercises. The two studies showed glycogen-depleted subjects the ventilatory threshold came at a lower exercise power output than when the same subjects were not glycogen depleted. In contrast to the VT is displaced left, the lactate response and hence, the Lactate Threshold (LT) is right-shifted and appears at a higher power output. Therefore, we can reasonably expect to see that in subjects who have lost body weight at altitude, the tendency for glycolysis to be accelerated will be counterbalanced by the effect of glycogen depletion.

29.2.3 Gender

In his recent review appearing in MSSE, Braun [29] addressed the mechanism of increased metabolic efficiency at altitude and produced an interpretation similar to that presented here. Using some of data previously shown above, Braun compared

metabolic responses at altitude in partially acclimatized men and women at altitude. In an altitude-induced shift to increased glucose use is evident, whereas this shift is not evident in women. Indeed, in a series of investigations by Braun and colleagues have shown a greater metabolic resilience in women compared to men at altitude. As well, independently we have shown differences in fatty acid [30] and glucose metabolism [31] in men and women during exercise and recovery from exercise at sea level.

29.2.4 Mechanism

Greater glucose disposal rates, but unchanged insulin levels imply increased insulin sensitivity at altitude [18, 19]. In subjects studied with dietary controls, Braun (ref) demonstrated increased insulin sensitivity in subjects 10 days after arrival on Pikes Peak (4300 m). However, by stimulating epinephrine, altitude exposure has the possibility to elicit insulin resistance. Such an effect may have been observed by Larsen et al. on Monte Rosa (4559 m) [32] in subjects studied 2 days after arrival at altitude exposure. Limited data are available on mechanism of hypoxia-stimulated muscle glucose uptake, but it appears that AMPKα2 is upregulated in hypoxia as it is during normoxic exercise [33].

Glycogenolysis, and hence lactate production during rest and exercise, is influenced by the sympathetic response to hypoxia. Epinephrine rises on exposure and is associated with increments in muscle glucose uptake [18] and lactate flux and accumulation [17] (Fig. 29.4). In our experience, the rise in epinephrine wanes with exposure, but it is apparent also that sensitivity to epinephrine rises over time with

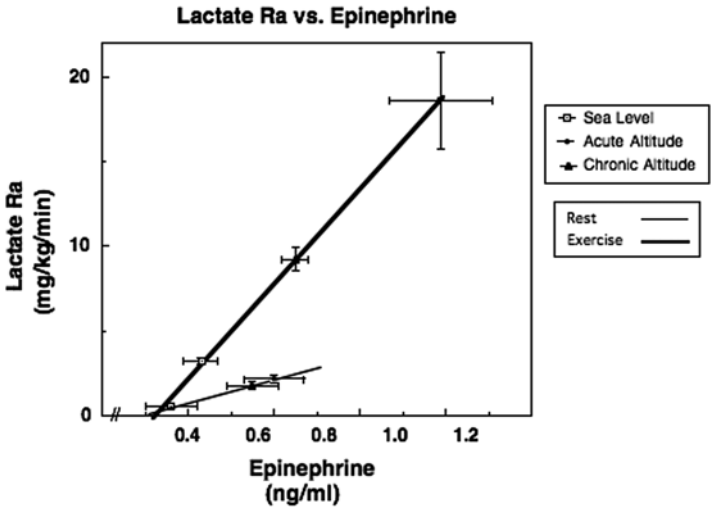

Fig. 29.4 Mean blood lactate rate of appearance (Ra) [±SEM] as a function of mean epinephrine concentration [±SEM] in men at sea level (*open squares*), upon acute exposure to 4300 m altitude (*diamonds*), and after 3 weeks of acclimatization to 4300 m (*triangles*). From Brooks et al. [46]

exposure to altitude. This effect of acclimatization was revealed in studies on Pikes Peak using β-blockade that increased glucose and decreased fatty acid uptake [18, 19]. In contrast to epinephrine, norepinephrine rises with acclimatization [17]. Hence, it is apparent that the changes in substrate utilization that occur over time correspond to epinephrine. It needs to be noted, however, that while the pattern of catecholamine response to altitude was similar in men and women, metabolic responses to altitude were less perturbed in women than men.

29.3 Why Lactate?

29.3.1 The Oxygen Deficit Shortfall

The conventional view that an oxygen deficit is responsible for lactate production and accumulation during rest or exercise is unsupported in the literature. As well, there is confusion equating lactate "accumulation" and "production," the mistake reflecting the assumption that lactate is a "dead end," as opposed to dynamic metabolic intermediate that servers multiple functions. And, even associating lactate production and accumulation, the conclusion that either or both are attributable to oxygen limitations is not supportable. Numerous times we [12, 34–38] and others have shown lactate production in resting individuals at sea level. Importantly, we have shown that the decline in blood [lactate] that accompanies exercise after training is largely attributable to enhanced clearance, and secondarily to better matching of glycolytic flux to energy demand. Whether at rest or during exercise, whether at sea level or 4300 m altitude, or during submaximal exercise at altitude with or without β-blockade, the circulation transports sufficient oxygen to maintain working muscle oxygen consumption [24, 38].

With regard to the state of tissue oxygenation as causal to working muscle lactate accumulation and net release, our results and interpretations are entirely consistent with those of Richardson, Wagner and associates who studied the effect of breathing gas containing 12 and 21 % O_2 at 1 ATM ambient pressure on circulating epinephrine and muscle oxygenation and lactate net release (efflux) during graded exercise [40]. The hypoxemia of breathing 12 % O_2 increased muscle lactate efflux while having a minimal effect on oxygenation which was 3–4 Torr and well above the critical mitochondrial PO_2 of ≈1.0 Torr. Instead of a deficiency in O_2 supply or muscle oxygenation, hypoxemia left shifted relationships between arterial epinephrine and working leg muscle VO_2, and between muscle lactate efflux and working leg muscle VO_2. As a result, net muscle lactate efflux was related to arterial epinephrine level.

In gaining an appreciation for the magnitude of lactate flux at rest, and the effects of exercise, acute hypoxia, and altitude acclimatization on it, it is useful to compare glucose and lactate fluxes determined simultaneously using dual tracers. From our first study on Pikes Peak, the resting 12-h glucose flux at sea level was ≈2 mg/kg/min, and the lactate flux about a third of the glucose value. On acute exposure, both fluxes rose

significantly, but not the lactate and glucose fluxes were approximately equal. And, after a 3-weeks sojourn at 4300 m, the lactate flux declined somewhat, as did epinephrine, but the glucose flux rose still further [36]. During 100 W leg cycle ergometer exercise at SL, glucose flux doubled over rest, and lactate flux approximated the glucose flux. On acute exposure to altitude, glucose flux increased over that at sea level, but the lactate flux increased many times more. With acclimatization, the glucose flux during exercise increased over that on initial exposure, while the lactate flux declined.

29.3.2 Stainsby and Other Effects

Our operating hypothesis is that of the Lactate Shuttle which now has Cell-Cell and Intracellular components. We and others have observed net release by particular tissues (e.g., working muscle at the onset of exercise) and net consumption by others (e.g., the heart) [41]. We know also that tissues such as a working muscle bed can switch from net consumption to release and that simultaneous production and utilization always occurs, thus obscuring the extent of turnover within cells and tissues. A prime example of this "Stainsby Effect" where by working muscle switches from lactate net release to uptake was seen in men studied at SL on Pikes Peak both before and after acclimatization (39 Brooks). The results are to be contrasted with the corresponding arterial blood levels, and example of which is found in observations that stable blood lactate levels were achieved during exercise with the lowest values being observed at SL, the highest on acute exposure, and with intermediate blood lactate levels after the 3-weeks acclimatization period. However, as seen in, regardless of altitude condition, lactate release is a feature after 5 min of exercise, with highest net release on acute exposure but declining net release transitioning in some cases to net uptake all the while arterial lactate concentration is elevated and pulmonary and working limb rates of oxygen consumption elevated over rest, but not different due to condition during exercise. That so many cells and tissues can produce and exchange lactate makes the task of understanding lactate from blood concentration (a-v) difference, biopsy, or flux measurements far more difficult than understanding glucose flux that under postabsorptive conditions is by the liver with one point of entry into the circulation.

29.4 A Different View of the Role of Lactate

29.4.1 The Lactate Shuttle Mechanism

The lactate shuttle works because lactate, the product of glycolysis, is the substrate for oxidative metabolism. Further, because MCTs are ubiquitous, lactate can shuttle down concentration and H^+ gradients, fluxing between cellular organelles (e.g.,

cytosol and mitochondria, and cytosol and peroxisomes), between cells in the same tissue (e.g., white Type 2b(x) and red Type 1 fibers), and between tissues and organs via vascular conductance (e.g., working muscle and heart). As mentioned earlier, there is utility in the formation and lactate exchange because lactate is an important energy source, the most important gluconeogenic precursor, and a lactormone [4]. The realization that lactate shuttles as it does lead to discovery that membrane permeation is via facilitated transport involving characteristics of saturation, stereo-specificity, competition, hydrogen ion co-transport, bi-directionality, and inhibition by specific molecules. These lactate transport proteins are now recognized to be products of a gene superfamily of solute transporters (SLC16), of which the first four (monocarboxylate transporters 1–4 (i.e., MCT1–MCT4)) are lactate-pyruvate transporters. Of these, MCT1 appears to be most ubiquitously expressed, but there being cell type and cell domain differences in MCT expression.

Having participated in discovery of the proteins now called MCTs and having also helped demonstrate their tissue and cell type expression and compartmentation, my bias is that lactate/pyruvate transporters are important. However, it is important to know that while they are lactate anion-proton symporters, MCTs are not the only means for cells to extrude protons. Further, so far as solute transport is concerned, the MCTs support facilitated exchange down concentration and pH gradients. More important, therefore, are the processes that establish the concentration gradients; here I speak of the circulation and mitochondrial respiration. The circulation is essential because it delivers oxygen for mitochondrial respiration and also because it is responsible for bulk flow delivery of lactate from cells and tissues of production to disposal sites.

29.4.2 Muscle Lactate Oxidation, the Mitochondrial Lactate Oxidation Complex, and Mitochondrial Dynamics

To reiterate, there are at least three biological functions of lactate (substrate oxidation, gluconeogenic precursor, and signaling molecule) [4]. Unfortunately, working muscle and whole-body lactate oxidation rates are less well worked out at altitude, but there are very good data for sea level. From Bergman et al. [33], Stanley et al. [34], Mazzeo et al. [35], and Brooks et al. [12], we know that lactate oxidation tracks disposal rate, and that working muscle, resting or working in the range of 45–65 % of VO_2max accounts for 50–60 % of lactate Rd. At altitude, we have as yet to measure pulmonary and working muscle $^{13}CO_2$ production following vascular infusion of ^{13}C-lactate tracer, but from efforts on Pikes Peak we know that arterial lactate concentration, tracer-measured lactate production and disposal, circulating epinephrine are highly correlated [36]. As well, from tracer-measured lactate fractional extraction during rest and during exercise at SL and after acute and chronic exposures to 4300 m we know that lactate extraction is concentration dependent [37].

29.4.3 The Mitochondrial Lactate Oxidation Complex (mLOC)

That working muscles and heart oxidize lactate raises the question as to how. Some have concluded that lactate is oxidized to pyruvate in working muscle, but cytosolic lactate oxidation is unlikely, and contrary to data available on humans and dogs as studied by Zinker et al. [21]. With dogs made hypoxic by breathing 11 % O_2 gas, they observed net lactate exchange and net and tracer-measure glucose uptake. They found that hypoxia increased glucose flux and working limb glucose and lactate uptake. More importantly, from carbon-labeled glucose they observed that half the glucose uptake observed was released as lactate. Hence, as with our experience with humans, the data of Zinker et al. are consistent with cytosolic glycolysis and contrary to cytosolic lactate oxidation. However, the data on running dogs is extraordinary showing simultaneous glycolysis to lactate and net lactate uptake.

Given our results and those of others, we took the obvious steps to investigate direct mitochondrial oxidation of lactate. We took several approaches to the problem. First, we [42] respired mitochondria from rat liver, heart, and skeletal muscle and found that respiration with each preparation was greater with lactate + malate than with pyruvate + malate. These findings were taken to mean that mitochondria require a lactate/pyruvate transporter and lactate dehydrogenase (LDH). To evaluate the potential role of LDH in mitochondrial lactate oxidation, we treated mitochondria with oxamate, and inhibitor of LDH, and found that lactate oxidation was inhibited whereas pyruvate oxidation was increased. To evaluate the potential role of MCT1, we treated mitochondria with cinnamate, a known MCT inhibitor, and respiration with both lactate and pyruvate was blocked. However, with the addition of either succinate or glutamate, the blockage to MCT inhibition was bypassed. Subsequently, a more careful reading of the literature revealed that these effects had been demonstrated previously, but the results were overlooked.

To demonstrate the presence of mitochondrial LDH (mLDH) and MCT1 (mMCT1), we isolated mitochondria and probed for the presence of mLDH and mMCT1 obtaining positive results. And when others failed to replicate our results, we sought to demonstrate mLDH and mMCT1 by electron microscopy (for LDH) and confocal laser scanning microscopy (LDH, MCT1 and other proteins). In both adult cells and cultured myocytes, mMCT1 and mLDH (Fig. 29.5) are clearly visible [43, 44]. Further, because the results were suggestive of the presence of a mitochondrial lactate oxidation complex (mLOC) comprised minimally of MCT1, LDH, Basigin, and cytochrome oxidase (COX). And, using combinations of cell fractionation, differential centrifugation and Western blotting, as well as immunocoprecipitation, we have verified presence of the mLOC in cultured myocytes and adult limb skeletal muscle. Realizing generality of our findings on muscle, we collaborated with Daniela Kaufer, an acclaimed neurobiologist and demonstrated the mLOC in primary neuronal cell cultures and adult rat brains [45]. Bolstered by publication of the MitoCarta [46] that contains the purported four mLOC proteins, at present we are utilizing muscle cell culture, pulse-chase tracer technology, and the most advanced techniques of proteomics to independently verify the existence of mLOC proteins, and to identify the factors responsible for mitochondrial biogenesis and protein turnover rates.

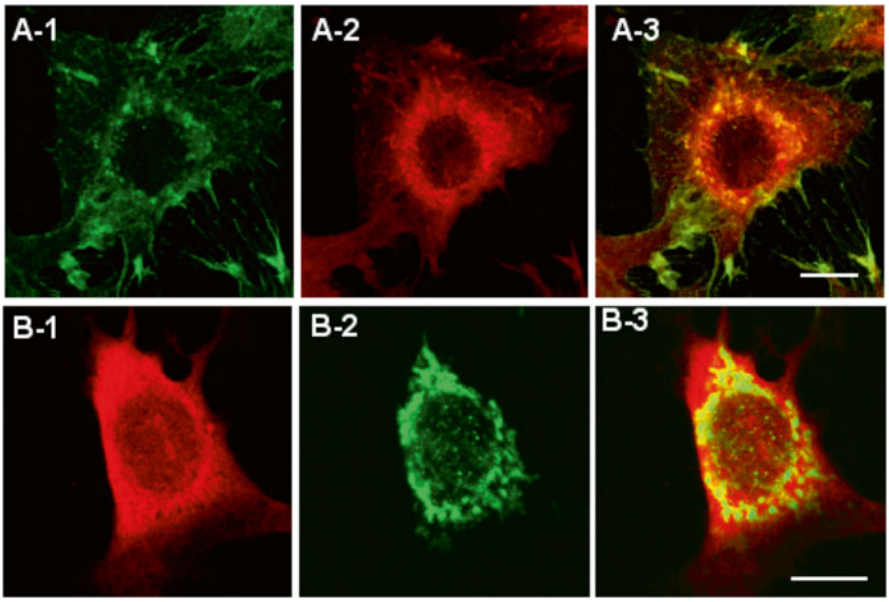

Fig. 29.5 Immunohistochemical images demonstrating some components of the lactate oxidation complex in L6, cultured rat muscle cells. This complex involves the mitochondrial constituent cytochrome oxidase, the lactate transport protein, lactate dehydrogenase (LDH), and other constituents. (**a**) Co-localization of the lactate transport protein (MCT1) and the mitochondrial reticulum. MCT1 was detected at both sarcolemmal and intracellular domains (A-1). The mitochondrial reticulum was extensively elaborated and detected at intracellular domains throughout L6 cells (A-2). When signals from probes for the lactate transporter (MCT1, *green*, A-1) and mitochondria (*red*, A-2) were merged, superposition of the signals (*yellow*) showed co-localization of MCT1 and components of the mitochondrial reticulum, particularly at perinuclear cell domains (A-3). In panel (**b**), Lactate dehydrogenase (LDH) (B-1) and mitochondrial cytochrome oxidase (COX) where most oxygen is actually used in cells (B-2) are imaged. Superposition of signals for LDH (*red*, B-1) and COX (*green*, B-2) shows co-localization of LDH in the mitochondrial reticulum (*yellow*) of cultured L6 rat muscle cells (B-3). Depth of field ~1 μm, scale bar = 10 μm. From Hashimoto et al. [44]

Getting close to closing, it is essential to mention a new field in which we are returning to an old theme that has been reinvigorated by new technology and discoveries. Today, it is passé to use terminology "mitochondrion" and "mitochondria" as terms mitochondrial "reticulum," "network," and "dynamics" have come into use.

The exploding field of mitochondrial dynamics has shown that structure of the mitochondrial reticulum constantly undergoes turnover. Fission involves the transmembrane protein fission-1 (Fis1) and dynamin-related (like) protein (Drp1 or DLP1). The mechanism has been reviewed by Yoon [47] who summarizes his work and that of others in this exciting and emerging field. Drp1 is a large GTPase (80–85 kDa, with multiple splicing variants) that assemble onto the outer surface of mitochondria during mitochondrial fission and sever the mitochondrial membrane via the GTP hydrolysis-mediated mechanical pinching. Mitochondrial scission appears to

involve Fis1, a small helix-rich mitochondrial outer membrane protein [48]. The Drp1-Fis1 complex apparently acts as a molecular noose pinching off sections of the mitochondrial reticulum in effect causing fission. A "normal," but as yet unknown rate of mitochondrial turnover is apparently necessary because Yoon [47] has shown mitochondrial dysfunction in cells overexpressing Fis1. In contrast, in recent case study [49], it was reported on the lethal effect in a newborn of a defect in Drp1 as rendering the protein ineffective. Significant current research includes our own focuses on Drp1 as it is the only mGTPase known to be regulated by phosphorylation (51). Following their lead, we are following up with studies of the effects of lactate, other ROS generators, and putative regulators of mitochondrial biogenesis.

29.5 Summary

This portrayal of what happens in working muscle and at the whole-body level is different from what might have been rendered a few years ago. If dietary CHO and nutrition are adequate, metabolism shifts toward glucose and total CHO oxidation. As given by the Crossover Concept, a percentage to total energy expenditure, lipid oxidation is greatest at exercise power outputs eliciting 45–50 % of VO_2max with greater intensities requiring relatively more CHO, and lesser lipid oxidation. At altitude, a given exercise power output is achieved at a greater relative intensity expressed as % VO_2max. Hence, exercise under conditions of hypoxia requires greater glycolytic flux, and lactate production than does the same effort at sea level, normoxic conditions. Glycolytic flux is further augmented at altitude by the effect of hypoxemia on sympathetic nervous system activity. Hence, augmented lactate production during exercise is adaptive. Over the short term, accelerated lactate flux provides ATP supporting muscle contraction and balances cytosolic redox. As well, lactate provides an energy substrate and gluconeogenic precursor. Over a longer term, via redox and ROS-generating mechanisms, lactate may affect adaptations in mitochondrial biogenesis and solute (glucose and lactate) transport.

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Chapter 30
Everest Physiology Pre-2008

John B. West

Abstract When Edmund Hillary and Tenzing Norgay reached the summit of Mt. Everest in 1953, it was the culmination of many attempts beginning in 1921. Alexander Kellas had actually predicted as early as 1920 that the mountain could be climbed, but the extreme altitude of 8848 m with the consequent oxygen deprivation had foiled previous attempts. One reason for the success of the 1953 expedition was the work done by the British physiologist Griffith Pugh in 1952 when he studied many of the physiological factors at high altitude including the oxygen requirements. Seven years later, Pugh and Hillary teamed up again for the Silver Hut Expedition in 1960–1961 that elucidated many of the problems of very high altitude. A group of physiologists spent several months at an altitude of 5800 m in a prefabricated hut and studied many aspects of exercise, pulmonary gas exchange, control of ventilation, and blood changes. Maximal exercise was measured as high as 7440 m and raised anew the question of whether Everest could ever be climbed without supplementary oxygen. The answer was shown to be yes in 1978 by Messner and Habeler, and 3 years later the American Medical Research Expedition to Everest clarified the physiological adaptations that allow humans to reach the highest point on earth. Five people reached the summit, the barometric pressure there was measured for the first time, and alveolar gas samples from the summit showed the critical importance of the extreme hyperventilation. However, the maximal oxygen consumption for the summit inspired PO_2 of 43 mmHg was shown to be only about 1 l min^{-1}. In other words, the highest point on earth is very close to the limit of human tolerance to oxygen deprivation. As we celebrate the anniversary of Charles Darwin, it would be nice to have an evolutionary explanation for this, but in fact it is a cosmic coincidence.

Keywords Extreme altitude • Severe hypoxia • Maximum oxygen uptake • Respiratory alkalosis • Ascent of Everest

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When Edmund Hillary and Tenzing Norgay reached the summit of Mt. Everest, altitude 8848 m, on May 29, 1953, the event was the culmination of a large number of attempts to reach the highest point on earth. Alexander Kellas (1868–1921) (Fig. 30.1) had made a study of some of the physiological problems associated with extreme altitude prior to the first Everest reconnaissance that took place in 1921, and he wrote an article entitled "A Consideration of the Possibility of Ascending Mt. Everest". His conclusion was as follows: "Mt. Everest could be ascended by a man of excellent physical and mental constitution in first-rate training, without adventitious aids [supplementary oxygen] if the physical difficulties of the mountain are not too great, and with the use of oxygen even if the mountain may be classed as difficult from the climbing point of view." This was a remarkable prediction in view of the paucity of information about the physiology of exercise under conditions of extreme hypoxia at the time that Kellas wrote. An interesting historical fact is that his manuscript was not published until 2001 [2].

One of the reasons for the success of the 1953 expedition was the work done by the physiologist L.G.C.E. Pugh (1909–1994) (Fig. 30.2) during an expedition to Cho Oyu, altitude 8153 m, in 1952. The principal objectives were to elucidate the physiological issues occurring at these extreme altitudes, including ventilation rates, effects of the supplementary oxygen, hydration, diet, appropriate clothing, etc. The lessons learned from the Cho Oyo expedition were applied in 1953 and some people believe that they were critical in the expedition's success. It is interesting that in the spring of 1952, a Swiss expedition very nearly reached the summit of Everest but they failed for two physiological reasons [4]. The first was that the oxygen equipment was inadequate in that oxygen could only been inhaled

Fig. 30.1 Alexander M. Kellas (1868–1921) who studied the physiology of extreme altitude in 1920 and predicted that Everest could be climbed without supplementary oxygen "if the physical difficulties of the mountain are not too great." From [3]

Fig. 30.2 Griffith Pugh (1909–1994) who studied aspects of the physiology of extreme altitude on Cho Oyu in 1952 and paved the way for the first ascent of Everest in 1953. From [3]

during rest periods, but not while climbing. The other physiological mishap was that the climbers were severely dehydrated at extreme altitude because they did not recognize the critical importance of maintaining adequate fluid balance. Part of the problem was that through a logistical mix-up, a stove was not available at the highest camp with the result that the climbers could not melt snow.

Shortly after the first Everest ascent, Pugh and Hillary discussed possible ways of obtaining more information about human physiology at extreme altitude when they were together on an Antarctic expedition in 1956–1957. The upshot was an expedition formally called the Himalayan Scientific and Mountaineering Expedition, 1960–1961, but now always referred to as the Silver Hut Expedition. This was in three parts. During the fall of 1960, a prefabricated hut was carried into a region about 10 miles south of Everest (Fig. 30.3). Then during the winter, a group of some seven physiologists spent several months at an altitude of 5800 m (19,000 ft) studying the response of lowlanders to this very high altitude. At that time, no one had previously spent such a long period at such a high altitude, and it was not clear that this could be tolerated. In fact, there were two slightly lower camps that expedition members could descend to if necessary [1].

The primary scientific objective of the Silver Hut Expedition was to elucidate the physiological changes that occur in lowlanders during exposure to an altitude of the 5800 m (barometric pressure 380 mmHg) for several months. Among the topics investigated were exercise including maximal oxygen consumption, maximal ventilation and cardiac output; pulmonary gas exchange including the arterial oxygen saturation during work and the diffusing capacity of lung for carbon monoxide; the control of ventilation at this very high altitude, blood changes, electrocardiogram, weight loss, and neuropsychological function.

At the end of the wintering period, the expedition moved across to Makalu, the fifth highest mountain in the world, altitude 8481 m, and attempted to climb this without using supplementary oxygen. Indeed if the climb had been success-

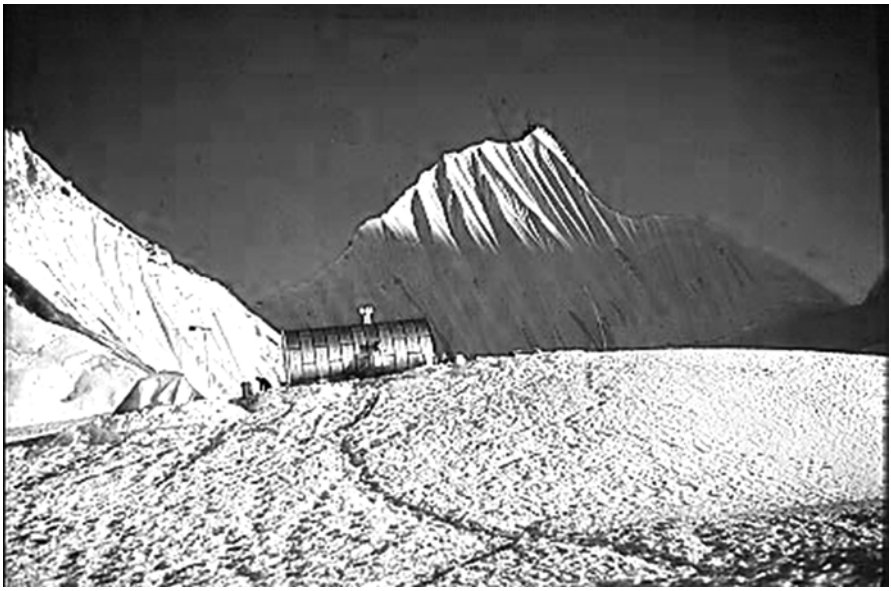

Fig. 30.3 The Silver Hut at an altitude of 5800 m during the Himalayan Scientific and Mountaineering Expedition of 1960–1961. From [3]

ful, this would have been the highest peak attained without oxygen to date. However, one of the climbers collapsed near the summit, and the climb was abandoned to return him to safety. Nevertheless, some measurements were made very high on Makalu. For example, measurements of maximal oxygen consumption were made at an altitude of 7440 m (24,400 ft) using a bicycle ergometer and these remained the highest measurements of work capacity in the field for over 40 years. In addition, alveolar gas samples were collected up to an altitude of 7830 m. The measurements of maximal oxygen consumption were extremely interesting because extrapolation of the line relating oxygen consumption to altitude raised a serious question about whether Everest could ever be climbed without supplementary oxygen (Fig. 30.4). The answer to this question had to wait another 17 years until Messner and Habeler astonished everybody by reaching the Everest summit without oxygen in 1978.

The feat of Messner and Habeler stimulated much speculation on how the human body could do any work at this extraordinary altitude and it was one of the factors that prompted the American Medical Research Expedition to Everest (AMREE) which took place 3 years later. One of its principal scientific objectives was to elucidate the physiological changes that allow humans to reach the highest point on earth. The design of the expedition was considerably influenced by the Silver Hut Expedition, and indeed three physiologists Sukhamay Lahiri, James Milledge, and John West were members of both. Two substantial laboratories were set up, one at the Base Camp, 5400 m, and the other high in the Western Cwm at an altitude of 6300 m. But the main objective was to try to obtain data from the summit and the

expedition was very fortunate. Five members reached the summit and a number of measurements were made there including the first direct measurement of barometric pressure. In addition, alveolar gas samples were collected on the summit, at an altitude of 8400 m, and also at 8050 m. The alveolar gas samples showed the critical importance of extreme hyperventilation at these enormous altitudes. For example, on the summit, Christopher Pizzo, MD who collected the alveolar gas samples there had an alveolar PCO_2 of 7–8 mmHg. Since the normal sea level value is 40 mmHg, this meant that he had increased his alveolar ventilation about fivefold. When the alveolar PO_2 and PCO_2 were plotted as altitude increased, they showed that the alveolar PO_2 declined up to an altitude of about 7000 m but thereafter was defended at a value of about 35 mmHg in the successful climbers (Fig. 30.5). This could only be done by enormously increasing the alveolar ventilation and driving the PCO_2 down to below 10 mmHg.

Another interesting feature of the measurements at the summit was that the arterial pH calculated from the alveolar PCO_2 and the base excess measured in venous blood the following morning was between 7.7 and 7.8. In other words, the climber had an extreme respiratory alkalosis. It turns out that this is beneficial because the resulting increase in the oxygen affinity of the hemoglobin assists the oxygen loading in the pulmonary capillary more than it interferes with the unloading in peripheral capillaries. Measurements were also made of maximal oxygen consumption for the inspired PO_2 on the summit and this came out at just over 1 l/min, a miserable value, equivalent to that seen in walking slowly on the level (Fig. 30.6).

The expedition therefore confirmed what had been suspected since the 1920s that is that the highest point on earth is very close to the limits of human tolerance to oxygen deprivation. As we celebrate Charles Darwin's anniversary this year, it would be nice to come up with evolutionary explanation for this, but in fact it is a cosmic coincidence like the moon being just large enough to hide the sun during a total solar eclipse.

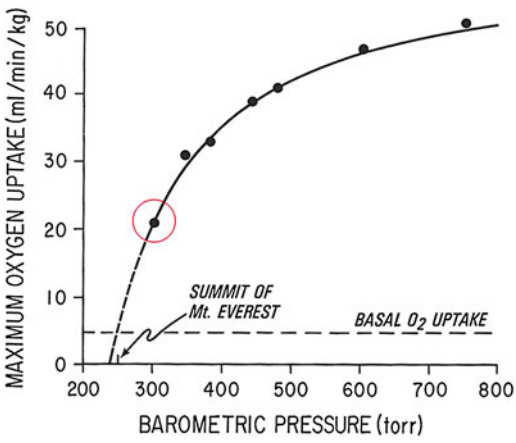

Fig. 30.4 Maximum oxygen consumption plotted against barometric pressure in acclimatized subjects. The *circled* data point was obtained on the Makalu Col, altitude 7440 m. From [3]

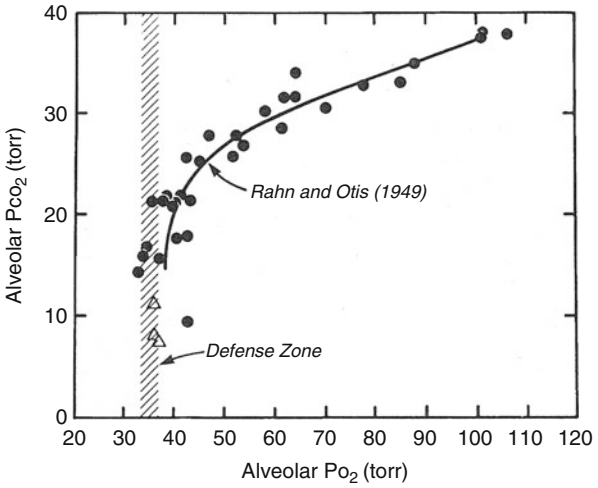

Fig. 30.5 Alveolar PO_2 and PCO_2 in climbers from sea level (*top right*) to the summit of Mt. Everest (*bottom left*). Note that after a certain altitude (about 7000 m) the alveolar PO_2 is defended at a value of about 35 mmHg by the extreme hyperventilation. From [3]

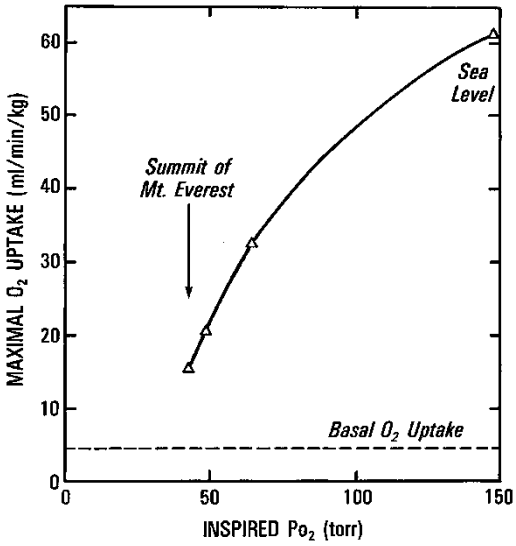

Fig. 30.6 Maximal oxygen uptake plotted against the inspired PO_2 from the American Medical Research Expedition to Everest. Note that for the PO_2 of the summit, the maximal oxygen consumption was only about l min^{-1}. From [3]

Author's Note This manuscript was written as an invited introduction to the presentations by Mike Grocott and Tom Hornbein. It makes no attempt to be a comprehensive coverage of the vast amount of work done on Mount Everest, and it is limited to field studies.

Acknowledgments This work was supported by NIH Grant R01 HL 60968.

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Abstracts from Hypoxia 2009–2015

48 HOURS SUSTAINED HYPOXIA INCREASES HYPERCAPNIC, BUT NOT HYPOXIC CEREBROVASCULAR RESPONSES. Erin A Krizay, Rui C Periera de Sa, Gordon K Prisk, Richard B Buxton, Miriam Scadeng, Frank L Powell, Susan R Hopkins, David J Dubowitz. UCSD. *Email: erin.krizay@gmail.com.* Physiological control of ventilation and cerebral blood flow (CBF) share similar sensitivity to hypoxia & hypercapnia. Previous studies have shown significant increases in hypoxic & hypercapnic ventilatory responses following acclimatization to sustained hypoxia. We investigated if similar mechanisms exist for control of CBF. We simultaneously measured CBF & ventilation using arterial spin labeling MRI (13 subjects, 5 male, 8 female, mean age 29). Data were acquired at 100%, 90%, 80% O_2 sat during isocapnia, and at 90% & 100% during mild hypercapnia ($ETCO_2$ +5 torr). MRI was repeated immediately following 48 hrs sustained hypoxia (3,800m). CBF & ventilation increased during hypoxia & hypercapnia. For 100-90% desaturation: Ventilation increased 0.39±0.24 mL/min/%sat, $p<0.01$, CBF increased 1.32±0.9 mL/100mL/min, $p<0.01$. For 90-80% desaturation: Ventilation increased 0.13±0.2 mL/min/%sat, $p<0.05$, CBF increased 1.11±0.47 mL/100mL/min, $p<0.001$. For hypercapnia@90%sat: Ventilation increased 1.5±0.63 mL/min/torr, $p<0.01$, CBF increased 3.19±2.58 mL/100mL/min/torr, $p<0.01$. During 15-20mins hypoxia there was hypoxic ventilatory decline (-1.58±2.39 mL/min @80%sat, $p<0.05$), but no hypoxic cerebrovascular decline - which if anything was augmented (+1.07±3.05 mL/100mL/min, $p=0.23$). After 48hrs hypoxia, ventilation showed typical acclimatization with increased sensitivity to hypercapnia and to hypoxia. CBF responses to hypercapnia & hypercapnic hypoxia also increased (+1.25 & +1.8 mL/100mL/min/torr, $p<0.05$). However, CBF responses to hypoxia showed no acclimatization (100-90% desaturation: -0.1±1.2 mL/100mL/min, $p=0.8$). Although the pattern of ventilatory & cerebrovascular responses to hypercapnia are similar, hypoxic ventilatory response shows ventilatory decline and acclimatization to 48hrs high altitude hypoxia, whereas CBF responses do not. This suggests a common $PaCO_2$ control for both ventilation & CBF, but independent controls for PaO_2. Supported by R01 NS05393. 2009.

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A COMPARISON OF AMS SCORING SYSTEMS: LAKE LOUISE AND VISUAL ANALOGUE. Adam S Booth, Brynn Bird, Faye Hext, Susannah Patey, Alison Stubbings, Alex Wright. BMRES. *Email: adambooth@doctors.org.uk.* Acute mountain sickness (AMS) is characterised by headache, dizziness, gastrointestinal disturbance, sleep problems and lethargy. There are many systems for assessing AMS, with the Lake Louise Self-Report Score (LLSRS) the most widely used and validated. It has been suggested that using a Visual Analogue Scale (VAS) may improve the sensitivity and specificity of current AMS scoring systems. METHODS: 24 healthy subjects participated in an expedition to the Chilean Altiplano. 48 hours were spent at sea level, 48 hours at 3500m and 4 days at 4400m. Each subject assessed their AMS twice daily using the standard LLSRS and a 10cm line VAS. Scores were calculated for each subject on both systems and were compared using Spearman's correlation coefficient. RESULTS: There was a strong positive correlation ($p<0.05$) between total LLSRS and VAS from sea level to 4400m. Correlations remained strong whether symptoms were minimal or severe: at sea level ($rs=0.91$, $p<0.01$) or 4400m ($rs=0.90$, $p<0.01$). Specifically scores for headache and weakness were also strongly correlated at all time points (sea level headache rs= 0.92, 4400m rs=0.93, sea level weakness rs=0.88, 4400m rs=0.94). Scores for GI, dizziness and sleep were best correlated when symptoms were worst (rs=0.83, 0.77, 0.93 respectively). At all times there was a positive correlation between the two scoring systems but at lower altitudes when symptoms were less, scores for GI, dizziness and sleep were weakly correlated and not highly significant ($rs<0.5$, $p>0.01$). CONCLUSION: Our study suggests that a VAS could provide a simple and more sensitive method for assessing AMS and our results showed strong correlations between VAS and LLSRS, but further studies are required for validation We have shown that VAS is appropriate for assessing both AMS and individual symptoms and propose that the scores could be classified into a "traffic light' warning system. FUNDING: The BMRES. The Arthur Thomson Trust. 2009.

A COMPARISON OF IN VIVO OXYHEMOGLOBIN DISSOCIATION CURVE (ODC) MEASUREMENTS WITH KELMAN MODEL PREDICTIONS. Dahlia Y Balaban, David Preiss, Alexandra Mardimae, Alex Vesely, Marat Slessarev, Richard Greene, Gustavo Zubieta, James Duffin, Joseph A Fisher. University of Toronto, University of British Columbia, University of New Mexico, Clinica IPPA. *Email: dahlia.balaban@utoronto.ca.* The Kelman model predicts SO_2 from blood PO_2, PCO_2, pH, and temperature. We have devised a method for using prospective end-tidal gas targeting to study the ODC in vivo. Our aim was to evaluate the method by comparing SpO_2 measured in vivo with SaO_2 predicted by the Kelman model. We provided conditions for measuring the ODC in vivo by prospectively targeting end-tidal PCO_2 ($PETCO_2$) and PO_2 ($PETO_2$) via a specialized gas blender (RespirAct™, Thornhill Research Inc., Toronto, Canada) and a sequential rebreathing circuit. At 3600 m, we studied 6 lowlanders (2 females) within 2 weeks of arrival at altitude and 8 healthy, chronically acclimatized Bolivian highlanders (3 females). We maintained $PETCO_2$ at each subject's resting level while targeting $PETO_2$s of 100, 70, 60, 50, 40 and 35 mm Hg, in steps lasting 2 min. During the last 30 s of each step,

an arterial sample was drawn and oxyhemoglobin saturation was recorded from a pulse oximeter (Onyx II, Nonin, Plymouth MN). The data of both the lowlanders and highlanders correlated well with predicted values (R2= 0.96 and 0.97, respectively). However, the data were heteroskedastic (diverging at lower saturations). A Bland and Altman analysis (predicted-measured) revealed a bias of -1.6 % (95% CI -2.5 to -0.8) for lowlanders with a slope of 0.17 ($p < 0.001$). In highlanders, the bias was -2.7 % (95% CI -3.6 to -1.9) with a slope of 0.26 ($p < 0.001$). The SaO_2 predicted by the Kelman model, which assumes normal acid-base status and 2,3-DPG levels, was consistently lower than the measured SpO_2. This is unexpected given the known increase in 2,3-DPG levels at altitude. Assuming the discrepancies between the model and measured values do not reflect a systematic difference between SpO_2 and SaO_2, they may be due to metabolic factors activated under hypoxic conditions. We thank CIHR and Thornhill Research Inc. for the generous support that made this research possible. 2009.

A FEASIBILITY STUDY FOR ESTABLISHING A DIPLOMA IN MOUNTAIN MEDICINE IN NEPAL. Suzy Stokes[1], Maniraj Neupane. [1]MEDEX. *Email: suzystokes@doctors.org.uk*. Introduction: The internationally recognised UIAA/ICAR/ISMM Diploma in Mountain Medicine is already running successfully in 7 different countries. Graduates use their skills on expeditions and in advisory roles around the world. As the Nepalese Himalaya become a more accessible and popular destination for tourist trekkers and mountaineers, so the importance of appropriately trained mountain medicine doctors increases. Facilitating a Diploma specifically tailored to the needs of Nepalese medics will allow autonomy in education and research and increase the accessibility of the speciality to Doctors from developing countries. In addition it would improve employment opportunities, encourage Nepalese trained doctors to stay in country for post-graduate training and foster further research into high-altitude medicine. Methods: In Oct-Nov 2010, the feasibility study took place in Kathmandu and the Khumbu region of Nepal. Assessments were made by visiting the potential course sites in person to see what facilities could be utilised. By discussing the proposal with local community leaders it was possible to identify mutual goals and benefits and following discussions with local doctors, the course structure and content could be devised to make it appealing and practical in content. Further meetings with an NGO ascertained some baseline costs. The aim was to identify potential barriers to a sustainable course that could be addressed promptly and necessary adaptations made, increasing the likelihood of a successful programme. Results: The response to the proposal was extremely positive from all involved parties. The significant hurdles that remain are mainly financial, although a sub-committee of the Mountain Medicine Society of Nepal (MMSN) are seeking solutions. Conclusion: With international support from mountaineering / medical organisations, and practical field support from international Diploma holders, it is hoped that a pilot course can be run in Fall 2011. In the long term, international education programs such as these will foster important medical and academic collaborations. Acknowledgements: Funding: MEDEX UK. 2011.

A NEW ANTI-TUMOR AGENT TARGETING THE HYPOXIA-INDUCIBLE FACTOR PATHWAY. Shaoman Yin[1], Stefan Kaluz[1], Narra S. Devi[1], Chalet Tan[1], Rita de Noronha[2], Kyriakos C. Nicolaou[2], Binghe Wang[3], Erwin G. Van Meir[1]. [1]Laboratory of Molecular Neuro-Oncology, Departments of Neurosurgery, Hematology/Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, [2]Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA, [3]Department of Chemistry and Center for Biotechnology and Drug Discovery, Georgia State University, Atlanta, Georgia, USA. *Email: dritsic@emory.edu*. Introduction: Hypoxia inducible factor 1 (HIF-1) pathway is a central mediator of tumor cell adaptation to the hypoxic microenvironment. HIF-1 is overexpressed in cancers and has become an important therapeutic target in solid tumors. Methods: To identify new small molecules inhibiting the HIF-1 pathway, we used a HIF-responsive reporter to screen on a 10,000-membered natural product-like chemical compound library. Results: This effort led to the discovery of KCN1, a novel and potent HIF-1 inhibitor. KCN1 markedly attenuates HIF-mediated transcription in tumor cells and down-regulates HIF-1 target genes, such as vascular endothelial growth factor (VEGF) and carbonic anhydrase IX (CA XI). Treatment with KCN1 inhibits the progression of human glioma cancer xenograft. Through co-immunoprecipitation, oligonucleotide pull-down and CHIP assays, we found that KCN1 significantly disrupts HIF-1α association with p300/CBP, an important co-factor in the formation of an active transcription complex. This provides a direct explanation as to why KCN1 can block the transcriptional activation of the HIF pathway. Conclusion: These results demonstrate that KCN1 is a promising therapeutic agent for cancer therapy and advance our understanding of its anti-tumor mechanism. Acknowledgements: Our research was supported in part by grants to E.G.V.M. from the National Institute of Health (DCB APRC supplement to CA86335, CA116804), the American Brain Tumor Association, the Brain Tumor Foundation for Children, the Southeastern Brain Tumor Foundation, and the Emory University Research Council. 2011.

A NEW ANTI-TUMOR AGENT TARGETING THE HYPOXIA-INDUCIBLE FACTOR PATHWAY. Shaoman Yin[1], Stefan Kaluz[1], Narra S. Devi[1], Chalet Tan[1], Rita de Noronha[2], Kyriakos C. Nicolaou[2], Binghe Wang[3], Erwin G. Van Meir[1]. [1]Laboratory of Molecular Neuro-Oncology, Departments of Neurosurgery, Hematology/Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, [2]Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA, [3]Department of Chemistry and Center for Biotechnology and Drug Discovery, Georgia State University, Atlanta, Georgia, USA. *Email: dritsic@emory.edu*. Introduction: Hypoxia inducible factor 1 (HIF-1) pathway is a central mediator of tumor cell adaptation to the hypoxic microenvironment. HIF-1 is overexpressed in cancers and has become an important therapeutic target in solid tumors. Methods: To identify new small molecules inhibiting the HIF-1 pathway, we used a HIF-

responsive reporter to screen on a 10,000-membered natural product-like chemical compound library. Results: This effort led to the discovery of KCN1, a novel and potent HIF-1 inhibitor. KCN1 markedly attenuates HIF-mediated transcription in tumor cells and down-regulates HIF-1 target genes, such as vascular endothelial growth factor (VEGF) and carbonic anhydrase IX (CA XI). Treatment with KCN1 inhibits the progression of human glioma cancer xenograft. Through co-immunoprecipitation, oligonucleotide pull-down and CHIP assays, we found that KCN1 significantly disrupts HIF-1α association with p300/CBP, an important co-factor in the formation of an active transcription complex. This provides a direct explanation as to why KCN1 can block the transcriptional activation of the HIF pathway. Conclusion: These results demonstrate that KCN1 is a promising therapeutic agent for cancer therapy and advance our understanding of its anti-tumor mechanism. Acknowledgements: Our research was supported in part by grants to E.G.V.M. from the National Institute of Health (DCB APRC supplement to CA86335, CA116804), the American Brain Tumor Association, the Brain Tumor Foundation for Children, the Southeastern Brain Tumor Foundation, and the Emory University Research Council. 2011.

A NOVEL ATTEMPT TO IMPROVE EXERCISE CAPACITY AT ALTITUDE. Robert A Jacobs, Martha C Tissot van Patot, Molly White, Ben Foreman, Robert W Gotshall, David C Irwin, Karyn L Hamilton. Colorado State University, University of Colorado Health Science Center. *Email: robert.jacobs@colostate.edu.* BACKGROUND: Sojourns to high altitudes result in a decrement to one's ability to exercise. Although acclimatization to high altitude eventually occurs allowing for some improvement in exercise capacity, some individuals may not be allowed the time necessary for those physiologic adaptations to occur. Thus, identification of an ergogenic aid that would minimize the loss of exercise capacity following rapid ascent would be of great benefit. PURPOSE: To determine the effect on exercise capacity at high altitude following the top-loading administration of a hemoglobin-based oxygen carrier (HBOC). METHODS: Conscious, male Sprague-Dawley rats were randomly treated with either Lactated Ringer's solution or HBOC solution and placed in either a hypobaric chamber with the barometric pressure reduced to the equivalent of 5,500 m or a normobaric environment of approximately 1,500 m. All animals were subjected to an exercise bout on a treadmill and time to exhaustion was monitored. RESULTS: Hypobaric hypoxia reduced time to exhaustion compared to animals that that ran at ambient conditions (195 and 2037 seconds, respectively; $p < 0.001$). When compared to the control animals, HBOC treatment did not improve time to exhaustion at hypobaric hypoxia (213 and 176 sec, respectively; $p = 0.912$) or ambient conditions (2104 and 1970 sec, respectively; $p=0.686$). CONCLUSION: There appears to be no effect of HBOC treatment on exercise capacity following acute exposure to hypobaric hypoxia. Although, the lack of improvement with the HBOC treated animals may have resulted from additional side effect of HBOC treatment. Supported by the Defense Advanced Research Projects Agency (DARPA) and the Army Research Office (ARO) contract number W911NF-06-1-0318. 2009.

A NOVEL EGLN1/PHD2 HIGH-FREQUENCY VARIANT IN TIBETANS PROTECTS AGAINST HYPOXIA-INDUCED POLYCYTHEMIA. Felipe Lorenzo[1], Chad Huff[2], Mikko Myllymäki[3], Sabina Swierczek[1], Jinchuan Xing[4], Lynn Jorde[2], Peppi Koivunen[3], Josef Prchal[1]. [1]Dept. of Med., Univ Utah School of Medicine Hematology, Salt Lake City, UT 84132, USA., [2]Eccles Institute Human Genetics, Univ Utah School of Medicine, Salt Lake City, UT 84132, USA., [3]Biocenter Oulu, Dept Medical Biochemistry and Molecular Biology, Oulu Center for Cell-Matrix Research, Univ Oulu, 90014 Oulu, Finland., [4]Dept. of Genetics, Rutgers, the State Univ New Jersey, Piscataway, NJ 08854, USA. *EMAIL: felipe.lorenzo@utah.edu* INTRODUCTION: The hypoxic response, mediated by hypoxia inducible transcription factors (HIFs), is central to the control and development of many essential biological functions, including erythropoiesis. As a high altitude population, Tibetans are genetically adapted to the environmental stress of high altitude, having normal hemoglobin concentration both at sea level and at high altitude. Several haplotypes have undergone positive selection in Tibetans, including variations at or near the EGLN locus, which encodes prolyl 4-hydroxylase (PHD2). PHD2 triggers the degradation of hypoxia-inducible factors (HIFs) that mediate many physiological responses to hypoxia, including erythropoiesis. METHODS: Blood samples from local Tibetan living in Salt Lake City were recruited for the study with inform consent, Affymetrix SNP array, screening of PHD2 gene, protein kinetics, gene expression and analysis of erythroid progenitors were carried out. RESULTS: We now describe two high-frequency Tibetan variants, PHD2D4E and PHD2C127S, that originated on the same haplotype about ~6,000 years ago and contribute functionally to the Tibetan high-altitude phenotype. The PHD2D4E,C127S variant exhibits a lower Km value for O_2, suggesting that it promotes HIF degradation under hypoxia. Because HIF directly stimulates erythropoiesis, the measured kinetic PHD2D4E,C127S properties were experimentally reproduced in native PHD2D4E,C127S erythroid progenitors that had decreased proliferation under hypoxic conditions, whereas wild-type progenitors have increased proliferation. Our results demonstrate that the Tibetan selected PHD2D4E,C127S variant contributes to protection from polycythemia at high altitude. CONCLUSION: We describe the first high-altitude adaptive mutations in Tibetans and demonstrate that they abrogate hypoxia-induced HIF-mediated augmentation of erythropoiesis, explaining Tibetan protection from polycythemia at high altitude. ACKNOWLEDGEMENTS: supported by NIH-P01CA108671, VA Merit Review Award and Univ Utah Seed Grant Program for studies of hypoxic adaptation. 2015.

A PROSPECTIVE EPIDEMIOLOGICAL STUDY OF ACUTE MOUNTAIN SICKNESS IN NEPALESE PILGRIMS ASCENDING TO GOSAINKUNDA (4380 M). Martin MacInnis[1], Eric Carter[1], Michael Freeman[2], Bidur Prasad Pandit[3], Ashmita Siwakoti[3], Ankita Subedi[3], Utsav Timalsina[3], Nadia Widmer[4], Ghan Bahadur Thapa[3], Michael Koehle[1], Jim Rupert[1]. [1]School of Kinesiology, Univ British Columbia, [2]Univ Glasgow, [3]Maharajgunj Medical Campus, Institute Medicine, Tribhuvan Univ, Nepal, [4]Faculty of Medicine, Univ British Columbia. *EMAIL: martin@alumni.ubc.ca* INTRODUCTION: Each year, thousands of pil-

grims travel to the Janai Purnima festival in Gosainkunda, Nepal (4380 m), ascending rapidly and often without the aid of pharmaceutical prophylaxis. METHODS: At the 2012 Janai Purnima festival, subjects were recruited in Dhunche (1950 m) and assessed in Gosainkunda (4380 m). Through interviews, subjects provided demographic information, ratings of AMS symptoms (Lake Louise Scores), ascent profiles, and strategies for prophylaxis. RESULTS: In the 501 (93%) subjects who completed the study, the incidence of AMS upon arrival at the lake was 34.0%. AMS was more common in females than in males (Relative Risk (RR) = 1.57; 95% CI = 1.23 – 2.00), and the AMS incidence was greater in subjects > 35 years compared to subjects ≤35 years (RR = 1.63; 95% CI = 1.36, 1.95). There was a greater incidence of AMS in subjects who chose to use garlic as a prophylactic compared to those who did not (RR = 1.69; 95% CI = 1.26 – 2.28). Although the LLS of brothers was correlated (intraclass correlation = 0.374, p = 0.028), sibling AMS status was a weak predictor of AMS. CONCLUSION: The incidence of AMS in Nepalese pilgrims upon reaching 4380 m was moderate (34%). Sex, age, and ascent rate were significant factors in the development of AMS, and traditional Nepalese remedies were ineffective in the prevention of AMS. ACKNOWLEDGEMENTS: Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC). MM was funded by an NSERC Canada Graduate Scholarship. We thank the Nepal Health Research Council for guidance on our study design, and the district health, public health, and chief district officers of Rasuwa district for permitting data collection in Dhunche and Gosainkunda. We are also thankful for logistical advice and assistance from members of the Himalayan Rescue Association. 2015.

A RANDOMIZED TRIAL OF ACETAZOLAMIDE VERSUS TEMAZEPAM IN HIGH-ALTITUDE SLEEP DISTURBANCE. John Tanner[1], Sarah Tanner[2], Ghan Thapa[3], Yuchiao Chang[1], Kirsty Watson[4], Eamon Staunton[2], Claire Howarth[2], Buddha Basnyat[2], N. Stuart Harris[1]. [1]Massachusetts General Hospital, Boston, Massachusetts, USA, [2]Himalayan Rescue Association, Kathmandu, Nepal, [3]Mountain Medicine Society of Nepal, Kathmandu, Nepal, [4]Royal Lancaster Infirmary, Lancaster, England. *EMAIL: john.barlow.tanner@gmail.com* INTRODUCTION: Although several medications are effective in treating high-altitude sleep disturbance, no head-to-head trials have been performed to determine superiority of any one medication over another. This study compared acetazolamide and temazepam. METHODS: Trekkers at 3540 meters were randomized to 125mg acetazolamide or 7.5mg temazepam. The primary outcome was sleep quality on a 100 mm visual analog scale (VAS). Secondary outcomes included the Groningen Sleep Quality Scale, Stanford Sleepiness Scale, self-reported Lake Louise Acute Mountain Sickness scores, sleep depth VAS, next-day drowsiness VAS, and measurements obtained from a sleep diary, actigraph, and pulse oximeter. RESULTS: 34 subjects were randomized to temazepam (N=16) or acetazolamide (N=18). Sleep quality by visual analog scale (VAS) was higher for temazepam (59.6, SD 20.1) than acetazolamide (46.2, SD 20.2; P=0.048). Temazepam demonstrated higher subjective sleep quality on the Groningen Sleep Quality Scale (3.5 vs 6.8, P=0.009) and sleep depth VAS (60.3 vs 41.4, P=0.028). The acetazolamide group reported

significantly more awakenings to urinate (1.8 vs 0.5, P=0.007). No difference was found with regards to mean oxygen saturation (84.1 vs 84.4, P=0.57), periodic breathing, relative desaturations, sleep onset latency, awakenings, wake after sleep onset, sleep efficiency, Stanford Sleepiness Scale average, daytime drowsiness, or change in self-reported Lake Louise Acute Mountain Sickness scores. CONCLUSION: Temazepam is associated with increased subjective sleep quality compared to acetazolamide. Improvements in sleep quality were not associated with lower oxygen saturation or next-day effects such as drowsiness or sleepiness. ACKNOWLEDGEMENTS: This study was funded by the Massachusetts General Hospital Dept Emergency Medicine. Technical support provided by Dr. Stephen Muza and USARIEM. 2015.

A SIMPLE AND PORTABLE BREATHING CIRCUIT DESIGNED FOR VENTILATORY MUSCLE ENDURANCE TRAINING (VMET). David Preiss, Dahlia Balaban, Rosemary Regan, Alexandra Mardimae, Marat Slessarev, Greg Wells, James Duffin, Steve Iscoe, Joseph Fisher. Boston University, Thornhill Research. *Email: davidapreiss@gmail.com.* Background: Ventilatory muscle endurance training (VMET) involves increasing minute ventilation (VE) against a low flow resistance at rest to simulate the hyperpnea of exercise. Ideally, VMET must maintain normocapnia over a wide range of VE. This can be achieved by providing a constant fresh gas flow to a sequential rebreathing circuit. The challenge to make VMET suitable for home use is to provide a source of constant fresh gas flow to the circuit without resorting to compressed gas. Methods: Our VMET circuit was based on a commercial sequential breathing circuit (Pulmanex Hi-Ox, Viasys Healthcare, Yorba Linda, CA USA). Air flow was provided either by a small battery-driven aquarium air pump or by the entrainment of air down a pressure gradient created by the recoil of a hanging bellows that was charged during each inhalation. In each case, fresh gas flow was adjusted to be just less than resting VE. Eight subjects then breathed from the circuit for three 10 minute periods consisting of relaxed breathing, breathing at 20 and then at 40 L/min. We monitored VE, end-tidal PCO_2 ($PETCO_2$) and pulse oximetry (SpO_2). Results: During hyperpnoea at 20 and 40 L/min, $PETCO_2$ did not differ significantly from resting levels with either method of supplying fresh gas. SpO_2 remained greater than 96% during all tests. Conclusions: Isocapnic VMET can be reliably accomplished with a simple self-regulating, sequential rebreathing circuit without the use of compressed gas. 2009.

A TRAINING PROGRAMME CONSISTING OF 4-12 SESSIONS AT A SIMULATED ALTITUDE OF 5,000 M IS NOT EFFECTIVE AS A PREVENTIVE STRATEGY AGAINST AMS. Gaizka Mejuto[1], Jose Francisco Aramendi[2], Aitziber Garcia[2], Juan Carlos Samaniego, Xabier Erro[3], Jose Ignacio Emparanza[4]. [1]Univ the Basque Country, Vitoria-Gasteiz. Spain, [2]Osasun Kirol Sport Clinic. Hondarribia, Spain, [3]Mendiak eta Herriak. Mountain Guides. Gaintza, Spain, [4]Donostia Univ Hospital. San Sebastian, Spain. *EMAIL: gaizka.mejuto@ehu.es* INTRODUCTION: Rapid ascent from low, to high altitude (and thus sudden exposures to a lower barometric pressure) make Acute Mountain Sickness (AMS) symptoms appear.

Therefore, in a randomized, single blind, placebo-controlled trial we aimed to test the efficacy of a normobaric hypoxic training programme on improving the symptoms of AMS during expeditions to high altitude. METHODS: Eighteen healthy participants performed 4-12 sessions consisting of 45 min walking on a treadmill at 80% HRmax, and at 15% grade, and carrying a backpack weighing 20% of their body mass. While exercising they were given a normobaric air mixture corresponding to an altitude of 3,000 m to breathe on the 1st day; the equivalent of 4,000 m on the 2nd day; and 5,000 m on the following days (HP group). A further eighteen healthy participants performed the same protocol under normoxic (masked) conditions (CON group). At altitude, AMS was assessed using the Lake Louise Score system (LLSA, LLSCL, EV) and SaO_2 was measured on arrival, after 6 and 12 h, and the following 3 consecutive mornings above 3,000 m. Clinical assessment of AMS was set as headache symptom + > 3 points in the LLS. RESULTS: 16 expeditions to an altitude of 4,340 ± 364 m took part in the present study. 8 participants in each group presented AMS symptoms (RR=1.00; CI of 95% 0.48 to 2.08). SaO_2, LLSA, LLSQ and EV showed no difference between groups (p>0.05; NS). LLS and SaO_2 were found to be inversely correlated. None of the observed correlations had predictive power. This single blind placebo-controlled study showed that a training programme consisting of 4-12 sessions at a simulated altitude of 5,000 m is not effective as a preventive strategy against AMS. However, the hypoxic training programme in the present study appeared to be effective at increasing the SaO_2 as the number of sessions increased. CONCLUSION: This hypoxic training programme provided no benefit in preventing AMS, but SaO_2 appeared to be higher as the hypoxic training sessions increased within HP group. ACKNOWLEDGEMENTS: We acknowledge the climbers who participated in this study. 2015.

ABNORMAL HYPOXIC PULMONARY VASCULAR RESPONSE ALONE IS NOT SUFFICIENT TO INDUCE HIGH ALTITUDE PULMONARY EDEMA. Christoph Dehnert[1], Sebastian Greiner[2], Dagmar Albers[1], Fabian Scheurlen[1], Derliz Mereles[2], Ekkehard Grünig[3], Peter Bärtsch[1]. [1]University Hospital Heidelberg, Internal Medicine VII, Sports Medicine, [2]University Hospital Heidelberg, Internal Medicine III, Cardiology, [3]University Hospital Heidelberg, Thoraxclinic, Centre of Pulmonary Arterial Hypertension. *Email: christoph.dehnert@uniklinik-ulm.de*. Introduction: High altitude pulmonary edema (HAPE) develops in 6% of otherwise healthy mountaineers after rapid ascent to 4559 m and in 62% of HAPE-susceptible mountaineers. HAPE-susceptibility is characterized by increased hypoxic pulmonary vascular response (HPVR) leading to systolic pulmonary artery pressures (PASP) over 40 mmHg during acute exposure to hypoxia (FiO_2 = 12%). Furthermore, it has been shown that about 10% in the population have increased HPVR of the same degree during hypoxic exposure. PURPOSE: To evaluate the incidence of HAPE after rapid ascent to 4559 m in individuals with HPVR > 40 mmHg. Based on previous data we expected an incidence of HAPE of 60%. Methods: Screening over 400 healthy individuals we found about 10% with echocardiographically determined PASP > 45 mmHg after 2 h exposure to FiO_2 = 12% (~4500 m) consistently in two independent exposures. 29 of them and 24 control subjects (PASP at FiO_2 < 40

mmHg in two exposures) ascended to 4559 m within 24 h where they stayed for 2 days. PASP was repeatedly determined at altitude and chest x-ray was taken before subjects descended or to confirm the diagnosis of HAPE if HAPE was clinically suspected. Results: In all subjects PASP at altitude 4 h after arrival at 4559 m was comparable to the value measured before in normobaric hypoxia equivalent to the same altitude. Values did not change significantly in the morning of the second day and before descend. In 4 subjects clinically or radiographically assured HAPE developed. HAPE was unequivocally diagnosed on clinical basis in one subject already at 3611 m and in the 3 others at 4559 m. Questionable radiographic signs of HAPE were found in 3 additional asymptomatic subjects at 4559 m. Thus, the incidence of clinically relevant HAPE was only 13%. Conclusion: PASP at high altitude can be predicted reliably by measurement after 2h exposure in normobaric hypoxia equivalent to the same altitude. Furthermore in our sample the incidence of HAPE in subjects with increased HPVR was considerably lower than observed with reexposure of HAPE-susceptible individuals and none occurred among controls. This indicates that an increased HPVR is necessary but not sufficient to induce HAPE and suggests that additional factors are necessary for its development. 2011.

ABNORMAL MUSCLE HISTOCHEMICAL AND BIOCHEMICAL PROPERTIES IN COPD. CASE STUDY OF TWO MALES. Howard J Green, Eric Bombardier, Margaret E Burnett, Christine L D'Arsigny, Sobia Iqbal, Kathy A Webb, Jing Ouyang, Denis E O'Donnell. University of Waterloo, Queen's University. *Email: green@healthy.uwaterloo.ca.* The purpose of this study was to assess the remodeling that occurs in vastus lateralis in 2 patients with COPD (COPD#1,FEV1/FVC=63%;COPD#2,FEV1/FVC=41%) exhibiting differences in muscle wasting as compared to healthy controls (CON, FEV1/FVC=111±2.2%, n=4). Type I fibers percentages were lower in both COPD#1 (16.7) and COPD#2 (24.9) compared to CON (57.3±5.2). Cross sectional area of the type I fibers of the patients ranged between 65-68% of CON and for the type II subtypes (IIA,IIAX, IIX) between 74 and 89% (COPD#1) and 17-32% (COPD#2). A lower number of capillary contacts were observed for all fiber types in COPD#1 but not COPD#2. Lower concentrations of ATP (24-26%) and phosphocreatine (18-20%) but not lactate occurred in COPD. In contrast to COPD#1, who displayed normal glucose transporter content, GLUT1 and GLUT4 were only 71 and 54%, respectively of CON in COPD#2. Lower monocarboxylate contents were found for MCT1 in both COPD#1 (63%) and COPD#2 (41%) and for MCT4 (78%) in COPD#1. Maximal oxidative enzyme activities (Vmax) for COPD#2 ranged between 37% (succinic dehydrogenase) and 70% (cytochrome c oxidase) of CON. For the cytosolic enzymes, Vmax ranged between 89% (hexokinase) to 31% (pyruvate kinase) of CON. Depressions were also observed in Vmax of the Na+-K+ -ATPase for COPD#1 (66% of CON) but not COPD#2 (92% of CON) while Vmax of the Ca2+-ATPase was near normal in COPD#1 (84% CON). It is concluded that disturbances can occur in muscle to a wide range of excitation, contraction and metabolic processes in COPD. Acknowledgements: Supported by the Department of Medicine (Queen’s University). 2009.

ACCLIMATION OF NOCTURAL PB AND RESPIRATORY EVENTS DURING MOUNTAIN CLIMBING IN HIMALAYA. Takeshi Yamauchi. Ishinomaki Senshu University. *Email: yamauchi@isenshu-u.ac.jp.* The introduction of commercial mountain guides has made it possible for many mountaineers to Himalayan tours above altitudes of 5000 m. One of the risk factors associated with high altitude mountain climbing is acute mountain sickness, which is closely related to sleep disturbance. Periodic breathing (PB) and apnea during sleep at altitude is considered to be the major cause of sleep disturbances. In the present study, to clarify the sleep acclimation during mountain climbing in himalaya, we investigated the nocturnal PB and respiratory events serially in one subject staying at above 4700m for 19 days. Nocturnal PB, respiratory events as well as in pulse oximetry (SpO_2) and urine samples were measured on day 1 (4700m) , day 3 (4700m), day 6 (5400 m), day 17 (5400 m) , and day 19 (4700 m). On the initial night after an ascent from 3700 m to 4700 m (day 1), the decrease in barometric pressure induced the respiratory instability during sleep. Apnea index (AI), apnea hypopnea index (AHI) and PB increased compared with the baseline data collected near sea level. And the frequency of these indices attenuated progressively with the lapse of time at 4700 m. The augmentation of SpO_2 during sleep could be involved with lessening of PB and apnea. There was an opposite phenomenon of respiratory events and PB at altitude of 5400 m. The incidence of AI, AHI and PB on day 17 increased in spite of the rise in SpO_2 during sleep compared with the data on the initial night after an ascent from 4700 m to 5400 m (day 6). These results suggest that the level of altitude could cause the different process of sleep acclimation. 2009.

ACETAZOLAMIDE AND N-ACETYLCYSTEINE IN TREATMENT OF ERYTHROCYTOSIS AND SYMPTOMS OF CHRONIC MOUNTAIN SICKNESS. Shailendra Sharma[1], Jane Gralla[2], Joyce Gonzalez[3], Maria-Elena Hurtado[3], Erik R. Swenson[4], Robert B. Schoene[5], Jackeline Pando Kelly[6], David Callacondo[7], Jeffrey Sirota[8], Richard Fuquay[9], Brian P. Jackson[10], Kai E. Swenson[11], Richard J. Johnson[1], Abdias Hurtado[3], Elizabeth Escudero[3]. [1]Division of Renal Diseases and Hypertension, University of Colorado Denver, USA, [2]Department of Pediatrics, University of Colorado Denver, USA, [3]Division of Nephrology, Hospital Arzobispo Loayza, Cayetano Heredia University, Lima, Peru, [4]Division of Pulmonary and Critical Care Medicine. University of Washington, Seattle, USA, [5]Alta Bates Summit Medical Center, Oakland, USA, [6]University College Cork, Cork, Ireland, [7]School of Medicine, Universidad Privada de Tacna, Tacna, Peru, [8]South Denver Nephrology Associates, Denver, USA, [9]Dallas Nephrology Associates, Texas, USA, [10]Trace Element Analysis Core, Dartmouth College, Hanover, USA, [11]Yale University School of Medicine, Connecticut, USA. *Email: Shailendra.Sharma@ucdenver.edu.* Introduction: We reported that miners in Cerro de Pasco (4200 M) with severe polycythemia and chronic mountain sickness (CMS) have low level cobalt toxicity. Here we tested whether CMS and polycythemia could be improved by N-acetylcysteine (NAC, an antioxidant that chelates cobalt) and/or Acetazolamide (ACZ, which stimulates respiration). Methods: 66 subjects with CMS score > 6 and hematocrit (HCT) ≥65 were randomized to receive Placebo,

NAC, ACZ, or NAC+ACZ for 6 weeks. Primary endpoint was change in HCT. Secondary outcomes were changes in CMS score, PaO_2, $PaCO_2$ and serum and urine cobalt levels. Linear mixed effect model was used to compare treatment arms and a paired t-test to compare changes between visits. Results: The mean (±SD) hematocrit, CMS score and serum cobalt level (μg/L) were 69 ± 4%, 9.8 ± 2.4 and 0.24 ± 0.15, respectively. ACZ reduced the HCT by 4.5 vs 1.9%, (p=0.044) and CMS score by 3.9 vs 1.5 (p=0.003) compared to placebo. This was accompanied by a decrease in $PaCO_2$ (-3.75 vs. + 0.16 mm Hg, p=0.004) and increase in PaO_2 (5.4 vs. 0.8 mm Hg, p=0.022). NAC reduced CMS score compared to placebo (3.4 vs 1.5, p= 0.019) but changes in other parameters did not reach significance (HCT, -3 vs. -1.9%; PaO_2 + 1.8 vs. 0.8 mm Hg). There were no differences in serum cobalt level and urinary cobalt excretion between treatment arms. Combination treatment was similar to ACZ alone, however a greater but statistically insignificant decrease in HCT (-5.3%) was observed. Conclusions: ACZ improves hematocrit and CMS score by increasing ventilation, whereas NAC improves CMS score without significantly lowering hematocrit. In prior studies low level cobalt toxicity (blood levels 1-5 ug/L) was observed in subjects with HCT>75; in this study, however, severe polycythemia (HCT>72) was observed in only 10 subjects, of which 4 had cobalt levels > 0.3ug/L. This may relate to the closing of the local rivers to fishing due to the contamination from the mines. 2015.

ACETAZOLAMIDE IMPROVES CEREBRAL TISSUE OXYGENATION DURING ACUTE HYPOXIA. Zachary Smith[1], Kang Wang[1], David Dubowitz[1]. [1]Univ California San Diego. *EMAIL: zmsmith@ucsd.edu* INTRODUCTION: We previously observed a paradoxical increase in cerebral O_2 metabolism ($CMRO_2$) during hypoxia. Acetazolamide accelerates acclimatization to high attitude hypoxia. We investigated how 250mg oral acetazolamide might affect $CMRO_2$ and how this impacted cerebral tissue oxygenation (PtO_2) during acute hypoxic conditions. METHODS: 6 healthy subjects (age 35 +/-9 yrs) participated. We made measurements of: SaO_2 using a pulse oximeter, and cerebral venous O_2 saturation and cerebral blood flow (CBF) using 3T MRI, to calculate $CMRO_2$, and changes in PtO_2 during both normoxia and 6 hours normobaric hypoxia at PIO_2 = 90 Torr. Measurements were repeated during oral Az therapy. RESULTS: During normoxia: $CMRO_2$ was 1.43+/-0.31 umol/g/min, PtO_2 25+/-1 torr (SaO_2 98+/-0.5%, $ETCO_2$ 39+/-2 torr, CBF 44+/-13 ml/100ml/min), and not significantly different with Az - $CMRO_2$ 1.48+/-0.27 umol/g/min, PtO_2 24+/-5 torr (SaO_2 98+/-1%, $ETCO_2$=38+/-2 torr, CBF 49+/-19 ml/100ml/min). Following hypoxia: $CMRO_2$ was 1.8+/- 0.25 umol/g/min*, PtO_2 8+/-8 torr* (SaO_2 78 +/- 3%*, $ETCO_2$ 36+/-4 torr, CBF 60+/-19 ml/100ml/min*) (*P<=0.05 relative to normoxia). With Az therapy during hypoxia - $CMRO_2$ was 1.4+/-0.1 umol/g/min**, PtO_2 14+/-5 torr** (SaO_2 81+/-6%, $ETCO_2$=33+/-5 torr, CBF 43+/-14 ml/100ml/min**) (**P<=0.05 relative to hypoxia with no acetazolamide). CONCLUSION: We hypothesize that increased tissue CO_2 from Acetazolamide helps mitigate some of the physiological disturbances during hypoxia. Ventilatory drive is increased, and $CMRO_2$ remains normal, which results in an improved PtO_2 in hypoxia. CBF also remains almost unchanged from normoxic

levels. Although this may limit any potential to improve PtO_2 from increasingO_2 delivery, this is overshadowed by the greater impact of maintaining normal $CMRO_2$. The symptomatic relief of cerebrally mediated symptoms of AMS by acetazolamide may relate to normalization (or partial normalization) of high CBF, high $CMRO_2$ and low PtO_2. ACKNOWLEDGEMENTS: Supported by NIH R01-NS053934(DJD) R21-NS075812(DJD). 2015.

ACONCAGUA ULTRAMARATHON, FIRST EDITION. Sebastián Donato[1], Miriam Patricia Pereiro[2], Rodrigo Duplessis[1], Alvaro Emilio Ortiz Naretto[3]. [1]Asociación Argentina de Medicina para la Altura (AAMpA), [2]Fiorito Hospital, Avellaneda, Buenos Aires, Argentina., [3]Muñiz Hospital, Buenos Aires City, Argentina. *Email: miriam.pereiro@gmail.com.* Introduction: A high altitude running competition of 25 and 50 km was held in the Aconcagua Provincial Park on November 29th 2014 with a cumulative elevation gain of 1590 meters. 25 K competitors had to acclimate during 24 h above 2000 masl before the start while 50 K ones, 48 h before the start. Because of technical, not weather related reasons, the official competition had to be stopped. However, most of runners decided to continue and complete their distance. Methods: 451 athletes participated. 255 were checked (64 females, 191 males). Age: 42 ±8.8 years; O_2sat 87% ±4.9; heart rate: 108 ± 17,8 bpm, Lake Louise Score: "0" points: 255, "1" points: 23; "2" points 4 and "3"points: 3. Results: Only one runner required medical aid because of dehydration and hypothermia without being necessary to be evacuated. There was no need to treat AMS on any person. Fatigue was the most common symptom. Conclusions: Both previous high altitude experience and time for acclimatization regulation requirements were enough for preventing any moderate nor severe high altitude illness. Acknowledgements: We thank the participants who contributed selflessly to the study. 2015.

ACUTE ASCORBIC ACID INFUSION IMPROVES PERIPHERAL HYPOXIC VASODILATION IN OLDER ADULTS. Jennifer Richards[1], Anne Crecelius[1], Gary Luckasen[2], Dennis Larson[2], Frank Dinenno[1]. [1]Colorado State Univ, Fort Collins, CO. [2]Medical Center of the Rockies Foundation, Loveland, CO. *EMAIL: Jennifer.Richards@Colostate.edu* INTRODUCTION: We tested the hypothesis that peripheral hypoxic vasodilation is impaired in older compared with young healthy adults and that local (brachial artery catheter) infusion of ascorbic acid (AA) would reverse the age-associated impairment. METHODS: Forearm blood flow was measured (Doppler Ultrasound) and vascular conductance (FVC) was calculated in 5 young (21±1 years) and 5 older adults (64±4 years) at rest and during systemic isocapnic hypoxia. Prior to the hypoxia trial, local sympathoadrenal blockade was performed in each subject via combined infusion of phentolamine and propranolol (α- and β-adrenergic receptor antagonists, respectively) to isolate local vascular control mechanisms. Subjects were exposed to 15 minutes of systemic isocapnic hypoxia (85% SpO_2); the first 5 min with infusion of saline (control) and the subsequent 10 min with AA infusion (8 mg/dL FAV/min). RESULTS: After 5 minutes of control hypoxia, older individuals exhibited a lower forearm vasodilatory response compared with young (ΔFVC = 14±3% vs 29±6%, $P<0.05$). AA infusion signifi-

cantly improved the dilatory response in older individuals (ΔFVC = 43±9% from steady-state hypoxia levels) whereas there was no significant change in the younger group (ΔFVC = 7±2%). CONCLUSION: These findings suggest that in the absence of sympathoadrenal influences on vascular tone, acute AA administration improves peripheral vasodilation during systemic hypoxia in older individuals such that the response is no longer impaired compared to young healthy humans. Although the mechanisms by which AA improves local dilatory signaling in older adults during hypoxia remain to be determined, prior work from our laboratory and others suggest this may be via improved nitric oxide bioavailability. ACKNOWLEDGEMENTS: Funded by NIH HL095573. 2015.

ACUTE EXPOSURE EFFECT OF MODERATE HYPOXIA ON CARDIOVASCULAR RESPONSES TO STRESS. Mari YOKOI, Astuko MASUDA, Shigeru MASUYAMA. Ryotokuji Univ. *Email: yokoi@ryotokuji-u.ac.jp.* Hypoxia is well known to induce sympathetic dominancy during exercise. In contrast to these accepted hypoxic physical effect, few studies have been performed to examine the combined effect of hypoxia and mental stress on cardiovascular changes. In this study, we hypothesized that acute moderate hypoxia can regulate the cardiovascular responses during mental task as well as exercise stress. Seven middle agers (4 men and 3 women) were exposed to normoxic and moderate hypoxic conditions (FIO_2=12.7%) on a separated day. On each exposure, they were kept in a resting for 10 min, and performed a mental test by color-ward stroop conflict test for 15 min. After a recovery from the stroop test, a graded cycle ergometer bout comprising 3 min by 30 W followed until exhaust. To investigate the indexes of cardiovascular autonomic activity, we assessed heart rate and blood pressure variability using power spectral analysis of RR intervals and continuous blood pressure wave forms, respectively. And spontaneous baroreflex sensitivity was estimated by sequence method. These data were evaluated continuously at rest, during mental task and during exercise and compared. 1) At rest, all cardiovascular indexes in hypoxic condition did not differ from their normoxic values. 2) Mental stress induced significant increases in HR, systolic BP and mean BP in both hypoxic and normoxic condition. 3) All indices of cardiac autonomic nerve activity during mental task were unchanged as compared with resting condition in both hypoxic and normoxic condition. In contrast, the vasomotor sympathetic nerve activity decreased significantly during mental stress in only hypoxic condition. These data suggest that acute normobaric moderate hypoxia during mental task in our subjects had negative effect on vasomotor sympathetic nerve activity was calculated by blood pressure variability, whereas HR and BPs during mental stress had been kept elevating in hypoxic condition equivalently in normoxic condition. 2009.

ACUTE HYPOXIA ALTERS THE COUPLING OF THE CBF/$CMRO_2$ RESPONSE TO NEURONAL ACTIVATION. Erin A Krizay, Richard B Buxton, David J Dubowitz. UCSD. *Email: dubowitz@ucsd.edu.* Neuronal activation is associated with coupled changes in cerebral blood flow (CBF) and cerebral oxygen metabolism ($CMRO_2$). Hypoxia is also known to alter baseline CBF. Using calibrated

Blood Oxygenation Level Dependent (BOLD) functional MRI, we investigated if the CBF/$CMRO_2$ response to neuronal activation is altered by this hypoxia-induced CBF change. Using 3T MRI, we made simultaneous measures of the BOLD and CBF response to an 8 Hz flickering checkerboard visual (neuronal) stimulus and an inspired 5% CO_2 stimulus. Measurements were repeated during a single MRI (30 mins normoxia, 30 mins poikilocapnic hypoxia at 90 torr P_IO_2, and 30 mins post-hypoxic normoxia) in 5 normal subjects (2 females, 3 males, mean age 28). The 5% CO_2 stimulus was used to calibrate the BOLD response to the visual stimulus and derive activation-induced changes in $CMRO_2$. Baseline CBF increased from 59.1±17.5 to 75.2±7.8 mL/100mL/min in response to hypoxia ($P<0.05$). %CBF response to neuronal activation decreased during hypoxia from 54.9±15.3% to 35.8±13.9%, although this did not reach significance ($P=0.054$). There was no significant change in %$CMRO_2$ response to neuronal activation (26.4±4.8%, 22.1±8.6%, P=NS). The coupling ratio between %CBF and %$CMRO_2$ decreased significantly during hypoxia (2.1±0.2 to 1.6±0.4, $P<0.01$). Additionally, during hypoxia, the CBF response to hypercapnia increased 23% (21.5±11.4 to 26.4±6.5 mL/100mL/min), however the CBF response to neuronal activation decreased 17% (32.1±10.8, 26.6±9.4 mL/100mL/min) although these did not reach significance. Acute 90 torr hypoxia changes the coupling between CBF and $CMRO_2$ responses to neuronal activation. Additionally, although the CBF response to hypercapnia is potentiated during hypoxia, the absence of any hypoxic potentiation of the neuronally-induced CBF increase warrants further investigation. This may indicate that these 2 CBF responses are under different control mechanisms. Support: R01-NS053934, R01-NS36722. 2009.

ACUTE MOUNTAIN SICKNESS AT 3450 M OF ALTITUDE IS NOT DIFFERENT BETWEEN CHILDREN AND ADULTS. Susi Kriemler, Birgit Soltermann, Christian Wick, Hans Peter Brunner-La Rocca. Inst of Exercise and Health Sciences, Cardiology. *Email: susi.kriemler@unibas.ch.* Background: There is little information about the prevalence of AMS in children and adolescents despite the fact that more and more children visit high altitude resorts for recreational reasons. Furthermore, it is not clear, whether a child adapted version of an AMS questionnaire would reveal different results. We therefore measured AMS in two related generations upon fast ascent to 3450m. Methods: Thirty six children and adolescents (13±2 y) and their parents (n=39, 48 ±6 y) participated. They ascended within 2 hours to 3540m of altitude and stayed there overnight. AMS was measured 8h after ascent, and on the following morning by 1. the adult Lake Louise Score (LLS), 2. the Sampson environmental questionnaire (ESQ), 3. a published child version of the LLS (LLAASS), and 4. an adapted version of the LLAASS. A score of >4 was defined as AMS. Results: The prevalence of AMS on day1 was 18-23% for children and adolescents and 33-41% for adults depending on the questionnaire used. On day 2 the prevalence was 8-14% and 24-30% for children and adults, respectively. The cumulative incidence was 27-38% and 33-54% for children and adults, respectively. There were no significant differences in prevalence and cumulative incidence between children and adults, between day 1 and day 2, nor among the different

questionnaires used. None of the subjects had to be evacuated and symptoms responded well to symptomatic treatment. Conclusion: The prevalence of AMS at 3450m of children and adolescents is relatively low, self limiting, and comparable to adults, irrespective of questionnaire used. This study was funded by the Swiss Federal Office of Sports. 2009.

ACUTE MOUNTAIN SICKNESS PREVENTION WITH A MOUTHPIECE. Michael Williams. *Email: drmikewilliams@gmail.com.* Introduction: Show that acute mountain sickness (AMS) may be prevented or improved by wearing a mouthpiece while sleeping. Methods: Five subjects were fitted with oral appliance mouthpieces to be used while sleeping at an average altitude of 9800 feet for 7 tests. The oral appliances were designed to advance the mandible, retain the tongue anteriorly and increase the pharyngeal space. Subjects used their mouthpiece at least one night. Subjects had a history of acute mountain sickness (AMS). Subjects reported their results according to the Lake Louise Self Assessment Scale (LLS) after sleeping with and without an oral appliance. Three of the tests were analyzed with pulse oximetry (Pox) during sleep plus LLS. Four tests reported LLS only. One subject was tested on 3 separate occasions. Results: When AMS was evaluated by LLS for the seven tests, all subjects reported an improvement in LLS of 2-5 points. Two subjects reported an increase in AMS symptoms after removing the mouthpiece following initial altitude use. SpO_2 desaturation events of 84% and below were reduced by 17, 44 and 52% while wearing the mouthpiece as compared to not sleeping with the appliance. Conclusion: Sleep hypoxia is associated with the severity of acute mountain sickness. While wearing the mouthpiece during sleep, subjects who were monitored with pulse oximetry showed an improvement in oxygen saturation as compared to measurements while sleeping with no mouthpiece. Subjects reported an improvement in AMS after wearing the mouthpiece and/or showed a reduction in desaturation events. A possible mechanism for this beneficial result in predisposed subjects is the reduction in upper airway resistance provided by the mouthpiece which incorporates the features of mandibular advancement, tongue retention and pharyngeal space increase. It is proposed that the wearing of an altitude mouthpiece may benefit persons predisposed to altitude sickness. 2011.

ACUTE MOUNTAIN SICKNESS PROPHYLAXIS: KNOWLEDGE, ATTITUDES, AND BEHAVIOURS. Thomas Kilner, Saptarshi Mukerji. University of Birmingham UK. *Email: thomk2000@googlemail.com.* Objective: To identify the proportion of high altitude travellers who use acute mountain sickness (AMS) prophylaxis in a way that is likely to be safe, and prevent high-altitude illness; and, to identify, assess, and understand the factors that affect acute mountain sickness prophylaxis usage at high-altitude Methods: Qualitative and quantitative methods were used. The qualitative component involved 20 one-to-one in-depth semi-structured interviews. Analysis was conducted using thematic analysis. The quantitative component involved conducting a questionnaire on 50 guides, and 300

trekkers at high altitude. Data was presented as a percentage mean +SE, and analysed using the Chi squared method, and the analysis of percentages method. $P<0.05$ was taken as significant. Results: Guides had a poor knowledge of prophylactic medication. Only 4% could identify the "recommended" dose (pre-defined as acetazolamide 250mg OD or 125mg bd), and none could correctly identify when it should be started. 54% carried acetazolamide prophylaxis for their clients. 29.33% of trekkers used prophylaxis. 25% of trekkers were taking acetazolamide, and only 15% were using the "recommended" dose. Poor knowledge amongst trekkers and guides was the main determinant of such poor uptake and inappropriate prophylaxis usage amongst trekkers. Trekkers' knowledge was primarily obtained from guide books and health professionals. Conclusions: This study found that 25 % of trekkers used acetazolamide as AMS prophylaxis. Poor knowledge amongst trekkers was found to be the main determinant of such poor uptake, and inappropriate prophylaxis usage. We believe that to improve prophylaxis uptake and use, and hence reduce trekkers' morbidity and mortality from AMS, policy makers must focus on delivering trekker targeted educational interventions in trekkers' home countries and in Nepal. Acknowledgements: Alex Wright. British Medical and Dental Students Trust. Sir Arthur Thomson Charitable Trust. 2009.

ADAPTATIONS FOR EXERCISING AT HIGH ALTITUDE IN THE FLIGHT MUSCLE OF BAR-HEADED GEESE. Graham R Scott, Stuart Egginton, Jeff G Richards, William K Milsom. University of British Columbia, University of Birmingham Medical School. *Email: scott@zoology.ubc.ca.* The bar-headed goose flies over the Himalayas on its migratory route between South and Central Asia, reaching altitudes of up to 9000m. To gain insight into their ability to exercise in such severe hypoxia, we examined the flight muscle of this species for possible physiological adaptations. The muscle phenotypes of bar-headed geese and low altitude birds were first compared using standard histological methods. Bar-headed geese had a pronounced alteration in muscle fiber composition, due to a greater density of oxidative fibers, and this was associated with more capillaries per muscle fiber. Although bar-headed geese had equivalent mitochondrial volume densities to low altitude birds, more of their mitochondria were situated in a subsarcolemmal location. These adaptations should increase the capacity for O_2 diffusion from the blood and reduce intracellular diffusion distance, respectively, and thus help maintain higher O_2 tensions at the mitochondria. Oxygen kinetics of isolated mitochondria were also compared between species using high-resolution respirometry. Mitochondrial respiration was dependent on O_2 tensions that are typical of the intracellular environment and there were no alterations in O_2 kinetics in bar-headed geese. These data emphasize the importance of increased capillarity and altered mitochondrial distribution for sustaining high O_2 delivery to mitochondria. We conclude that the flight muscle of bar-headed geese has evolved for exercise in severe hypoxia and that enhanced muscle O_2 supply is essential for this species' exceptional ability to fly high. (Supported by NSERC of Canada). 2009.

ALTERATIONS IN CEREBROVASCULAR CO_2 REACTIVITY AND CENTRAL SLEEP APNEA AT HIGH ALTITUDE; INFLUENCE OF PARTIAL ACCLIMATIZATION. Samuel J Lucas, Keith R Burgess, Rishi Basnyat, Kate N Thomas, Jui-Lin Fan, Andrew Dawson, Kelly Shepherd, Marianne Swart, Joseph Donnelly, Rebekah A Lucas, Karen C Peebles, James D Cotter, Philip N Ainslie. University of Otago, University of Sydney, Nepal International Clinic, Peninsula Sleep Laboratory. *Email: sam.lucas@otago.ac.nz.* We tested the hypothesis that reductions in cerebral blood flow (CBF)-CO2 reactivity and related elevations in VE-sensitivity would be related to the progressive occurrence of central sleep apnea (CSA) at high altitude (5,050 m). At sea level, and during 1-3 and 12-15 days of living at high altitude, we measured middle cerebral artery blood flow velocity (CBFv) and ventilation (VE) in 12 participants under conditions of eucapnia (room air), hypocapnia (voluntary hyperventilation), and steady state hyperoxic hypercapnia (7% CO_2). Sleep was also studied using full polysomnography at each time point. Cerebrovascular and VE reactivity to CO_2 were characterized following their steady state change to hyper- or hypocapnia. Upon arrival to high altitude, CBFv was elevated ($P<0.01$) compared to sea-level (+25±17%), but had returned to normal after 12-15 days. Upon initial arrival, whereas cerebrovascular reactivity to hypercapnia was reduced ($P<0.05$) compared to sea-level (3.3 v 4.6 cm/s/mmHg), hypocapnic reactivity was enhanced (9.5 v 3.7 cm/s/mmHg, $P<0.01$); VE-CO_2 sensitivity was also elevated (4.4 v 2.0 L/min/mmHg, $P<0.05$) and the development of marked CSA was apparent (77±49 events/h). However, after 12-15 days, although CBFv reactivity to hypercapnia had returned towards sea-level values (3.9 cm/s/mmHg, $P=0.22$), VE-sensitivity remained elevated (3.1 L/min/mmHg, $P=0.07$), and the occurrence of CSA was further increased (116±20 events/h; $P<0.05$ v initial). Elevations in CBF reactivity to hypocapnia were still evident ($P<0.01$) after 12-15 days. CSA, at 12-15 days only, was related to the CBFv reactivity to hypocapnia ($r=0.66$, $P<0.05$). Such findings, if extrapolated to sleep, indicate that changes in CBF reactivity to hypocapnia may play a critical role in further exacerbating breathing instability and CSA following partial acclimatization to high altitude. 2009.

ALTERATIONS OF SYSTEMIC AND MUSCLE IRON METABOLISM IN HUMAN SUBJECTS TREATED WITH LOW DOSE RECOMBINANT ERYTHROPOIETIN. Paul Robach, Stefania Recalcati, Domenico Girelli, Cecilia Gelfi, Agnese Vigano, Paolo Santambrogio, Tibor Kempf, Kai C Wollert, Stephane Moutereau, Carsten Lundby, Gaetano Cairo. Ecole Nationale de Ski et d'Alpinisme, University of Milan, University of Verona, H.S. Raffaele, Hannover Medical School, Hôpital Henri-Mondor, Copenhagen Muscle Research Centre. *Email: paul.robach@jeunesse-sports.gouv.fr.* The high iron demand associated with enhanced erythropoiesis during high-altitude hypoxia leads to skeletal muscle iron mobilisation and decrease in myoglobin protein levels (Blood 109: 4724-31, 2007). In order to investigate the effect of enhanced erythropoiesis on systemic and muscle iron metabolism under non-hypoxic conditions, eight healthy volunteers were treated with recombinant erythropoietin (rhEpo) for one month. As expected, the treatment efficiently increased erythropoiesis and stimulated bone marrow iron use. It was also

associated with a prompt and considerable decrease in urinary hepcidin, and a slight transient increase in GDF-15. The increased iron use and reduced hepcidin levels suggested increased iron mobilisation, but the treatment was associated with increased muscle iron and L ferritin levels. The muscle expression of transferrin receptor and ferroportin was up-regulated by rhEpo administration, whereas there was no appreciable change in myoglobin levels, which suggests unaltered muscle oxygen homeostasis. In conclusion, under rhEpo stimulation, the changes in the expression of muscle iron proteins indicate the occurrence of skeletal muscle iron accumulation despite the remarkable hepcidin suppression that may be mediated by several factors, such as rhEpo and/or decreased transferrin saturation. 2009.

ALTERED SYSTEMIC METABOLISM OF NITRITE AND S-NITROSOHEMOGLOBIN IN AMS. Damian M Bailey, Kevin A Evans, Lewis Fall, Philip E James, Philip N Ainslie. University of Glamorgan, Cardiff University, University of Otago. *Email: dbailey1@glam.ac.uk.* Objective: Nitrite ($NO_2^{\cdot}$) reduction and S-nitrosohaemoglobin (SNO-Hb) formation have been identified as potential nitric oxide (NO) reservoirs that are capable of preserving NO bioactivity and facilitating hypoxic vasodilatation. Since subjects with AMS (AMS+) are typically more hypoxemic than their healthy (AMS-) counterparts, we examined if this was due to a functional impairment in the systemic metabolism of $NO_2^{\cdot}$ and SNO-Hb during acute exposure to inspiratory hypoxia. Methods: Eighteen healthy males (aged 26 ± 6 years) were examined in normoxia and following 6h passive exposure to 12% O_2. AMS was diagnozed if subjects presented with a Lake Louise (self + clinical) score of ≥5 points combined with a Cerebral Symptoms Score ≥0.7 points. The plasma concentrations of $NO_2^{\cdot}$ and SNO-Hb in venous blood were assessed via ozone-based chemiluminescence. Arterial hemoglobin oxygen saturation (SaO_2) was measured by pulse oximetry. Data were analyzed using paired samples t-tests and expressed as mean ± SD. Results: Nine subjects (50%) developed clinical AMS and were more hypoxemic (hypoxia minus normoxia) relative to healthy controls (AMS+: -18 ± 5 vs. AMS-: -13 ± 5%, $P < 0.05$). There was a tendency towards a greater increase (hypoxia minus normoxia) in plasma $NO_2^{\cdot}$ (AMS+: +50 ± 180 vs. AMS-: -71 ± 214 nmol) and SNO-Hb (AMS: +28.9 ± 29.7 vs. AMS-: +9.6 ± 38.3 nmol) in AMS+ though the differences did not attain statistical significance. Conclusions: These findings tentatively suggest that AMS is associated with (a tendency towards) an increased systemic bioavailability of $NO_2^{\cdot}$ and SNO-Hb that may represent functional "back-pockets" of NO that serve to defend vascular O_2 delivery in the face of prevailing arterial hypoxemia. Moreover, these noxious agents may result in direct activation of the trigeminovascular system that may prove the primary mechanism that causes AMS. 2009.

ALTERED TEAR FILM LEADS TO EVAPORATIVE DRY EYE SYNDROME AT HIGH ALTITUDE. Gabriel Willmann[1], Andreas Schatz[1], Manuel Dominik Fischer[1], Kai Schommer[2], Eberhart Zrenner[1], Karl-Ulrich Bartz-Schmidt[1], Florian Gekeler[1]. [1]Centre for Ophthalmology, Univ Tübingen, Tübingen, Germany, [2]Dept Sports Medicine, Medical Clinic, Univ Hospital Heidelberg, Heidelberg, Germany.

EMAIL: Gabriel.Willmann@googlemail.com INTRODUCTION: This study investigated the quality of the tear film during high altitude exposure in healthy subjects. This work is related to the Tübingen High Altitude Ophthalmology (THAO) study. METHODS: Tear film osmolarity (TFO), tear film break up time (TBUT) and the Schirmer test without anesthesia were used to measure the quality of the tear film under standardized conditions in 14 healthy subjects on day 1, 2 and 4 during acute exposure to high altitude at the Capanna Margherita (CM; 4559m, Italy) compared to baseline measurements in Tübingen (341 m, Germany) before (BL1) and after (BL2) exposure. In addition, a Spectralis® anterior segment module (Heidelberg Engineering, Heidelberg, Germany) was used to measure tear film thickness on day 1 and 3 at high altitude. Intra-individual ratios were calculated using MANOVA with a significance level of $p < 0.05$. RESULTS: Upon arrival at CM, a significant increase in intra-individual ratios of tear film osmolarity (day1: 8.0%, 95% confidence interval (CI) 2.1-13.8%; day2: 9.0%, CI 2.3-15.6%; day4: 7.1%, CI 1.8-12.4%) and a significant decrease of TBUT (day1: 31.2%, CI 4.8-57.7%; day2: 46.1%, CI 74.1-26.0%; day4: 55.2%, CI 43.5-66.8%) was noted compared to baseline recordings. Schirmer test at high altitude remained non significant compared to baseline. High resolution imaging of the tear film showed a clear trend towards decreased tear film thickness without reaching statistical significance on both days measured. Measurements at BL1 and BL2 showed no statistically significant differences and recordings of right and left eyes for TBUT and Schirmer did not differ significantly on each day measured. CONCLUSION: High altitude exposure leads to an altered tear film resulting in a evaporative dry eye syndrome. Tear film osmolarity followed by TBUT were found to be the most informative and reliable markers. This is of clinical importance to trekkers and mountaineers exposed to high altitude since possible disturbance of vision with dry eye symptoms may be a potential risk factor at high altitude. ACKNOWLEDGEMENTS: Wilderness Medical Society (WMS) and TearLab. 2015.

ALTITUDE EFFECT ON SLEEP QUALITY AND SERUM MELATONIN IN CHILEAN MINING WORKERS. Rodrigo Calderon-Jofré[1], Andres Robles-Hidalgo[1], Andres Pedreros[1], Fernando A Moraga[1]. [1]Laboratorio Fisiología, Hipoxia y Función Vascular, Dpto Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte. *Email: fmoraga@ucn.cl.* Introduction: Mining operations in high altitude are a very important business in Chile but reduced availability of oxygen affects the sleep quality, increasing the risk of accidents. One important regulator of sleep-wake cycle is the hormone Melatonin, produced by pineal gland as a sleep inductor. Methods: The aim of this study is to correlate the effect of altitude (1600, 2500, 3500 and 4500 m.a.s.l.) in sleep quality of 229 volunteers (using surveys of sleepiness and sleep quality and measurement of sleep apnea using nocturnal oximetry) with serum levels of melatonin. Results: While apnea Index increased from 3500 m.a.s.l. and scoring of surveys from 1600 m.a.s.l. comparing to sea level, melatonin levels rise at 1600 m.a.s.l. but remain constant to all other altitudes. Conclusion: This data suggest that although apnea is increasing in altitude, the elevation in melatonin levels are not due directly to altitude, but probably due to work

shift system or to other factors not studied here. Acknowledgements: Proyecto Biomarcadores 07CN13ISM-152. 2015.

ALTITUDE MEDICINE: REPORT FROM THE FRONTLINE. Piotr Szawarski, Stephen Halvorson, Kirstie Nicol, Leon Wren. South East School of Anaesthesia UK, Himalayan Rescue Association. *Email: piotr.szawarski@gmail.com.* Adventure travel to mountain regions across the globe is increasingly popular. In the Nepal Himalaya, voluntary organisations such as Himalayan Rescue Association (HRA) are a source of health care to both travellers and local population. We have performed a retrospective analysis of HRA clinic records for the autumn 2007 season with the aim of highlighting the magnitude and nature of problems encountered by volunteer physicians. At the Pheriche Clinic (4200m) - on the Everest B.C. trek within the Khumbu region - in a two-month period, 404 patients were seen. 50% were trekkers and 50% Nepali. 21.5% of attendances were altitude-related. 15 individuals required helicopter evacuation, 26 inpatient treatment. Two required hyperbaric therapy as well as oxygen. There was one house call and one fatality. Among Nepali patients 64% were porters or seasonal low altitude Nepali workers. Among trekkers those participating in organised treks appeared to be more likely to suffer from severe forms of altitude illness. At the Manang Clinic (3570m) - on the Annapurna trekking circuit - 347 patients were seen. 48% were trekkers and 52% Nepali. 16.7% of complaints were altitude-related. 13 patients required evacuation, 8 inpatient treatment. There were 13 house calls. The majority of complaints at both clinics were not altitude-related and of respiratory or gastro-intestinal nature. 2009.

ALTITUDE, LIFE EXPECTANCY, AND MORTALITY FROM ISCHEMIC HEART DISEASE AND COPD: POPULATION BASED ANALYSIS IN THE UNITED STATES. Ben Honigman, Mara E Horwitz, Deborah Thomas, Ari B Friedman, Robert Roach, Timothy Clark, Christopher J Murray, Majid Ezzati. Altitude Research Center, University of Colorado Denver, University of Washington, University of Colorado, Harvard School of Public Health, US Army Corps of Engineers. *Email: benjamin.honigman@ucdenver.edu.* Background: Of the twenty counties with the highest life expectancy in the United States, eleven for men and five for women are in Colorado and Utah, all at mean elevations of 1,819 m or higher. We examined the relationship between elevation of the county of residence and life expectancy, and how the effects of altitude on major causes of death may explain this relationship. Methods: We used the National Elevation Dataset to estimate mean elevation and mortality statistics from the National Center for Health Statistics (NCHS) to estimate county cause-specific death rates. We analyzed the crude association between altitude and life expectancy, and mortality from cancers, cardiovascular diseases, and COPD. We also adjusted for socio-demographic factors and cumulative exposure to smoking in multivariable regression analysis. Results: Male and female life expectancies in 500 meter bands above 1,500 m were 71.2-75.9 and 76.4-77.2 years, respectively. These were longer than those within 100 m of sea level, by 3.3-8.0 years for men and 1.0-1.8 years for women. The asso-

ciation between altitude and overall life expectancy became non-significant or negative after adjustment for socio-demographic factors and smoking. Disease specific analysis after socio-demographic adjustments demonstrated that counties above 1,500 m had 10-14 fewer ischemic heart disease (IHD) deaths but 1-5 more COPD deaths per 10,000 people than those within 100 m of sea level. There was a dose-response relationship for both diseases. The adjusted associations for stroke and most cancers (except prostate and colon cancers) were non-significant. Conclusions: There may be a protective effect of living at higher altitudes on IHD and a harmful effect on COPD, with no net effect on overall life expectancy after adjustment for important confounding factors. 2009.

ALTITUDEOMICS: EFFECT OF HIGH ALTITUDE ACCLIMATISATION AND SUBSEQUENT RE-EXPOSURE ON CEREBROVASCULAR FUNCTION AND VENTILATORY CONTROL. Jui-Lin Fan[1], Andrew Subudhi[2], Oghenero Evero[2], Nicolas Bourdillon[1], Bengt Kayser[1], Andrew Lovering[3], Robert Roach[2]. [1]Univ Geneva, Geneva, Switzerland, [2]Univ Colorado, Denvor, USA, [3]Univ Oregon, Eugene, USA. *EMAIL: Jui-Lin.Fan@unige.ch* INTRODUCTION: The effect of high altitude acclimatisation and re-exposure to altitude on the cerebral blood flow and ventilatory responses to CO_2 has not been well described. METHODS: Using the steady-state method (clamping $PETCO_2$), we assessed the changes in middle cerebral artery velocity (MCAv) and ventilatory (VE) responses to CO_2 at sea-level (SL), during initial exposure to 5,260m (ALT1), after 16 days of acclimatisation (ALT16) and upon re-exposure 7 (POST7) or 21 days (POST21) of deacclimatisation. RESULTS: During initial exposure to altitude, the MCAv and VE responses to CO_2 at ALT1 were elevated compared to SL (by 65.1±63.6% and 14.7±11.9 L/min, respectively, $P<0.05$). With acclimatisation at ALT16, both MCAv and VE responses were further elevated compared to ALT1 (by 93±79% and 47.3±31.3 L/min, respectively, $P<0.05$). Upon re-exposure to altitude, the MCAv-CO_2 slopes remained higher at POST7 (by 117±84%) and POST21 (by 106±45%, $P<0.05$) compared to SL, but were lowered compared to ALT16 ($P<0.05$). Likewise, VE at 40 mmHg $PETCO_2$ remained higher at POST7 and POST21 compared to SL (by 29.3±12.8 and 25.7±6.5 L/min, respectively, $P<0.05$), but lower compared to ALT16 ($P<0.05$). These changes in CO_2 responses correlated with the changes in resting arterial bicarbonate concentrations with acclimatisation and upon re-exposure ($P<0.05$, pooled data). CONCLUSION: We found cerebrovascular and ventilatory responsiveness to CO_2 to be concurrently elevated with altitude acclimatisation. These increases might be accounted for, by the changes in acid-base balance associated with high altitude exposure. We found the effect of acclimatisation on these physiological parameters is partly retained despite a deacclimatisation period of 7 and 21 days, thus highlighting the potential benefits of pre-exposure for subsequent altitude sojourns. An enhanced cerebrovascular CO_2 reactivity could help maintain cerebral O_2 delivery during perturbations in breathing stability at high altitude. ACKNOWLEDGEMENTS: Support: Dept Defense #W81XWH-11-2-0040(RCR). 2015.

ALTITUDEOMICS: INTRAPULMONARY & INTRACARDIAC SHUNT IS ASSOCIATED WITH GREATER LAKE LOUISE SCORE AT 5300M. Julia Kern[1], Kara Beasley[1], Jonathan Elliott[1], Steven Laurie[1], Randall Goodman[2], Andrew Subudhi[3], Robert Roach[4], Andrew Lovering[1]. [1]Univ Oregon Dept Human Physiology, Eugene, OR, USA, [2]Oregon Heart and Vascular Institute, Springfield, OR, USA, [3]Altitude Research Center, Univ Colorado-Denver Anschutz Medical Campus, Aurora, CO; Univ Colorado-Colorado Springs, Dept Biology, Colorado Springs, CO, [4]Altitude Research Center, Univ Colorado-Denver Anschutz Medical Campus, Aurora, CO. *EMAIL: jkern@uoregon.edu* INTRODUCTION: It has been shown that lower SaO_2 after 30-60min breathing hypoxic gas is either predictive of, or associated with, greater acute mountain sickness (AMS) susceptibility upon further ascent. Right-to-left shunt has been suggested to contribute to lower SaO_2 experienced by AMS susceptible subjects. We hypothesized that the degrees of blood flow through intrapulmonary arteriovenous anastomoses (IPAVA) and patent foramen ovale (PFO) while breathing a FIO_2=0.14 for 30min would be predictive of AMS susceptibility at 5300m. METHODS: At sea level, subjects were screened for PFO using saline contrast echocardiography (SCE), 10 were PFO+ and 11 were PFO-. All subjects breathed a FIO_2=0.14 for 30min and SCE was performed to assess the degree of intrapulmonary and intracardiac shunt, and bubble scores of 0-5 were assigned. Subjects were subsequently divided into High ($\geq$3, n=10) and Low Score (<3, n=11) groups. After $\geq$ 1 month, subjects ascended from 1525m to 5260m in ~4hrs and AMS score was determined using the Lake Louise Symptoms Questionnaire (LLS) upon arrival (ALT1), day 2 (ALT2), day 5 (ALT5), and day 16 (ALT16). RESULTS: There were no significant differences between the Low and High Score groups for AMS. LLS score was not different between the High and Low Score groups at ALT1, ALT2, or ALT5; there was a trend for higher LLS in the High Shunt group at all time points. At ALT16, LLS was significantly higher in the High Shunt group (p=0.003). At ALT1 and ALT5, 100 and 40% of PFO+ subjects had AMS, respectively. At ALT1 and ALT5, only 64% and 10%, of PFO- subjects had AMS, respectively. CONCLUSION: These data suggest that blood flow through IPAVA and a PFO play a role in determining LLS severity and susceptibility to AMS, respectively. ACKNOWLEDGEMENTS: Support: Dept Defense #W81XWH-11-2-0040(RCR). 2015.

ALTITUDEOMICS: PERSISTENCE OF HIGH ALTITUDE ACCLIMATIZATION ON SUBSEQUENT RE-EXPOSURE: EFFECTS ON SUBMAXIMAL EXERCISE PERFORMANCE. Oghenero Evero[1], Andrew Subudhi[1], Jui-Lin Fan[2], Nicolas Bourdillon[2], Bengt Kayser[2], Andrew Lovering[3], Robert Roach[1]. [1]Univ Colorado, Denver, USA; [2]Univ Geneva, Geneva, Switzerland; [3]Univ Oregon, Eugene, USA. *EMAIL: oghenero.evero@ucdenver.edu.* INTRODUCTION: Maximal exercise performance is not improved with acclimatization. However, submaximal performance may be improved due to acclimatization-related increase in PaO2 and a tighter defense of SaO2. METHODS: In a repeated-measures fashion, 21 healthy, habitually active subjects (20.8±1.4 years; 175.8±7.9 cm; 69.7±9.0 kg),

born and residing at sea level completed a timed road run on a 3.2km course with 305m of elevation gain. We recorded the time to completion and estimated VO2 at sea-level (BL; 130m, PB=749mmHg), 1 day after initial exposure to 5260m on Mt. Chacaltaya, Bolivia (ALT2; 5260m, PB=406mmHg), after 17 days of acclimatization (ALT17) and upon re-exposure after subjects spent 7 (POST7) or 21 days (POST21) at a lower altitude (1525m, PB=639mmHg). RESULTS: Relative to BL, acute exposure to high altitude reduced average run speed 44 ± 6% (P<0.001). After acclimatization, run speed improved by 12 ± 22 % (P<0.001) from ALT2, but was still 39 ± 5% (P<0.001) slower than at BL. The improvement in run speed with acclimatization was retained at POST7 (P=0.762) but tended to decrease at POST21 (P = 0.094). Changes in average run speed paralleled changes in VO2 estimated from average run speed and the course grade. Compared to ALT2, subjects performed the road run at a higher percentage of their BL VO2max at ALT17 (ALT2 vs. ALT17, 62 ± 8% vs. 65 ± 7%, P<0.001), but there was no difference between ALT17 and POST7 (ALT17 vs. POST7, 65 ± 7% vs. 65 ± 8%, P=0.706). CONCLUSION: As has been previously described, high altitude submaximal performance decrements can be overcome with 17 days of acclimatization. in this stuy we show for the first time that some cardiorespiratory benefits of acclimatization are maintained for 7 and potentially 21 days when subjects are rapidly re-exposed to high altitude. ACKNOWLEDGEMENTS: Support: Dept Defense #W81XWH-11-2-0040(RCR). 2015.

ALTITUDEOMICS: REDUCED BLOOD FLOW THROUGH INTRAPULMONARY ARTERIOVENOUS ANASTOMOSES FOLLOWING 16 DAYS OF ACCLIMATIZATION TO 5300M. Jonathan Elliott[1], Steven Laurie[1], Randall Goodman[2], Kara Beasley[1], Julia Kern[1], Andrew Subudhi[3], Robert Roach[4], Andrew Lovering[1]. [1]Univ Oregon, Dept Human Physiology, Eugene, OR, [2]Oregon Heart & Vascular Institute, Echocardiography, Springfield, OR, [3]Altitude Research Center, Univ Colorado-Denver Anschutz Medical Campus, Aurora, CO; Univ Colorado-Colorado Springs, Dept Biology, Colorado Springs, CO, [4]Altitude Research Center, Univ Colorado-Denver Anschutz Medical Campus, Aurora, CO. *EMAIL: elliott.jon.e@gmail.com* INTRODUCTION: Acute hypoxia increases blood flow through intrapulmonary arteriovenous anastomoses (IPAVA) at rest and during exercise as detected via transthoracic saline contrast echocardiography. Pulmonary gas exchange efficiency, quantified by the alveolar-to-arterial PO_2 difference ($AaDO_2$), is known to worsen in acute hypoxia during exercise at iso-workloads. However, blood flow through IPAVA and concomitant changes in $AaDO_2$ at iso-workload after high-altitude acclimatization (HAA) in healthy lowlanders has never been studied. Accordingly, we sought to investigate if changes in blood flow through IPAVA are associated with changes in $AaDO_2$ that occur following HAA in healthy lowlanders. METHODS: Twenty-one healthy lowlanders were studied at rest and at 70, 100, 130, and 160W of cycle ergometer exercise at sea level (SL), in acute hypoxia at 5260m (ALT1), and after 16 days of acclimatization to 5260m (ALT16). During each 3 min workload metabolic data and radial arterial blood samples (body temperature and tonometry corrected) were collected and transthoracic saline con-

trast echocardiography was performed. Data were analyzed at the highest iso-workload achieved at SL, ALT1 and ALT16. RESULTS: The $AaDO_2$ during exercise at ALT1 was 13.6±2.9 mmHg, which decreased to 9.1±4.0 mmHg at ALT16 ($p<0.05$). Similarly, bubble scores based on the number and spatial distribution of saline contrast in the left ventricle, decreased during exercise from ALT1 to ALT16 ($p<0.05$). The total venous admixture (QVA/QT) was 19.5±4.3% at ALT1, which decreased to 12.7±3.6% at ALT16 ($p<0.05$). CONCLUSION: Blood flow through IPAVA decreases after HAA, which may in part explain the improvement in pulmonary gas exchange efficiency during exercise that accompanies HAA in healthy lowlanders. ACKNOWLEDGEMENTS: Support: Dept Defense #W81XWH-11-2-0040 (RCR), #W81XWH-10-2-0114 (AL), Evonuk Memorial Graduate Fellowship (SL), American Heart Association Predoctoral Fellowship (JE). 2015.

AMBULATORY BLOOD PRESSURE MONITORING REVEALS INCREASED SLEEPING BLOOD PRESSURE IN HYPERTENSIVE INDIVIDUALS AT HIGH ALTITUDE. David S. Young[1], Charles Duke[2], Jennifer Starling[1], David Twillman[1], Buddha Basnyat[3], Devlin Cole[4], Luke Mather[5], Theodore McConnell[6], Matthew McElwee[4], Sushil Pant[7], Purshotam Paudel[8], Benoit Phelan[9], Nirajam Regmi[10], T. Douglas Sallade[11], Alison Sheets[12], Linda E. Keyes[1]. [1]University of Colorado, [2]University of Tennessee, [3]Nepal International Clinic, [4]Case Western University, [5]University of Washington, [6]McGill University, 7Kunde Hospital, 8Tribhuwan University, [9]Dalhousie University, [10]Mountain Medicine Society of Nepal, [11]Philadelphia College of Osteopathic Medicine, [12]Longmont United Hospital. *Email: daveyoung222@gmail.com.* Introduction: For those traveling to high altitude, it has been postulated that blood pressure (BP) changes when compared to living at sea level. In this study, we aimed to measure continuous sleep and awake blood pressures in both normotensive and hypertensive Himalayan trekkers. Methods: Blood pressure was measured with Welch-Allyn 6100 24h ambulatory BP monitors (24h-ABPM) every 30min while awake and every 60min while asleep. Baseline demographic data was attained on arrival at 2860m and 24h-ABPM measured between 2860m-3440m and 3440m-4400m. Hypertensive subjects were defined by self-reported diagnosis of HTN. AMS was measured by Lake Louise Score (LLS). Results: Eight subjects were enrolled, 4 with pre-existing treated hypertension (HTN). Data was insufficient or incomplete for 2 with HTN. Between 2860m and 3400m, the 4 normotensive subjects (NTN) had the expected decrease in BP during sleep. MAP decreased from an average of 95mmHg (95% CI 89.76-100.24mmHg) while awake to 82.5mmHg (95% CI 74.72-90.28mmHg). The 2 with HTN had an increase in BP from awake hours (95.5mmHg [95% CI 84.81-106.11mmHg]) to sleep (112.5mmHg [95% CI 100.48-124.52mmHg]). The observed pattern in awake vs. sleep BP was similar between 3440-4400m, (N=4, 3 NTN, 1 HTN). The NTN had an average awake MAP of 94.67mmHg (95% CI 90.1-99.24mmHg) that decreased to 86mmHg (95% CI 74.03-97.97mmHg) and the HTN subject had an increase from awake MAP (90mmHg) to sleep MAP (106mmHg). No subject developed AMS at any altitude. No differences in HR, maximum or minimum BP, or number of BPs above normal were found between

NTN or HTN groups at altitude. Conclusions: These findings suggest that those with baseline HTN may have an increase in BP while sleeping compared to normotensive individuals at high altitude. The clinical significance of these findings remains to be studied. Acknowledgements: Wilderness Medical Society, University of Colorado, Nepal International Clinic. 2015.

AMP-ACTIVATED KINASE AND AKT KINASE IN RESPONSE TO OXYGEN AVAILABILITY IN THE ANOXIA TOLERANT CRUCIAN CARP (CARASSIUS CARASSIUS) HEART AND BRAIN. Kaere-Olav Stenslokken, Stian Ellefsen, Jonathan Stecyk, Mai Britt Dahl, G¯ran E Nilsson, Jarle Vaage. University of Oslo, University College of Lillehammer. *Email: k.o.stenslokken@imbv.uio.no.* Very few vertebrates can tolerate long periods of oxygen deprivation; one exception being the crucian carp (Carassius carasius), a North European freshwater fish. It readily survives months of anoxia at cold temperature, surviving solely on glycolysis and high glycogen stores, and avoids acidosis by converting lactate to ethanol. We recently showed that the crucian carp maintains cardiac output even after five days of anoxia. As mammalian heart and brain are especially anoxia-intolerant, the molecular background to anoxia tolerance is particularly interesting. We therefore investigated if two protein kinases critical for mammalian cells during energy deficiency, AMPK and AKT, are equally important for hypoxic/anoxic survival in the extremely anoxia-tolerant crucian carp. We report that phosphorylation of AMPK and AKT in heart and brain showed small changes after 10 days of severe hypoxia (0.3mg O_2/l at 9 ° C). In contrast, anoxia exposure (<0.01mg O_2/l at 8°C) substantially increased AMPK phosphorylation, but decreased AKT phosphorylation in carp heart and brain, indicating activation of AMPK and deactivation of AKT. In agreement, blocking the activity of AMPK in anoxic fish in vivo with 20 mg/kg Compound C resulted in an elevated metabolic rate (as indicated by increased ethanol production) and tended to reduce energy charge (ATP/ADP AMP ratio). This is the first in vivo experiment with Compound C in a non-mammalian vertebrate and it appears that AMPK plays a role in mediating anoxic metabolic depression in crucian carp. In the heart, expression of the regulatory γ_2 subunit increased in heart during anoxia. In the brain, expression of the α_1, α_2 and γ_1 subunits decreased with anoxia exposure, but expression of the γ_2 subunit remained constant. Combined, our findings suggest that AMPK and AKT may play important, but opposing roles for hypoxia/anoxia survival in the anoxia-tolerant crucian carp. 2009.

AN OBSERVATIONAL STUDY OF THE PRESENTATION AND CHARACTERISTICS OF HAPE IN PHERICHE, NEPAL (4243M). Suzy Stokes, Suzi Mackenzie. *Email: suzystokes@doctors.org.uk.* Introduction: An observational study of the presentation and characteristics of HAPE in Pheriche, Nepal (4243m) Methods: Between September and November 2010 at the Himalayan Rescue Association's (HRA) rescue post in Pheriche (4243m), 31 patients who fulfilled the diagnostic criteria for High Altitude Pulmonary Edema (HAPE) were analysed. Their age, gender, nationality, ascent profile, symptoms, predominant symptom, height of onset, co-morbidities, medications, treatment and evacuation

method were ascertained from paper records. In addition their physiological data including, pulse, blood pressure, oxygen saturations, post-oxygen saturations and Lake Louise Score (LLS) were also recorded. For comparison, data was also collected from 33 patients with mild-moderate Acute Mountain Sickness (AMS) and 32 asymptomatic trekkers. Results: Of 31 in the HAPE group 23 were men (74.2%), mean age 39 (range 12 -63). The average height of symptom onset was 4768m vs. 4367m in the AMS group which consisted of 17M (51.5%), mean age 38 (range 19-62). The control group contained 22 men, mean age 35 (range 15-65). The HAPE subjects most commonly reported breathlessness (11/24) and fatigue (18/26) as their predominant symptom and six who reported fatigue denied breathlessness. Other symptoms included cough (26), sputum (4), haemoptysis (2), fever (1), chest pain (5) and difficulty sleeping (26). All 31 had crackles on auscultation and four also had wheeze. Mean saturation in HAPE patients was significantly lower (67.3% vs 88.6% vs 88.9%), pulse significantly higher (112 vs.88 vs.87) although MAP was similar in all three groups (97.7mmHg vs. 98.0mmHg vs. 96.5mmHg). Thirty received Nifedipine without adverse event. Sixteen were evacuated by helicopter. Conclusion: HAPE can manifest in a variable fashion, and we wish to highlight this aberrant group of patients who do not develop dyspnoea despite marked hypoxaemia. It is important for expedition doctors to consider HAPE even in the absence of breathlessness. 2011.

ANGIOGENIC RESPONSES TO INTERMITTENT NORMOBARIC HYPOXIA IN HUMAN SKELETAL MUSCLE. Jung Hoon Lee[1], Jun Bong Yoon[1], Hyung Tae Kwon[1], He Long Quan[1], Yu Nah Jeon[1], Hyo Jeong Kim[2], Chang Keun Kim[3]. [1]Korea National Sport Univ, [2]Aging Research Center, Korea National Sport Univ, [3]Altitude Research Center, Korea National Sport Univ. *EMAIL: ckkim@knsu.ac.kr*
INTRODUCTION: Continuous residence at moderate altitudes (<3,000m) improves the oxygen transport capacity by an erythropoietin-induced increase in the red cell volume. An increase in the hemoglobin concentration has been shown to augment maximal O_2 consumption, although improved oxygen transport capacity is achieved after altitude training, because acute mountain sickness, problems with acclimatization and detraining due to decreased intensity are believed to influence the effectiveness of altitude training. Therefore, the effects of altitude training on sea-level performance show contradictory results. METHODS: The purpose of the present study is to investigate the angiogenic response to intermittent normobaric hypoxia exposure (INH). Twelve leisure cyclists participated in the study. INH group have completed 8 hours of INH per day for three weeks (n=6) and trained a routine workout together with the control group (CON, n=6) in normobaric normoxic condition. Blood and muscle sample obtained from arm vein and vastus lateralis muscle before and after INH and compared to CON group. RBC, Hb, Hct unchanged in both INH and CON groups after training. Hypoxia inducible factor-1a, vascular endothelial growth factor total /165, angiopoeitin1 and 2 proteins were measured. RESULTS: HIF-1a significantly increased by 23% ($p<.05$) and 27% ($p<.001$) in both INH and CON groups after training, respectively. VEGF total significantly increased by 17% ($p<.05$) in INH group, whereas unchanged in CON group, and VEGF165 signifi-

cantly increased by 15% ($p<.05$) in INH group, whereas unchanged in CON groups after training. Angiopoeitin 1 significantly increased by 39% ($p<.01$) in INH group after training, whereas unchanged in CON group, and angiopoeitin 2 unchanged in both INH and CON groups after training. CONCLUSION: The present study showed that three weeks of intermittent normobaric hypoxia exposure did increase in angiogenic factors and did additive effect by INH exposure. 2015.

APPLICATION OF ACCELEROMETRY TO MEASURE INTENSITY AND DURATION OF ACTIVITY DURING HIGH ALTITUDE EXPEDITIONS. Walter Hailes, Brent Ruby. University of Montana. *Email: walter.hailes@gmail.com.* Objective: Researchers investigating high altitude physiology lack a reliable tool for measuring the intensity of activity during mountainous expeditions. The purpose of the present investigation was to describe the intensity and duration of activity during a climbing expedition to 6194m using accelerometry. Method: Eight individuals (38.4 ± 11.7 yrs, 79.8 ± 7.7 kg) served as subjects. Climbers reached the summit of Denali (6194 m) via the same route but participating in two different climbing expeditions. Activity data were compared using the climbing seasons (2007 {n=4} vs. 2008 {n=4}). Mean activity (counts·min^{-1}, non-dominant writs worn activity monitors) was divided into four ascent stages and analyzed during walking and sleeping time periods for the duration of each expedition. Results: While duration and distance of travel during each stage of the expeditions were similar, the 2008 expedition demonstrated higher activity compared to the 2007 expedition. The mean activity while walking decreased during each ascent stage above 3395 meters. Sleep activity was similar independent of expedition year. Discussion: The novel findings of the current project are that mean walking activity was greater during the 2008 compared to 2007 expedition. Independent of expedition year, walking activity decreased as altitude increased over 3395 meters until 5212 meters was reached. The 2007 expedition experienced several days of fresh snow while the 2008 had no snow accumulation. It is our hypothesis that the fresh snow of 2007 made traveling more difficult as well as reducing the exercise intensity recorded by the activity monitors. Therefore within one environment, activity monitors can measure activity intensity, but caution must be taken when comparing activity measured with varied ground conditions. 2009.

ARE ALL PULSE OXIMETER RELIABLE AT SEVERE HYPOXIC CONDITION?. Nao Kurita[1], Shigeru Masuyama[1], Shinji Fukushima[1], Atsuo Hamada[1]. [1]Travelers Medical Center, Tokyo Medical University Hospital, Tokyo Japan. *Email:* drkuriko0318@gmail.com. Introduction: Pulse oximeters(PO) are now the most popular tools to monitor hypoxia level at altitude study. A lot of models of PO arrive on the commercial market, while many researchers have noticed considerable variation of SpO_2 among the models especially at high altitude. Are all of them reliable? Our purpose is to evaluate model dependent accuracy of PO at altitude. Methods: Nine models of PO, which are commonly used at medical institutions in Japan, by ten healthy non-smoking volunteer males (from 21 to 43 years old) were monitored at hypoxic room. The SpO_2 values were compared with the

arterial SaO_2 sampled at FiO_2=0.21, 0.15, 0.13, 0.12, and 0.10 and analyzed by Rapid lab 1265, Bayer Health Care of Laboratory Center of Tokyo Medical University Hospital, Tokyo Japan. SpO_2 accuracy was evaluated by their bias from SaO_2 and the standard deviation (S.D.) of SpO_2. Results: At severe hypoxic range of SaO_2 less than 80%, the bias and S.D. were greater than those at moderate SaO_2 between 80 and 100% in all models. Four out of 9 models showed greater S.D. of SpO_2 than 2% and five out of 9 models showed larger than 2.0 ARMS calculated by taking the square root of the sum of the square of the SpO_2 bias from SaO_2 plus the square of the S.D. of the SpO_2. Conclusions: It was suggested that PO under severe hypoxia were not always so accurate as under moderate hypoxia and that some models of PO could not keep their accuracy at severe hypoxia without sustained $PaCO_2$. We need to carefully choose the models of PO when we try them as a scientific devices at altitude study. 2015.

ARTERIAL OXYGEN CONTENT ON ASCENT TO ALTITUDE. Daniel Martin, Denny Levett, Mike Grocott, for the Caudwell Xtreme Everest Investigator Group . University College London. *Email: dan.s.martin@gmail.com.* Objective: Arterial oxygen content (CaO_2) may return to sea level values after sufficient acclimatisation, but the time-course of this response is unclear. We report changes in CaO_2 with graduated exposure to hypoxia in three groups of healthy volunteers with different profiles of ascent to altitude. Methods: Measurements of peripheral oxygen saturation (SpO_2), using a pulse oximeter, and haemoglobin concentration (Hb), using a hand held photometric device, were made in 222 subjects at sea level, 1300m, 3500m, 4200m, and 5300m. CaO_2 (Hb x SpO_2 x 1.39) was calculated. There were 3 subject groups: Group 1: Trek to 5300m over 11 days (n=180) Group 2: Trek to 5300m over 13 days and staying at 5300m until day 70 (n=9) Group 3: Trek to 5300m over 13 days and climbing higher until day 70 (n=14) Subjects were tested 1 - 2 (group 1) or 2 - 4 (groups 2 and 3) days after arrival at each altitude. Results: In group 1 CaO_2 fell from baseline (197.1±16.1 ml/l) with ascent to 3500m (181.8±17.3 ml/l), 4300m (182.8±19.1 ml/l) and had not recovered at 5300m on day 12 (170.9±20.9 ml/l) ($p<0.001$ for all comparisons). In groups 2 and 3 CaO_2 remained unchanged throughout ascent on arrival at 5300m on day 16. Prolonged stay at 5300m (group 2) produced no further rise in CaO_2; 181.7±11.5 ml/l at sea level, 206.9± 27.2 ml/l on day 70. Higher ascent (group 3) resulted in a further increase of CaO_2; 193.9±10.1 ml/l at sea level, 234.6±16.5 ml/l at day 72 ($p<0.001$). Conclusions: Oxygen content on ascent to altitude depends, in part, on duration and pattern of acclimatisation. Oxygen content may exceed sea-level values following sufficient duration and extent of altitude exposure. 2009.

ARTIFICIAL OXYGEN CARRIERS AND VASODILATORS: THE ANSWER TO EXERCISE AT ALTITUDE? Robert A Jacobs, Martha C Tissot van Patot, Molly White, Ben Foreman, Robert W Gotshall, David C Irwin, Karyn L Hamilton. Colorado State University, University of Colorado Health Science Center. *Email: robert.jacobs@colostate.edu.* BACKGROUND: Reduced oxygen availability at

high altitudes can severely decrease exercise capacity. Hemoglobin-based oxygen carriers (HBOC) have been shown to increase oxygen carrying capacity in a hypoxic environment, however overall delivery of oxygen to tissue was reduced as a consequence of attendant increases in systemic and pulmonary vasoconstriction ultimately resulting in diminished blood flow. If the associated reductions in blood flow could be offset, then HBOC administration may be able to conserve exercise capacity following rapid ascent. PURPOSE: To investigate the effectiveness of supplementing a vasodilator with the administration of HBOC on exercise capacity at an approximate simulated elevation of 4,300 m. METHODS: Conscious, male Sprague-Dawley rats were randomly assigned to one of five groups; a group treated with Lactated Ringerís solution which served as the control (LR), a group treated with HBOC (HBOC), a group treated with sildenafil and HBOC (SILOX), a group treated with s-nitrosylated HBOC (SNO), and a group treated with sildenafil (SIL). Following treatment, all animals were placed in a hypobaric chamber with the barometric pressure reduced to the equivalent of approximately 4,300 m. At altitude, all animals were subjected to an exercise bout on a treadmill and time to exhaustion was monitored. RESULTS: The HBOC group exhibited significantly quicker time to exhaustion when compared to both the LR and SIL groups ($p = 0.019$ and 0.023, respectively). There were no other significant differences observed between groups. CONCLUSIONS: There appears to be no benefit of HBOC administration on exercise capacity in hypoxia despite attempts to compensate for HBOC-specific vasoconstriction. In fact, HBOC administration seems to exacerbate hypoxia-mediated impairments to exercise capacity. Supported by the Defense Advanced Research Projects Agency (DARPA) and the Army Research Office (ARO) contract number W911NF-06-1-0318. 2009.

ASSESSMENT OF C-REACTIVE PROTEIN WITH ACUTE EXPOSURE TO ALTITUDE FROM SEA LEVEL TO 3417M. Paul Visich[1], James Parrish[1], Chad Lyons[1], Erin Flatley[1], Lexie Basiliere[1], Rebecca Miles[1], Annelise Donahue[1], Shireen Rahman[1]. [1]University of New England, Biddeford, ME. *Email: pvisich@une.edu.* Introduction: C-reactive protein (CRP) is considered an inflammatory marker that can rise when the body is exposed to acute or chronic inflammatory conditions such as tissue injury, bacterial infections, diabetes, hypertension, CVD and other inflammatory diseases. Acute exposure to elevation has also observed changes in CRP but inconsistent, based on the level of altitude exposure. Exposure to 3458m have observed significant increases in CRP whereas acute exposure to 2590m produced a decline in CRP. Objective: Assess CRP, AMS and hemodynamic changes to acute exposure to 3417m in healthy subjects that live at sea level. Methods: 12 healthy college students living at sea level from Portland, ME traveled to Vail Pass, CO where they lived for 5 days at 3417m. Saliva samples were collected in the morning prior to leaving for CO, then re-assessed after 24 and 96h of altitude exposure. AMS survey was completed by all subjects at each time point along with blood pressure (BP), heart rate (HR) and oxygen saturation (O_2S). Saliva samples were were analyzed by Salimetrics, Inc. for CRP determination. Results: Two subjects were eliminated from the study because they became ill from a viral infection. In the 10

subjects (20.7±.65y of age) there was no significant difference in CRP between sea level samples and 24 and 96h of altitude exposure. In addition, there was no relationship between CRP levels and AMS survey scores. At 24h of altitude exposure vs. baseline, there was a decline in systolic and diastolic BP ($p<.03$ and $<.00$, respectively), O_2S ($p<.00$) and increase in HR ($p<.03$). However the only difference at 96h Vs baseline was a decline in O_2S ($p<.00$). Conclusion: Though there were hemodynamic changes with acute altitude exposure at 3417m, this elevation appears not to promote an increase in inflammatory markers. 2015.

AT HIGH ALTITUDE SHERPAS HAVE LOWER PULMONARY ARTERY PRESSURES THAN WESTERNERS. Buddha Basnyat, Jenny Hargrove, Peter S. Holck, Soni Srivastav, Kshitiz Alekh, Laxmi V. Ghimire , Kaushal Pandey , Anna Griffith, Ravi Shankar, Komal Kaul, Asmita Paudyal, David Stasiuk, Rose Basnyat, Christopher Davis, Andrew Southard, Cathleen Robinson, Thomas Shandley, Dan W. Johnson, Ken Zafren Sarah Williams, Eric A. Weiss, Jeremy J. Farrar, and Erik R. Swenson. Stanford University, Nepal International Clinic, Institute for Altitude Medicine, University of Washington. *Email: zafren@alaska.com.* Background: It has been hypothesized, but never proven, that people with Tibetan ancestry, like Sherpas, have lower pulmonary artery systolic pressure (PASP) at high altitude than lowlanders who have traveled to the same elevation. Objective: To compare PASP in Sherpa individuals with two groups of lowland residents, non-Sherpa Nepalis and westerners traveling to Everest Base Camp. Methods: Cardiac ultrasonography was used to measure the velocity of tricuspid regurgitation and estimate PASP in 26 Sherpas, 27 non-Sherpa Nepalis, and 164 Westerners who were healthy and did not suffer from high altitude pulmonary edema at 4900 m at Lobuje in the Everest region. Results: The mean PASP values (mean /-SD) for the three groups were 28.2 /-4.9 mm Hg for the Sherpas, 31.9 /-8.7 mm Hg for the non Sherpa Nepalis, and 32.6 /-9.7 mm Hg for Westerners. The difference between Sherpas and Westerners was statistically significant ($p< 0.025$). There were 26 Westerners with PASP > 48 mm Hg, although none had high altitude pulmonary edema. The highest pressure in the Sherpa group was 38 mm Hg. Conclusion: This study demonstrates that Sherpas had lower PASP when compared to Westerners and non-Sherpa Nepalis at 4900m in Lobuje, although the measured differences were small. Further study is necessary to investigate the mechanism behind these observed differences. 2009.

AUTONOMIC REGULATION IN ADOLESCENTS AFTER RAPID ASCENT TO 3,454 M. Thomas Stuber, Stefano R Rimoldi, Phang B Lim, Sophie Garcin, Herve Duplain, Jonathan Bloch, Claudio Sartori, Urs Scherrer, Yves Allemann, Nicholas S Peters. Triemlispital Zurich, Swiss Cardiovascular Center, Imperial College Healthcare NHS, St.Marys Hospital, Dept Internal Medicine and Botnar Center for Extreme Medicine. *Email: tstuber@inside.net.* Increasing numbers of children and adolescents are travelling to destinations situated at high altitude and are subsequently exposed to hypoxia and its potential health risks. Rapid ascent to high-altitude triggers sympathetic activation in adults, which can be detected by measurements of heart rate variability and contributes when exaggerated in the

pathogenesis of high-altitude pulmonary edema. Data in children on autonomic regulation after rapid ascent to high altitude are lacking. The aim of this study is to characterise autonomic regulation in healthy children during short time exposure to high altitude. 97 healthy children (mean±SD age, 11.9±2.2 years, range 7-17 years, 47 girls and 50 boys) participated in the study. HRV was measured from 5 Minutes ECG recording at low altitude (450m) and 24h after arriving at the high altitude research station of the Jungfraujoch in Switzerland (3454m asl). Time domain analysis demonstrated an increase in heart rate in all subjects from low to high altitude (74±9 vs. 97±13 bpm, P<.0001). Frequency domain analysis showed a decrease in the total, high- and low frequency powers calculated with linear and non-linear models as the subjects travelled from low to high altitude. In normalized units (nu) there was a significant increase of low frequency powers on ascent to high altitude (43.9±17.3 vs. 56.9±21.0, P<0.0001). The same was observed with non-linear (auto regressive) model, where the amount of LF-powers in normalised units (nu) increased on ascent to high altitude (51.1±23.3 vs. 64.3±20.1, P<0.0001) and LF/HF ratio increased significantly from low to high altitude (2.04±2.36 vs. 4.72±7.10, P=0.0007). We report here the first study on autonomic regulation in a large group of healthy low altitude resident children after rapid ascent to high altitude. Children respond similar to adults with sympathetic activation, an important mechanism in the pathogenesis of high-altitude pulmonary edema. 2009.

AUTONOMOUS NERVOUS SYSTEM (ANS) ACTIVITY AND MOOD STATE IN ELITE ATHLETES FOLLOWING 4-WEEK OF LIVE HIGH – TRAIN LOW: A DOUBLE BLINDED PLACEBO CONTROLLED STUDY. Aurélien Pichon[1], Laurent Schmitt[2], Christoph Siebenmann[3], Peter Rasmussen[3], Robert A Jacobs[3], Victor Diaz[3], Paul Robach[4], Carsten Lundby[3]. [1]Laboratoire 'Réponses cellulaires et fonctionnelles à l'hypoxie', Université Paris 13, Bobigny, France, [2]Centre National de Ski Nordique, Prémanon, France, [3]Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland, [4]Ecole Nationale de Ski et d'Alpinisme, Chamonix, France. *Email: aurelien.pichon@univ-paris13.fr*. Introduction: During a Live-High-Training-Low (LHTL) training camp adaptations to hypoxia interact with the autonomous nervous system (ANS) adaptations to training. Moreover mood has been shown to be closely linked to ANS modulations. Therefore the aim of this study was to assess the effect of a 4-week double-blinded placebo controlled LHTL session on ANS activity and mood state in elite athletes. Methods: Sixteen subjects submitted to the same training at about 1100m were assigned to LHTL (n=10, 3000m) or placebo groups (PLA, n=6, 1100m) and remained in their chamber a minimum 16h/day. ANS activity was assessed during an orthostatic test performed on the morning before, during and after the training camp by heart rate variability (HRV) and Poincaré Plot analysis giving short parasympathetic (SD1) and long term sympathetic (SD2) variabilities. The Profile of Mood State questionnaire was filled out concomitantly. Results: In the supine position, SD2 increase during the first week of hypoxic exposition in the LHTL group as compared to baseline. However, PLA group showed a larger SD2 than the LHTL group after 2 weeks. In the upright position, we observed a main group effect with-

out effect of intervention with lower total HRV, high frequency power, SD1 and SD2 in LHTL group as compared to PLA group. The Vigor scores decreased for both groups at the end of the training camp as compared to baseline or to the beginning of the camp. The PLA group also displayed less Vigor scores as compared to the LHTL group for the whole study. This decrease in Vigor scores in PLA subjects was significantly correlated to the decrease in total and low frequency powers (ms2), and SD2 in supine ($R>0.49$, $P<0.01$). Conclusion: A LHTL training camp induced an increase in sympathetic modulation only during the first week of the hypoxic exposure. The decrease in Vigor in PLA group during the period seems to be related to a decrease in sympathetic modulation without hypoxic stimulation. 2011.

AYSMMETRY OF CEREBRAL BLOOD FLOW VELOCITY IS ASSOCIATED WITH DAYTIME AND OVERNIGHT DESATURATION IN SICKLE CELL ANEMIA. Joseph Collinson[1], Julie Makani[2], Fenella J Kirkham[3]. [1]University of Oxford, Oxfordshire, UK, [2]Mhumbili University, Dar-es-Salaam, Tanzania, [3]UCL Institute of Child Health, London, UK. *EMAIL: joseph.collinson@some.ox.ac.uk* INTRODUCTION: Following acclimatisation to altitude, healthy adults and children are predisposed to low daytime and overnight haemoglobin oxygen saturation (SpO_2). Low SpO_2 is associated with changes in cerebral blood flow velocity (CBFV) as measured by transcranial doppler (TCD) ultrasound, which appear to be different for right and left sides (Gavlak et al. 2013). Daytime and overnight desaturation is also common in sickle cell anemia (SCA) even at sea level but there are currently no data on any association between CBFV asymmetry and low SpO_2. Our aim was to investigate associations between asymmetry in CBFV in the MCA, ACA, and internal carotid artery (ICA), measured as the ratio between left and right CBFV, and daytime and overnight SpO_2 in children with SCA. METHODS: Daytime and overnight SpO_2, vital signs and TCD studies were undertaken in 127 Kenyan children aged 1-16, 109 with SCA and 18 controls. Left and right MCA, ACA, and ICA CBFV were obtained. Correlations were examined between asymmetry in CBFV (ratio of Right:Left MCA, ACA and ICA) and daytime and overnight SpO_2 (SPSSv17). RESULTS: We found significant correlations between the degree of asymmetry in the ICA and the median overnight SpO_2 ($r=-0.292$, $p<0.05$) and between asymmetry in MCA velocity and mean daytime SpO_2 ($r=-0.313$, $p<0.05$). CONCLUSION: Our data suggests that asymmetry of CBFV is associated with haemoglobin oxygen desaturation in SCA. Individuals with asymmetrical flow in MCA and ICA were more likely to have reduced daytime and overnight saturations respectively. Further work is needed to establish whether there are differences in the determinant of right and left sided CBFV (Gavlak et al 2013). 2015.

BETA-BLOCKERS, HYPERTENSION AND TOLERANCE TO HIGH ALTITUDE. Linda Keyes[1,2], François Lhuissier[1], Jean-Paul Richalet[1]. [1]Université Paris 13, Bobigny, France, [2]Univ Colorado School of Medicine, Aurora, CO. *EMAIL: linda.keyes@aya.yale.edu* INTRODUCTION: Hypertension is common amongst visitors to high altitude. Although hypertension does not increase the risk for acute mountain sickness (AMS), beta-blockers, a common treatment for hyper-

tension, could impact acclimatization by blunting the adrenergic response to hypoxia. We asked: 1) Does chronic beta-blocker use alter chemoreceptor sensitivity to hypoxia as measured by hypoxic exercise test? 2) Do beta-blockers increase the risk of AMS? METHODS: We used the hypoxic exercise test developed in our department at Avicenne hospital to measure the ventilatory and cardiac responses to hypoxia at exercise. This test provides independent predictors of altitude illness and improves an altitude-illness risk prediction model. We compared the physiological parameters of response to hypoxia and the clinical tolerance to high altitude (questionnaire) in a group of 81 patients taking beta-blockers (Beta-) for any indication with a control group (Ctrl) of 1177 age-matched subjects (mean 54±6 yrs). RESULTS: Anthropometric variables were comparable between groups. Cardiac response to hypoxia at exercise (HCR) was blunted in Beta- (HCR men 0.55±0.26 vs 0.72±0.28; HCR women 0.46 ± 0.29 vs 0.65±0.32, $p<0.001$ for both) but ventilatory response to hypoxia (HVR) was similar (HVR men 0.81±0.32 vs 0.78±0.35; HVR women 0.64±0.32 vs 0.68±0.33, ns). Hypoxia-induced desaturation at exercise was similar in the two groups. The prevalence of altitude illness was similar (23.7% in Beta- vs 24.0% in Crtl). Results did not differ in a sub-group of 44 patients with hypertension. CONCLUSION: The use of beta-blockers expectedly limits the cardiac response to hypoxia but does not impair the ventilatory response. The use of beta-blockers at high altitude does not modify the susceptibility to high altitude illnesses. 2015.

BLOOD FLOW THROUGH HYPOXIA-INDUCED INTRAPULMONARY ARTERIOVENOUS ANASTOMOSES PREDICTS ARTERIAL OXYGEN DESATURATION. Kara Beasley[1], Steven Laurie[1], Jonathan Elliott[1], Jerold Hawn[2], Andrew Lovering[1]. [1]University of Oregon, [2]Oregon Heart and Vascular Institute. *Email: kbeasley@uoregon.edu*. Introduction: Arterial O_2 desaturation is associated with an increased susceptibility to high altitude illnesses. Therefore, our aim was to determine if blood flowing through hypoxia-induced intrapulmonary arteriovenous anastomoses (IPAVAs) could be used to predict arterial O_2 desaturation in hypoxic conditions. Methods: Thirteen subjects (6 female) breathed 4 FIO_2s (0.10, 0.12, 0.14 & 0.16) for 30 min each in either ascending or descending order. Forehead saturation was measured and saline contrast echocardiography was performed to detect blood flow through IPAVAs. Bubble scores (0-5, low to high) were assigned based on the density and spatial distribution of bubbles in the left ventricle. Results: Subjects with the highest bubble scores at the highest saturations had the greatest degree of arterial O_2 desaturation after breathing an FIO_2=0.10. 6/13 subjects had a bubble score of ≥ 2 at a saturation of 93.8+/-3.0% (mean+/-SD) and after breathing an FIO_2=0.10, saturation decreased to 63.0+/-5.4% (desaturators). In contrast, 5/13 subjects did not achieve a bubble score of ≥ 2 until they desaturated to 85.3+/-3.0% and after breathing an FIO_2=0.10, their saturation only decreased to 76.2+/-7.4% (non-desaturators). Conclusions: Eight-five percent of the subjects could be classified as either “desaturators” or “non-desaturators” while breathing severe hypoxic gas mixtures based solely on their bubble scores while breathing mild hypoxic gas mixtures. These data support the idea that blood flow through IPAVAs is detrimental

to pulmonary gas exchange efficiency. Accordingly, detecting blood flow through IPAVAs may be used to identify those who are potentially susceptible to high altitude illnesses. Acknowledgements: APS Giles F. Filley Memorial Award; Oregon Health & Science University MRF Grant #0820; Dept of Defense #DM102758 TATRC. 2011.

BLOOD FLOW THROUGH INTRAPULMONARY & INTRACARDIAC SHUNTS PREDICTS AMS SUSCEPTIBILITY. Kara Beasley[1], Julia Kern[1], Tyler Mangum[1], Jonathan Elliott[1], Randall Goodman[2], Jerold Hawn[2], Andrew Lovering[1]. [1]Univ Oregon, Eugene, OR, USA, [2]Oregon Heart and Vascular Institute, Springfield, OR, USA. *EMAIL: kbeasley@uoregon.edu* INTRODUCTION: It has been shown that lower SaO_2 after 30-60min breathing hypoxic gas is either predictive of, or associated with, greater acute mountain sickness (AMS) susceptibility upon further ascent. Right-to-left shunt has been suggested to contribute to lower SaO_2 experienced by AMS susceptible subjects. We hypothesized that the degrees of blood flow though intrapulmonary arteriovenous anastomoses (IPAVA) and patent foramen ovale (PFO), while breathing a FIO_2=0.14 for 30min would predict AMS susceptibility in subjects breathing severe hypoxia, FIO_2=0.115 for 10hrs. METHODS: Subjects were screened for a PFO and divided into PFO+ (n=16), and PFO- (n=12) groups. On Day #1 subjects breathed FIO_2=0.16, 0.14, 0.12, 0.10 for 30min each. Measurements of arterial blood gases and bubble scores (0-5, based on density and spatial distribution) using saline contrast echocardiography to assess intrapulmonary and intracardiac shunt were made at baseline and after 15 and 30min of breathing each hypoxic gas. On Day #2 (≥7 days later), subjects breathed a FIO_2=0.115 (4760m equivalent) for 10hrs in an environmental chamber to determine AMS susceptibility using the Lake Louise Symptoms Questionnaire. RESULTS: After 30min breathing FIO_2=0.14, SaO_2 was significantly lower in AMS susceptible (AMS+) compared to AMS resistant (AMS-) subjects (87.7±3.2 vs. 90.0±2.0, p=0.03). Subjects with bubble scores ≥3 and <3 breathing a FIO_2=0.14 were predicted to be AMS+ and AMS-, respectively. Using bubble scores obtained on Day #1 breathing a FIO_2=0.14, we were able to predict AMS susceptibility on Day #2 with 82% accuracy in both PFO+/- groups. PFO+ subjects had a 47% chance of developing AMS and PFO- subjects had only a 36% chance. CONCLUSION: This study suggests that blood flow through IPAVA and a PFO play a role in the development of AMS and can be used to predict AMS susceptibility. ACKNOWLEDGEMENTS: Support: Defense of Defense, #W81XWH-10-2-0114/#DM1027581JTCG5TATRC. 2015.

BLOOD PRESSURE VS ALTITUDE IN HYPERTENSIVE AND NON-HYPERTENSIVE HIMALAYAN TREKKERS. T. Douglas Sallade[1], Alison Sheets[2], Jennifer Starling[3], David Young[3], David Twillman[3], Nirajan Regmi[4], Benoit Phelan[5], Purshotam Paudel[6], Sushil Pant[7], Matthew McElwee[8], Theodore McConnell[9], Luke Mather[10], Charles Duke[11], Devlin Cole[8], Buddha Basnyat[4], Linda E. Keyes (presenting author)[3]. [1]Philadelphia College of Osteopathic Medicine, [2]Longmont United Hospital, [3]University of Colorado, [4]Nepal International Clinic,

[5]Dalhousie University, [6]Tribhuvan University, [7]Kunde Hospital, [8]Case Western Reserve University, [9]McGill University, [10]University of Washington, [11]University of Tennessee. *Email: thomassal@pcom.edu.* Introduction: Determine blood pressure (BP) response to changes in altitude in Himalayan trekkers with and without hypertension (HTN). Methods: BP was measured in Lukla (2800m), Namche (3400m), and either Pheriche or Dingboche (4400m) on ascent and descent. Hypertensive subjects were defined by self-reported diagnosis of HTN. Results: Trekkers had HTN (H, n=60) or no HTN (NH, n=604). Of those with HTN, 50 (83%) took one or more BP medications including ACEIs/ARBs (n=35, 48%), Ca++ channel blockers (n=15, 22%), beta-blockers (n=9, 13%), thiazide diuretics (n=7, 10%), and others (n=5, 7%). At 2800m, systolic BP (SBP) and diastolic BP (DBP) were greater in the H group than in the NH group [mean SBP= 151mmHg (95% CI 145.4-155.7) vs 127mmHg (95% CI 125.5-128.0); mean DBP=88mmHg (95% CI 85.1-91.7) vs 80mmHg (95% CI 79.3-80.8)] and remained higher at both 3400m [mean SBP=150mmHg (95% CI 143.7-156.9) vs 127mmHg (95% CI 125.8-128.5); mean DBP=88mmHg (95% CI 84.3-90.8) vs 82mmHg (95% CI 80.7-82.5)] and 4400m [mean SBP=144mmHg (95% CI 136.7-151.7) vs 128mmHg (95% CI 126.4-129.5); mean DBP=87mmHg (95% CI 83.2-91.7) vs 82mmHg (95% CI 81.3-83.2)]. Between 2800m and 3400m, BP increased in 37% of trekkers, decreased in 25%, and did not change in 38%; from 3400m to 4400m, BP increased in 35% of trekkers, decreased in 26%, and did not change in 40%. Prevalence of severe hypertension (BP>180/120mmHg) was similar across altitudes but higher in the H group (9%; 10%; 8% vs 0.7%; 0.6%, 0.3%) at 2800m, 3400m, and 4400m, respectively. No subjects reported symptoms of hypertensive emergency (chest pain, stroke, etc.). Conclusion: Blood pressure response to altitude is variable. High prevalence of severe hypertension in hypertensive trekkers warrants further study regarding BP control at high altitude. Acknowledgements: Wilderness Medicine Society, Nepal International Clinic. 2015.

BLOOD-BASED BIOMARKERS IDENTIFY NOVEL PATHWAYS FOR RESISTANCE TO ACUTE MOUNTAIN SICKNESS. Colleen G. Julian1; Andrew Subudhi1,2; Megan J. Wilson1; Andrew Dimmen1; Travis Pecha1; Robert C. Roach1.1University of Colorado Denver, Aurora, CO, 2 University of Colorado at Colorado Springs, CO. *Email:colleen.julian@ucdenver.edu.* Introduction: Acute mountain sickness (AMS) is marked by headache onset within hours after exposure to hypobaric hypoxia (HH). Increased brain volume with HH is thought to play a key role in the pathophysiology of AMS. One explanation for increased brain volume is that biochemical changes in response to HH disrupt the blood brain barrier (BBB) and permit abnormal movement of fluid into the brain parenchyma. This report evaluates whether blood-based biomarkers known to influence BBB function differ prior to or during HH exposure between AMS susceptible and AMS resistant individuals. Methods: Twenty subjects aged 18-45 were included for study. The study consisted of three arms, distinguished by pre-treatment with placebo, acetazolamide or dexamethasone, and conducted at least 3 weeks apart. Peripheral blood was collected under Denver normobaric conditions (PB= 625 mmHg) and

during 10h of HH (PB= 425 mmHg) to measure anti-inflammatory and/or anti-permeability (IL1ra, HSP70 and ADM), proinflammatory (IL6, IL8, IL2, IL1beta and substance P), and angiogenic or chemotactic factors (MIP1beta, VEGF, TNFalpha, MCP1 and MMP9). AMS status ["AMS-susceptible" (AMS·S) or "AMS-resistant" (NO·AMS)] was assessed using the Lake Louise Questionnaire (LLQ). Results: IL1ra, HSP70 and ADM were greater in NO·AMS than AMS·S on placebo, and increased with dexamethasone (ADM) or acetazolamide (IL1ra and HSP70) in AMS·S. MIP1beta was higher in AMS·S than NO·AMS subjects after 4h of HH; this difference was minimized with dexamethasone. Proinflammatory factors were unrelated to AMS. Conclusion: AMS susceptibility may be driven more by weak defensive, responses to HH than by an exaggerated inflammatory response. Acknowledgements: We would like to acknowledge the research subjects for their participation, and Alison Anderson, Jason Chapman, Maggie Crawford, Ruth Johnson, Barbara Lommen, Nick Robbins, and Janet Uhde for their technical and administrative expertise. Funding was provided by a National Heart, Lung and Blood Institute Grant (HL-070362), the Maren Foundation and the Altitude Research Center, University of Colorado Denver.

BLUNTED CHEMORECEPTOR AND CEREBROVASCULAR RESPONSIVENESS IN SHERPAS AT HIGH ALTITUDE. Jui-Lin (Mickey) J Fan, Kate N Thomas, Samuel J Lucas, Keith R Burgess, Karen C Peebles, Joseph Donnelly, Rebekah A Lucas, James D Cotter, Philip N Ainslie. University of Otago. *Email: mickeyfanny@hotmail.com.* Sherpas are known for their performance at extreme altitudes. However, it remains unclear whether they have blunted ventilatory responses to hypoxia and lowered cerebral blood flow. Such alterations would allow for greater ventilatory and cerebrovascular reserve and subsequent capacity to perform work at extreme altitude. Therefore, we reasoned that Sherpas would have blunted chemoreceptor and cerebrovascular responsiveness compared with newcomers to high altitude. We measured ventilation (VE) and middle cerebral artery blood flow velocity (MCAv) in conditions of eucapnia (baseline) and during both hyperoxic and hypoxic rebreathing to examine central and peripheral chemoreceptor responsiveness, respectively. Arterial blood samples were obtained during baseline to measure partial pressure of arterial CO_2 ($PaCO_2$) and O_2 (PaO_2). Twelve healthy lowland subjects were tested at sea-level (SL), and at high altitude (HA, 5050 m) along with eight Sherpas. During baseline for newcomers, VE was elevated, while PaO_2 and $PaCO_2$ were lowered, at HA compared with SL ($P<0.01$). In the Sherpas, MCAv was lower, whilst $PaCO_2$ were higher during baseline ($P<0.05$) compared with newcomers. In the newcomers, VE response during hyperoxic rebreathing tended to be higher at HA compared to SL ($P=0.59$), whilst both MCAv and VE response was elevated during hypoxic rebreathing ($P<0.01$). Sherpas had blunted MCAv and VE responses during both hyperoxic and hypoxic rebreathing compared to newcomers ($P<0.05$). These findings support the notion that Sherpas have blunted chemoreceptor and cerebrovascular responsiveness at high altitude. Such alterations may represent an efficient strategy to cope with altitude-induced hypoxia. This study was supported by the Otago Medical Research

Foundation, Peninsula Health Care p/l, Air Liquide p/l and the Italian National Research Council who kindly provided use of the EV-K2-CNR research laboratory. 2009.

BOLD MRI: A NON-INVASIVE METHOD TO EVALUATE RENAL HYPOXIA IN PATIENTS WITH CHRONIC KIDNEY DISEASE. Pottumarthi Prasad[1], Lu-Ping Li[1], Muhammad Haque[1], Stuart Sprague[1]. [1]NorthShore Univ HealthSystem, Evanston, IL, USA. *EMAIL: pprasad@northshore.org* INTRODUCTION: Hypoxia is well established to play a key role in the progression of chronic kidney disease and blood oxygenation level dependent (BOLD) MRI is currently the only demonstrated non-invasive method to monitor renal hypoxia in humans. METHODS: 47 subjects participated to-date: healthy control (n=10), anemic (n=7), CKD stage 2 (n=10), 3 (n=10), 4 (n=7), and 5 (n=3) by eGFR. BOLD MRI data was acquired using breath-hold multiple gradient echo sequence at baseline and after iv administration of 20 mg of furosemide to inhibit Na reabsorption along the medullary thick ascending limbs which accounts for about 65% of renalO_2 consumption. Rate constant R2* was used as the BOLD MRI parameter, which represents the level of hypoxia. RESULTS: Both renal medulla and cortex showed increased levels of hypoxia in subjects with CKD compared to controls (healthy + anemic): cortex R2* (25.5±5.5 s-1 vs. 21.4±3.6 s-1, p = 0.006), medulla R2* (45.7±9.7 s-1 vs. 40.2±7.0 s-1, p = 0.03). When separated in to different stages of CKD, stages 2 and 3 showed increased medullary hypoxia compared to healthy controls (R2* = 46.4±2.6 and 46.8±2.9 vs. 40.1±2.4 s-1, p<0.05) and showed reduced response to furosemide (DeltaR2* = 5.1±1.9 (p=0.07) and 3.2±1.0 (p=0.01) vs. 10.5±2.8 s-1). CONCLUSION: BOLD MRI results do support the feasibility of monitoring renal hypoxia in patients with CKD and future studies are necessary to demonstrate if these increased levels of hypoxia observed at early stages are predictive of progression. ACKNOWLEDGEMENTS: Work was supported in part by a grant from the National Institutes of Health, R21DK079080. 2015.

BRAIN TUMOR - NOCTURNAL HYPOXIA AND CHEYNE-STOKES VENTILATION. Michael Laub. Respiratory Centre East, Rigshospitalet, Copenhagen, Denmark. *Email: ml@laub.dk*. Introduction: Periodic breathing and Cheyne-Stokes ventilation (CSV) is characterized by crescending-descending oscillatory respiratory tidal volume during sleep. In periodic breathing central hypopneas are present while central apneas appear in Cheyne-Stokes ventilation. The phenomena was originally thought to arise from serious neurologic or cardiovascular disease and therefore to carry a poor outlook. It is now known to occur in more normal persons during sleep, typically in patients with congestive heart failure. Methods: In this case story we report a 54-year old male with symptoms as progressive headache and cognitive and behavioral impairment during six months. Results: CT-scan and MRI revealed a neoplasm 5 cm in diameter in the left temporal hemisphere. The patient's spouse had the last month observed nocturnal abnormal and irregular breathing. A sleep study (cardiorespiratory monitoring) showed an apnea-

hypopnea index (AHI) of 32 per hour and oxygen desaturations to 80%. More than 90 % of the apneas and hypopneas were seen during periodic breathing and CSV. The patient started treatment with nocturnal adaptive servoventilation (ASV) delivered by an AutoSet CS2 machine (ResMed) and full face mask. After two nights AHI was reduced to 4 and the spO_2 curve was almost normal. Five days later surgical resection of the tumor was carried out. Histological examination showed glioblastoma multiforme. Three weeks later radiotherapy was started followed by chemotherapy. Now five months after surgery the patient is still content with using nocturnal ASV. He once discontinued this treatment for four nights which resulted in daytime headache and increased tiredness. Conclusion: Cheyne-Stokes ventilation is known to occur primarily in congestive heart failure, but should be considered in other cases, especially in patients with neurological disorders. More evidence suggests ASV as the treatment of choice. 2011.

CALCULATED ALVEOLAR-ARTERIAL OXYGEN DIFFERENCE ON ASCENT TO EXTREME ALTITUDE. Daniel Martin, Denny Levett, Sundeep Dhillon, Chris Imray, Mike Grocott, for the Caudwell Xtreme Everest Investigator Group. University College London, Wrwick Medical School. *Email: dan.s.martin@gmail.com.* Objective: In order to gain further understanding of pulmonary gas exchange in climbers ascending Mt Everest we used arterial blood gas (ABG) data taken during studies at altitude(1) and calculated the alveolar-arterial oxygen difference ($AaDO_2$). Methods: Subjects were all volunteers climbing Mount Everest and partaking in studies requiring arterial blood gases to be taken at rest. Samples were obtained at 75m (n=13), 5300m (n=12), 6400m (n=10), 7100m (n=6) and 8400m (n=4). At 8400m, supplemental oxygen had been removed for 20 minutes prior to sampling. ABG's were measured using a specially validated blood gas analyser. Inspired partial pressure of oxygen (PIO_2) was calculated by subtracting the saturated vapour pressure of water from ambient barometric pressure. Alveolar partial pressure of oxygen (PAO_2) was calculated using the alveolar gas equation, with resting respiratory quotient measured using a validated respiratory gas analysis system. Results: Mean PIO_2, PAO_2 and PaO_2 fell with increasing altitude; values at 8400m have been previously published(1). Mean $AaDO_2$ showed a biphasic response to altitude; from a sea-level mean of 1.14 kPa it fell to 0.16 kPa at 5300m and 0.08 kPa at 6400m then rose to 0.25 kPa at 7100m and finally 0.72 kPa at 8400m. Conclusions: In this cohort of subjects, resting $AaDO_2$ fell whilst ascending to moderate altitude then rose again above a threshold of 7100m. Decreased PIO_2 leads to a reduction in $AaDO_2$ whereas exercise has the opposite effect. The increasing $AaDO_2$ observed above 7100m therefore represents a deviation from what one would normally predict. We speculate that this may be due to subclinical pulmonary oedema that develops secondary to severe physical exertion and fails to resolve at rest. Pulmonary end-capillary diffusion disequilibrium may also contribute. 1. MPW Grocott, DS Martin, DZH Levett, R McMorrow, J Windsor, H Montgomery. Arterial blood gases and oxygen content in climbers on Mount Everest. NEJM 2009;360;30-39. 2009.

CALCULATION OF PULMONARY ARTERY BLOOD FLOW DURING VENO-VENOUS BYPASS WITH CENTRAL VENOUS RETURN. Roland CE Francis[1], Steffen Weber-Carstens[1], Willehad Boemke[1], Philipp A Pickerodt[1]. [1]Charité - Universitätsmedizin Berlin, Dept Anesthesiology and Intensive Care Medicine. *EMAIL: roland.francis@charite.de* INTRODUCTION: Veno-venous extracorporeal membrane oxygenation (ECMO) can be used to treat refractory hypoxemia in patients with the acute respiratory distress syndrome. Typically, a fraction of the patient's cardiac output is drained from the inferior vena cava (IVC), bypassed through a heated membrane oxygenator, and then directly returned to the central vein compartment via the superior vena cava (SVC). Concomitant fluctuations in central venous blood temperature impair the validity of standard thermodilution techniques in assessing pulmonary artery blood flow (Q_L). We aim at deducing a simple calculation of Q_L in patients on veno-venous ECMO. METHODS: We algebraically modeled the mixing of recombinant streams of blood entering the pulmonary artery using the principle of conservation of mass. RESULTS: The mass flow of O_2 molecules entering the pulmonary artery (I_{PA}) is partitioned into the streams of blood originating from the central venous infusion cannula of the ECMO (I_E) and from the IVC and SVC combined (I_{CV}). $I_{PA}=I_E+I_{CV}$. I is the product of the oxygen content C and the blood flow Q of the respective partition, i.e. I=CxQ. We obtain : $C_{PA}xQ_L=C_ExQ_E+C_{CV}xQ_{CV}$, rearranging to: $Q_L=Q_Ex(C_E-C_{CV})/(C_{PA}-C_{CV})$. We suggest that C_{CV}, i.e. the oxygen content of central venous blood, approximates the afferent ECMO blood (C_A) which is drained from the central vein compartment via a cannula introduced through the IVC. This gives : $Q_L=Q_Ex(C_E-C_A)/(C_{PA}-C_A)$. C_E and C_A can be measured in blood efferent from and afferent to the membrane oxygenator, C_{PA} can be measured in a mixed-venous blood sample collected through a pulmonary artery catheter. Q_E is typically measured by an ultrasound flow probe included in the ECMO bypass. CONCLUSION: We have deduced a simple formula to calculate pulmonary artery blood flow during veno-venous ECMO. This formula needs to be validated against more invasive and cumbersome means of cardiac output assessment.

CAN COMBINED EFFECTS OF DECREASED NASAL POTENTIAL AND EXAGGERATED HYPOXIC PULMONARY VASOCONSTRICTION EXPLAIN HAPE-SUSCEPTIBILITY? Theresa Betz[1], Heimo Mairbäurl[1], Christoph Dehnert[1], Kai Schommer[1], Peter Bärtsch[1]. [1]Medical Clinic VII, Sports Medicine, Univ Heidelberg, Germany. *EMAIL: heimo.mairbaeurl@med.uni-heidelberg.de* INTRODUCTION: High altitude pulmonary edema (HAPE) appears to be caused by alveolar leakage due to exaggerated pulmonary arterial pressure (PAP) and/or high capillary blood flow due to inhomogeneous hypoxic pulmonary vasoconstriction. However, only a small proportion of altitude-naïve individuals with exaggerated hypoxic pulmonary vasoconstriction detected in normobaric hypoxia developed HAPE upon ascent to 4559m, which indicates that the elevated PAP alone is not sufficient to induce HAPE. Impaired Na-reabsorption has been found in individuals who developed HAPE at 4559m {Sartori et al., NEJM, 2002; Mairbäurl et al., AJRCCM, 2003}. It is thus possible that the combination of elevated PAP and altered ion transport might explain HAPE susceptibility. METHODS: To test this

hypothesis we measured nasal potential differences (NPD) in normoxia of individuals who had never developed HAPE (controls; n=14), individuals with well documented HAPE and high PAP at 4559 m (HP+HAPE; n=11), and altitude naïve subjects with high PAP in hypoxia but without HAPE at 4559 m (HP-no-HAPE; n=14). RESULTS: Our results confirm earlier findings of a decreased NPD in HP+HAPE. However, as reported earlier, there was only a minute difference in the amiloride-sensitive NPD between controls and HP+HAPE ($p<0.075$) indicating a difference in ion transport but likely not in Na-transport between these groups. Values for HP-no-HAPE were between controls and HP+HAPE but were statistically not different from either group. Interestingly, those individuals with high PAP in hypoxia tended to have an increased chloride-conductance ($p< 0.06$). CONCLUSION: Taken together our results, although obtained on relatively small groups only, indicate that nasal potentials did not differ significantly between individuals with high PAP in hypoxia but who didn't develop HAPE at 4559 m and those with HAPE, indicating that altered sodium transport does not identify HAPE susceptibility in individuals with exaggerated PAP in hypoxia. 2015.

CAN PEOPLE WITH RAYNAUD'S PHENOMENON TRAVEL TO HIGH ALTITUDE? Andrew M Luks, Dominique Jean, Colin K Grissom, Erik R Swenson. University of Washington, Centre Hospitalier Universitaire, Intermountain Medical Center. *Email: andrew_luks@yahoo.com.* Objective: Patients with Raynaud's phenomenon (RP) are sometimes counseled to avoid mountain travel but little data exist to support this advice. Our goal was to determine whether high altitude travel adversely affects mountain enthusiasts with RP Methods: Volunteers with RP were recruited to complete an on-line survey using announcements disseminated by organizations dedicated to climbing or wilderness travel and internet discussion boards with a focus on mountain activities. Survey questions addressed demographic variables, aspects of their RP and features of their mountain activities. Respondents compared experiences with RP between high (>2,440 m / 8,000 ft) and low elevations and rated agreement with statements concerning their disease and the effects of high altitude. Results: 142 people, 98% of whom had primary RP, completed the questionnaire. Respondents spent 5-7 days/month at elevations above 2,440 m and engaged in 5.4 ± 2.0 different activities. Eighty-nine percent of respondents engaged in winter sports and only 22% reported changing their activities due to RP. Respondents reported that the frequency and severity of attacks are greater during the winter months than during the summer months at high altitude. Respondents reported a variety of tactics to prevent and treat Raynaud's attacks but only 12% used prophylactic medications. Fifteen percent of respondents reported an episode of frostbite following a RP attack at high altitude. There was considerable heterogeneity in participants' perceptions of the frequency, duration and severity of attacks at high altitude compared to their home elevation. Conclusions: Motivated individuals with primary RP, employing various prevention and treatment strategies, can engage in different activities, including winter sports, at altitudes above 2,440 m. Frostbite may be common in this population at high altitude and care must be taken to prevent its occurrence. 2009.

CAPILLARY BLOOD VOLUME RESPONSE TO EXERCISE IN ENDURANCE-TRAINED ATHLETES VS. SEDENTARY NON-ATHLETE MALES. Vincent Tedjasaputra[1], Melissa Bouwsema[1], Michael Stickland[2]. [1]Faculty of Physical Education and Recreation, University of Alberta, Edmonton, AB Canada, [2]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB Canada. *Email: tedjasap@ualberta.ca.* Introduction: Endurance-trained athletes exhibit enhanced cardiovascular function compared to non-athletes, but it is generally accepted that exercise training does not affect the pulmonary system. Recent work has shown that increased resting pulmonary capillary blood volume (VC) is associated with a higher VO_2max, but there have been no studies to date examining how fitness affects the VC response to exercise. Based on previous work, we hypothesized that endurance-trained athletes will have greater VC compared to non-athletes during cycling exercise. Methods: Fifteen endurance-trained athletes (HI: VO_2max 64.6±6.9 mL/kg/min) and 14 controls (LO: VO_2max 45.6±4.1ml/kg/min) were matched for age and height. Hemoglobin-corrected diffusion capacity (DLCO), pulmonary capillary blood volume (Vc) and diffusion membrane capacity (Dm) were determined using the multiple FIO_2-DLCO method at rest and during incremental cycle exercise up to 90% of peak O_2 consumption. Results: At rest, athletes had 12% higher DLCO (HI: 37.6±4; LO: 34.1±7 mL/min/mmHg, p=0.047) and 19% higher VC (HI: 101±13; LO: 85±15 mL, p=0.005), but no difference in Dm. During exercise at 70% of VO_2max, there were no between-group differences in VO_2, DLCO, Vc, or Dm. At 90% of VO_2max, athletes had superior VO_2 (HI: 4.51± 0.71; LO: 3.54±0.59 L/min, p<0.01), 16% larger DLCO (HI: 58.8±10.1 mL/min/mmHg; LO: 50.9±8.2 mL/min/mmHg, p=0.026) and 34% greater Dm (HI: 136.7±40.5; LO: 99.1±24.1 mL/min/mmHg, p=0.006) compared to non-athletes; however, Vc was not different between groups (HI: 154±28; LO: 141±25 mL, p=0.54). Conclusion: Contrary to our hypothesis, exercise Vc was not increased in endurance-trained subjects compared to controls; rather, the increased DLCO in athletes at peak exercise was secondary to an enhanced Dm. These findings suggest that endurance-trained athletes appear to have differences within the pulmonary membrane that facilitate the increased O_2 delivery needed for high-level exercise. Acknowledgements: NSERC. 2015.

CARBONIC ANHYDRASE INHIBITION DOES NOT ENHANCE NITRITE TO NITRIC OXIDE REDUCTION DURING ALVEOLAR HYPOXIA IN PIGS. Philipp A. Pickerodt[1], Sebastian Kronfeldt[1], Katja Vorbrodt[1], Philipp Lother[1], Roland CE Francis[1], Thilo Busch[2], Willehad Boemke[1], Erik R Swenson[3]. [1]Department of Anesthesiology and Intensive Care Medicine Campus Charité Mitte and Campus Virchow-Klinikum, Charité – Universitätsmedizin Berlin, [2]Department of Anesthesiology and Intensive Care Medicine, University of Leipzig, Germany, [3]Department of Pulmonary and Critical Care Medicine, University of Washington, Seattle, Washington; and Veterans Affairs Puget Sound Health Care System, Seattle, Washington. *Email: philipp.pickerodt@charite.de.* Introduction: Inhibition of carbonic anhydrase (CA) and reduction of nitrite to nitric oxide (NO) both prevent hypoxic pulmonary vasoconstriction (HPV).[1,2] Aamand et al. showed a nitrite-reductase activity of CA in vitro, surprisingly augmented by inhibition of

CA.[3] Here, we tested the physiological relevance of augmented nitrite reduction with CA inhibition during alveolar hypoxia in vivo. Methods: Anesthetized pigs (24.2±2.1 kg; n=27) were ventilated and exhaled NO (NOex) was measured via ozone-based chemiluminiscense. Standard respiratory, hemodynamic and blood gas measurements were performed. After a one hour, inspired partial pressure of oxygen was reduced from 320 mmHg (baseline) to 92-110 mmHg for three hours (hypoxia I, II and III). Four groups were investigated: 1) Controls. 2) Inhaled sodium nitrite ($iNaNO_2$): 450 mg nebulized over 30 min .3) Acetazolamide intravenously (ACZ i.v.): 2mg/kg BW i.v. bolus followed by 2 mg kg^{-1} x h^{-1} i.v.). 4) $iNaNO_2$ + ACZ i.v. Results: During hypoxia, PaO_2 was comparable among all groups (range: 49-54 mmHg) In controls, mean pulmonary artery pressure (MPAP) increased from 14±2 to 28±4 mmHg and pulmonary vascular resistance (PVR) from 493±120 to 1045±314 dyn x sec^{-1} x cm^{-5} after hypoxia III. $iNANO_2$ prevented HPV during hypoxia I only and MPAP and PVR were comparable to controls after hypoxia III (29±3 mm Hg and 890±237 dyn x sec^{-1} cm^{-5}) despite sustained increases in NOex. ACZ i.v. significantly prevented HPV with no change in NOex. MPAP and PVR did not further increase during hypoxia I-III in $iNaNO_2$ + ACZ, while NOex was fully comparable to $iNaNO_2$ treated animals. Conclusion: Our results argue against a physiological relevant nitrite-reductase capacity of carbonic anhydrase in vivo in the lung. Acknowledgements: This study was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) to Drs. Pickerodt, Boemke and Swenson. 2015.

CARDIOVASCULAR AND CEREBROVASCULAR RESPONSES TO TRANSIENT TESTS OF THE PERIPHERAL CHEMOREFLEX IN HUMANS. Jamie Pfoh[1], Maria Abrosimova[1], Michael Tymko[2], Anthony Bain[2], Philip Ainslie[2], Liliana Cardona[1], Trevor Day[1]. [1]Mount Royal University, [2]University of British Columbia Okanagan. *Email: tday@mtroyal.ca.* Introduction: Carotid chemoreceptors detect arterial hypoxemia and hypercapnia, eliciting a fast peripheral chemoreflex (PCR). Steady-state manipulation of inspired gases are routinely utilized to isolate the PCR over 10-20 min of hypoxia, but the cardiovascular and cerebrovascular responses during these tests may contaminate the responses due to CO2 washout from central chemoreceptors. Transient tests of the PCR have been previously developed, but have not gained wide acceptance, nor have they been fully characterized. We aimed to characterize the cardiovascular and cerebrovascular responses of two separate transient PCR tests and hypothesized that the cardiovascular and cerebrovascular responses would be negligible. Methods: We tested 20 healthy adults (25yrs; BMI 24 kg/m2; 8 males) with two separate transient PCR tests: a three-breath 100% N2 test (TT-N2) and a single-breath 13% CO2 (in air) test (TT-CO2). Peak ventilatory responses were quantified from a mean of five trials for the TT-N2 test (1.04±0.2 L/min/-% SpO_2) and the TT-CO2 test (1.09±0.2 L/min/Torr CO2). Results: The heart rate (HR; ECG) and mean arterial pressure (MAP; finometry) responses to the TT-N2 were 8.9±1.2% bpm and 5.0±0.5% mmHg, respectively (both $P<0.001$). For the TT-CO2 tests, these changes were 3.5±0.8% bpm and 2.5±0.5% mmHg, respectively (both $P<0.001$). The middle (MCAv) and

posterior (PCAv) cerebral artery velocity responses (TCD) to the TT-N2 were 3.1±0.9% cm/s and 2.7±0.8% cm/s, respectively (both P=0.003). For the TT-CO2 tests these changes were 9.2±1.3% cm/s and 8.6±0.9% cm/s, respectively (both P<0.001). Conclusion: We suggest that transient tests of the PCR may have broader utility than previously accepted, as the cardiovascular and cerebrovascular responses are small and short in duration, reducing or eliminating the confounds of these responses when using steady-state tests. Acknowledgements: Alberta Innovates Health Solutions Summer Studentship, Faculty of Science and Technology Innovation Grant and an MRU Internal Research Grant. 2015.

CENTRAL ALTERATIONS DURING PROLONGED EXERCISE IN NORMOXIA AND HYPOXIA. Samuel Verges[1], Marc Jubeau[1], Thomas Rupp[1], Stephane Perrey[2], François Esteve[3], Bernard Wuyam[4], Patrick Levy[4], Guillaume Millet[5]. [1]HP2 laboratory, INSERM U1042, Joseph Fourier Univ, Grenoble, France, [2]Movement To Health (M2H), Montpellier-1 Univ, Euromov, Montpellier, France, [3]Grenoble Institute Neurosciences, INSERM U836, Grenoble, France, [4]HP2 laboratory, INSERM U1042, Joseph Fourier Univ, Grenoble, France; 2 Lyon Univ, Saint-Etienne, France, [5]Lyon Univ, Saint-Etienne, France; HP2 laboratory, INSERM U1042, Joseph Fourier Univ, Grenoble, France; 2 Lyon Univ, Saint-Etienne, France. *EMAIL: sverges@chu-grenoble.fr* INTRODUCTION: Prolonged cycling exercise in normoxia (N) induces fatigue due to both peripheral and central alterations. It has been reported that cerebral perturbations are greater during short-duration/intense exercise at the same power output in hypoxia (H) compared to N[1]. The purpose of this study was to test the hypothesis that central alterations are accentuated in H compared to N during prolonged whole-body exercise at the same relative intensity. METHODS: Ten subjects performed two sessions consisting of 3 x 80-min cycling bouts at 45% of their relative maximal aerobic power in N and H (FiO_2 = 12%). Before exercise and after each bout, transcranial magnetic stimulation was used to assess corticospinal excitability (motor evoked potential; MEP) and intracortical inhibition (cortical silent period; CSP) of knee extensors. Peripheral electrical stimulation of the femoral nerve was used to measure muscle characteristics. Voluntary activation was also assessed with both types of stimulation. RESULTS: A significant maximum voluntary torque reduction was observed at the end of the exercise, the reduction being similar in H and N. With the exception of CSP, a significant time effect was observed for all parameters. CSP was longer and the reduction of peripheral peak twitch torque was lower in H than in N. Maximum voluntary activation was reduced to a similar extent in N and H. All other parameters did not differ between sessions. CONCLUSION: Central fatigue was found to be similar between N and H when exercise is performed at the same relative intensity, i.e. at lower absolute power output in hypoxia. Even if the brain is importantly affected by hypoxia[2] as shown by the greater intracortical inhibition, this does not appear to further affect central motor drive. ACKNOWLEDGEMENTS: 1. Goodall et al. J Physiol 590: 2767–2782 (2012) 2. Verges et al. Am J Physiol Regul Integr Comp Physiol 302: R903-R916 (2012). Financial support was provided by the French National Research Agency (grant number NT09_653348). 2015.

CENTRAL-PERIPHERAL CHEMORECEPTOR INTERACTION: A NOVEL 3-D PERSPECTIVE ON CLASSICAL RESPIRATORY CHEMOREFLEXES. Trevor Day[1], Richard Wilson[2]. [1]Mount Royal University, Calgary, Alberta, Canada, [2]University of Calgary, Calgary, Alberta, Canada. *Email: tday@mtroyal.ca*. Introduction: We recently demonstrated the existence of a negative (i.e., hypoadditive) interaction between central (brainstem) and peripheral (carotid body) chemoreceptors. Specifically, when the central chemoreceptors are relatively hypocapnic, the peripheral chemoreflex magnitude is larger (Day and Wilson, 2007 and 2009). Based on these data, we hypothesize that the known multiplicative relationship between O_2 and CO_2 stimuli measured in the peripheral chemoreflex is modulated by the level of central chemoreceptor CO_2. Methods: We employed a previously developed and characterized artificially-perfused decerebrate, vagotomised rodent preparation where the central and peripheral chemoreceptors are independently perfused with defined media containing precisely controlled gas concentrations (Day and Wilson, 2005). The phrenic neurogram is recorded as a neural index of ventilation. Results: In random order, we stimulated the peripheral chemoreceptors simultaneously with oxygen (400, 100 or 40 Torr PO_2) and carbon dioxide (15 or 55 Torr PCO_2) mixtures at various levels of central chemoreceptor activation (25, 35 or 50 Torr PCO_2). We observed that the multiplicative interaction between peripheral O_2 and CO_2 was modulated by the level of brainstem CO_2 in a dose-dependent manner. The more hypocapnic the brainstem, the larger the magnitude of the peripheral chemoreflex multiplicative O_2-CO_2 interaction. When the brainstem was relatively hypercapnic, the peripheral chemoreflex O_2-CO_2 interaction was eliminated. Conclusion: These data demonstrate multiple chemoreceptor interactions within the respiratory control system. The known multiplicative O_2-CO_2 stimulus interaction measured in the peripheral chemoreflex is modulated by the level of central chemoreceptor CO_2. Our study adds a novel three-dimensional perspective to classical models of respiratory chemoreflexes. Acknowledgements: This project was funded by a CIHR operating grant. Salary support was provided by AIHS (Alberta Innovates Health Solutions; RJAW), a CIHR Doctoral award and the Mount Royal University Internal Research Grants Fund (TAD). 2011.

CEREBRAL AND BRACHIAL BLOOD FLOW RESPONSES TO 60 MIN OF ISOCAPNIC HYPEROXIA IN HEALTHY HUMANS. J.S. Vantanajal, J.C. Ashmead, T.J. Anderson, R.T. Hepple and M.J. Poulin. University of Calgary. *Email: poulin@ucalgary.ca.* We previously demonstrated a greater sensitivity of cerebral blood flow (CBF) than brachial blood flow (BBF) to hypoxia. However, little is known about the vasoactive responses of cerebral and brachial circulations to hyperoxia, or about potential differences in the sensitivities of CBF and BBF to hyperoxia. We hypothesized that the sensitivity of CBF to isocapnic hyperoxia (IH) would be greater than that of BBF. Nine males (30.1±5.2 years, mean±SD) underwent two IH exposures separated by 60 min. A dynamic end-tidal forcing system was used to hold end-tidal PCO_2 ($PETCO_2$) constant at eucapnia (1.5 Torr above normal value) whilst controlling the end-tidal PO_2 ($PETO_2$) at the desired level. The protocol started with 10 min eucapnic euoxia ($PETO_2$= 88 Torr). Then, $PETO_2$ was increased rapidly to ~460 Torr

(75% O_2) and held constant for 60 min followed by a returned to euoxia for 10 min. Transcranial Doppler ultrasound was used to measure beat-by-beat peak blood flow velocity (VP) in the right middle cerebral artery. BBF (right arm) was measured using echo Doppler ultrasound. The VP responses to hyperoxia were fitted to a simple mathematical model (gain term (i.e., sensitivity), two time constants, baseline, time delay). The sensitivity of BBF to hyperoxia was determined by the slope of the relationship between BBF and arterial O_2 saturation (calculated from $PETO_2$). IH elicited decreases of 11.8±5.1 and 12.9±5.9 % in VP and BBF, respectively. VP and BBF remained lower during IH with no evidence of adaptation. The sensitivities of VP and BBF to IH were similar (3.75±1.11 vs. 3.30±1.60 %·% saturation^{-1}; P>0.05, ANOVA). The mechanisms by which isocapnic hyperoxia elicits similar decreases in CBF and BBF remain to be elucidated. Supported by AHFMR and HSFA. 2009.

CEREBRAL AND CORONARY BLOOD FLOW SENSITIVITY TO HYPERCAPNIA AND HYPOXIA IN HUMANS. Andrew Beaudin, Julien V Brugniaux, Matthias Vohringer, Jacqueline Flewitt, Jordin D Green, Matthias D Friedrich, Marc J Poulin. University of Calgary, Foothills Medical Centre, Siemens Healthcare. *Email: abeaudin@ucalgary.ca.* Cerebral vascular responses to CO_2 and O_2 are well documented, but little is known about the myocardial vasculature responses. Few studies have assessed differences between these two vasculatures. We compared cerebral (middle cerebral artery blood velocity; VP) and coronary sinus blood flow (CSBF) sensitivities to euoxic hypercapnia (EH) and isocapnic hypoxia (IH) in healthy volunteers. Participants (n=15) were tested at rest on two occasions. On visit one, $\dot{V}P$ (transcranial Doppler ultrasound) and arterial oxyhemoglobin saturation (SaO_2; pulse oximetry) were measured during EH and IH. On visit two, cardiac magnetic resonance imaging (1.5 Tesla system; 6 element coils) was used to measure CSBF responses to EH and IH. CSBF was normalized to heart workload by using the rate pressure product (nCSBF). Both testing protocols began with 5-min euoxic isocapnia (end-tidal PO_2 ($PETO_2$) = 88 Torr; end-tidal PCO_2 ($PETCO_2$) = +1 Torr above rest). EH involved two 5-min increments in $PETCO_2$ (40 and 45 Torr), while $PETO_2$ remained euoxic. IH involved three 5-min decrements in $PETO_2$ from 88 to 60, 52, and 45 Torr, while $PETCO_2$ was held isocapnic. VP, CSBF, and nCSBF were normalized to isocapnic euoxic conditions and indexed against $PETCO_2$ and % desaturation. Blood flow sensitivity to EH (%Δ·Torr-1) was not different between VP and CSBF (p=0.179), but nCSBF sensitivity was significantly lower than VP and CSBF ($p \leq 0.019$). Conversely, CSBF sensitivity to IH (%Δ·% desaturation-1) was significantly greater than $\dot{V}P$ (p=0.008) and nCSBF (p=0.012), but VP was not different from nCSBF (p=0.216). These findings demonstrate cerebral and coronary vascular sensitivities to hypercapnia are similar, but myocardium blood flow has a greater sensitivity to hypoxia. When heart workload is considered, compared to cerebral circulation, the coronary circulation is less sensitive to hypercapnia, but similarly sensitive to hypoxia. Acknowledgements: Supported by the Heart and Stroke Association of Alberta, Alberta Heritage Foundation for Medical Research and the Husky Energy Program for the Early Detection of Cardiovascular Disease. 2009.

CEREBRAL BLOOD FLOW AND METABOLISM DURING EXERCISE AT HIGH ALTITUDE. Smith KJ[1], MacLeod D[2], Willie CK[1], Lewis NCS[1], Murphy K[1], Hoiland R[1], Ray[1] L, Smirl J [1], Ikeda K[2], Tymko M[3], Donelly J[4], Day TA[3], Dumanoir G[5], Foster GE[6], MacLeod N[7], Lucas SEJ[4], Ainslie PN[1]. [1] Centre for Heart Lung and Vascular Health, School of Health and Exercise Science, Univ. of British Columbia, Kelowna, BC, Can, [2]Dept. of Anesthesiology, Duke Univ. Medical Center, Durham, NC, USA, [3]Dept Biology, Mount Royal Univ., Calgary, AB, Can, [4]Univ Otago, Dunedin, NZ, [5]Dept. Human Kinetics Diploma Program, Okanagan College, Penticton, B.C, Can, [6]School of Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada, [7]Carolina Friends School, Durham, NC, USA. INTRODUCTION: We tested the hypothesis that at rest and during exercise: 1) increases in cerebral metabolic rate for oxygen ($CMRO_2$) at high altitude (HA; 5050 m) would be reflected in elevations in cerebral blood flow (CBF); and 2) trans-cerebral exchange of substrate availability of oxygen, glucose and lactate would also be altered. METHODS: At SL and HA, arterial (a) – jugular venous (v) differences for PO_2, PCO_2, O_2 content (CaO_2; CvO_2), glucose, and lactate were sampled at rest and supine exercise at 20, 40, 60, 80 and 100% of the maximum workload (%Wmax). Blood flow in the internal carotid and vertebral arteries, and velocities in the middle [MCAv] and posterior [PCAv] cerebral arteries were measured using Doppler ultrasound. $CMRO_2$ was calculated as: CBF*(CaO_2-CvO_2). RESULTS: Rest: Compared to sea-level (SL), HA reduced PaO_2 (42±3 vs. 92 ±7 mm Hg), $PaCO_2$ (24±4 vs. 38±3 mm Hg) and CaO_2 (18±2 vs. 21±2 $ml.dl^{-1}$) and increased global CBF (748±140 vs. 641±110 $ml.min^{-1}$), and $CMRO_2$ (+26%) (all: $P<0.05$). Glucose and lactate a-v differences were unchanged at HA. Exercise: At HA, %Wmax was reduced by 50%. Both MCAv (range: 11-14%) and PCAv (7-11%) increased up to 40%Wmax at SL and HA. At SL, MCAv, PCAv and global CBF decreasing towards baseline >60 %Wmax. Conversely, at HA, MCAv (+23±10%), PCAv (+19±6%) and global CBF increased after 40%Wmax. The $CMRO_2$ was elevated by ~50-60% throughout exercise at HA (vs. SL). Arterial lactate concentration was 50% lower at 100% Wmax at HA, and cerebral lactate extraction was also reduced (1±0.7 [HA] vs 4±1 $\mu mol.L^{-1}$ [SL]) ($P<0.05$). Cerebral glucose extraction was comparable during exercise at SL and HA. CONCLUSION: Reductions in exercise capacity at HA attenuate arterial lactate concentrations and trans-cerebral lactate extraction. Nevertheless, the apparent elevations in $CMRO_2$ and CBF may reflect a compensatory and advantageous response to maintain substrate availability. ACKNOWLEDGEMENTS: This study was carried out within the framework of the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology as foreseen by the Memorandum of Understanding between Nepal and Italy, and thanks to contributions from the Italian National Research Council. Supported by NSERC and CRC. 2015.

CEREBRAL BLOOD FLOW VELOCITY IS NOT ASSOCIATED WITH ACUTE MOUNTAIN SICKNESS DURING EXPOSURE TO NORMOBARIC HYPOXIA. Eric A Carter[1], Martin J MacInnis[1], Pei Wang[1], Danielle Robson[1], Brendan J Boere[1], James L Rupert[1], Michael S Koehle[1]. [1]University of British Columbia. *Email: ecarter1@interchange.ubc.ca*. Introduction: The role of cerebral

blood flow velocity in acute mountain sickness (AMS) is unclear, with some reports suggesting it is elevated in subjects with AMS and others showing no such relationship. The purpose of this study was to assess the contribution of cerebral blood flow velocity to the development of AMS following rapid ascent to a simulated altitude of 4500 metres. Methods: Seventeen healthy male climbers and skiers were exposed to an FiO_2 of 12.5% while resting quietly in a normobaric hypoxic chamber for six hours. Lake Louise Score (LLS), Visual Analogue Score (VAS) for high-altitude headache, oxygen saturation (SPO_2), and heart rate (HR) were measured before entry and every hour upon entering the chamber. Blood flow velocity in the middle cerebral artery (Vmca) was estimated using trans-cranial Doppler ultrasound immediately prior to, and every two hours during, the exposure. Diffusion capacity of carbon monoxide (DLco) was measured pre- and post- exposure. Results: Nine subjects (52.9%) were diagnosed with AMS at one or more time points during their exposure. Average VAS was significantly correlated to average LLS (r2= 0.80) at each time point. No significant differences were noted in SPO_2 or Vmca between subjects with and without AMS; however, HR was found to be significantly higher at times when subjects had AMS (p= 0.001). A significant reduction in DLco (M= 31.7 and 30.4, respectively, p= 0.002) occurred after the hypoxic exposure. Conclusion: Changes in cerebral blood flow were not predictive of AMS, but the change in diffusion capacity associated with hypoxia exposure is worthy of additional investigations into the underlying pathology of AMS. 2011.

CEREBRAL EDEMA IN CHRONIC MOUNTAIN SICKNESS: A NEW FINDING. Haihua Bao[1], Xipeng Zhao Zhao[1], Peter Hackett[2], Ri-Li Ge[3]. [1]Dept Medical Imaging, Affiliated Hospital of Qinghai Univ, Xining, Qinghai China, [2]Institute for Altitude Medicine, Telluride, CO, USA, [3]Research Center for High Altitude Medicine, Qinghai Univ in Xining, Qinghai, China. *EMAIL: geriligao@hotmail.com* INTRODUCTION: With acute ascent to high altitude, some people develop high-altitude cerebral edema. With prolonged altitude exposure, some residents develop chronic mountain sickness (CMS), which may present with predominantly neurological symptoms. To determine if cerebral edema is present in such patients, and to assess cerebral hemodynamics, we performed CT and MRI in a sample of CMS patients. METHODS: We studied a convenience sample of 17 patients with CMS and 15 control subjects; all resided permanently at 3000- 4000m in China. Measurements at 2260m within 48 hours of descent included symptom score, MSCT, perfusion CT imaging, pulse oximetry (SaO_2%), hemoglobin concentration (Hb), hematocrit (Hct), and pulmonary function. RESULTS: 9 of 17 CMS subjects showed diffuse cerebral edema, 8 by CT and one by MRI; no controls did. CSF pressure was elevated in those with edema (20.1±1.4 mmHg). Blood density (HU) of bilateral middle cerebral artery and superior sagittal sinus was higher in entire CMS group compared to controls, and highly correlated with Hb. Mean cerebral blood flow (CBF) in CMS (31.4±4.9 ml/100g) was significantly lower than in control group (40.5±3.9 ml/100g, $p<0.001$), and was inversely correlated with Hb ($r= -0.653$, $p<0.01$) in grey matter. Mean transit time in both grey and white matter was prolonged in CMS group ($p<0.01$). Subjects with cerebral edema had higher

Hb and CMS scores, and lower SaO_2 and CBF compared to CMS subjects without edema. CONCLUSION: Cerebral edema, which is a previously unrecognized complication of CMS, may be common in CMS patients with prominent neurological symptoms and signs, and is associated with exaggerated polycythemia and hypoxemia, and with lower and sluggish CBF. ACKNOWLEDGEMENTS: This work supported by Supported by 973(No.2012CB518200), Program of IS&T (No.0S2012GR0195), NNSFC (No.30393133). 2015.

CEREBRAL HEMODYNAMICS AND SLEEP MONITORING DURING ASCENT OF MT. KILIMANJARO. Mike Patz[1], Erika Williams[2], Ally Eran[2], Pamella Basto[2], Defang Zhang[2], Gary Strangman[3], Stephen Muza[4], Stuart Harris[3]. [1]Harvard Medical School, [2]Harvard-MIT Health Science and Technology (HST), [3]Massachusetts General Hospital, Harvard Medical School, [4]U.S. Army Research Institute for Environmental Medicine(USARIEM). *Email: mpatzd@gmail.com.* Introduction: The pathogenesis of Acute Mountain Sickness (AMS) is poorly understood. One hypothesis suggests that hypoxia and hyperventilation-associated hypocapnia results in cerebral hemodynamic dysregulation and possibly vasogenic cerebral edema. Desaturation events and pathologic Cheynes-Stokes respirations during sleep may contribute, but thespecific hemodynamic response to these events have not been characterized. Methods: To investigate relevant physiological changes associated with ascent and descent, several parameters were monitored on seven climbers during a 6-day ascent of Mt Kilimanjaro (19,341ft), 1-day descent, and several days of recovery. Cerebral hemodynamic responses to a serial-sevens cognitive task, breath hold, re-breathing, Valsalva maneuver, and Mueller maneuver were monitored daily using a portable near-infrared neuro-imaging device. Sleep was monitored in four subjects using portable polysomnagraphy, and sleep was monitored with actigraphy, and peripheral oxygen saturation (SaO2) in all seven subjects. One subject who had polysomnaghraphic monitoring each night also underwent non-invasive cerebral hemodynamic monitoring during sleep. AMS Lake-Louise scores were assessed twice daily, actigraphy was collected continuously, and other physiologic parameters were recorded once daily to assess the possible roles of cerebral hemodynamic changes, and periodic breathing during sleep in the pathogenesis of AMS. Results: Most subjects did not develop AMS, but one subject who desaturated to 57% during sleep at the highest camp became acutely ill with an AMS c-score of 2.63 on summit day. Periodic breathing during sleep was mild but increased with ascent. Several respiratory events were observed in subjects while undergoing cerebral hemodynamic monitoring and polysomnography at the two highest camps. Conclusion: Preliminary data from cerebral hemodynamic monitoring during sleep and wake will be discussed. 2011.

CEREBRAL HEMODYNAMICS AT ALTITUDE. Matthew Sanborn[1], Meeri Kim[2], Heng Yow[3], Daniel Martin[3], Paula Meale[3], Mark Edsell[4]. [1]Hospital of the University of Pennsylvania, Philadelphia, PA, USA, [2]University of Pennsylvania, Philadelphia, PA, [3]Centre for Altitude, Space and Extreme Environment Medicine, University College London, London, England, [4]St. George's Hospital, London,

England. *Email: Matthew.Sanborn@uphs.upenn.edu*. Introduction: Alterations in cerebral blood flow have been implicated in a host of altitude-associated pathologies. We used transcranial Doppler (TCD) and a novel technology called diffuse-correlation spectroscopy (DCS) to assess the cerebrovascular response to hyperventilation during acclimatization. Methods: 11 subjects were studied at 75m above sea level and then 2 and 7 days after ascent to 4,559m. Transcranial Doppler was used to measure vessel diameter and peak systolic velocity (PSV) in the middle cerebral artery (MCA). Diffuse correlation spectroscopy, a novel technology that provides an index of cerebral blood flow and has been previously validated against Xenon CT, was used to measure a flow index in a discreet area of the left frontal lobe. After obtaining baseline measurements subjects were asked to hyperventilate to reduce their baseline end-tidal carbon dioxide ($PETCO_2$) by 50%. After 3 minutes of maintaining this $PETCO_2$ a further set of measurements were obtained. Results: At sea level PSV was reduced from 82.16+/-16 to 54.74+/-10.84 cm/s following hyperventilation. At day 2 of altitude, baseline PSV had increased to 100.9+/-34.13 cm/s before decreasing to 63.86+/-17.38 cm/s following hyperventilation. After 7 days at 4,559m PSV decreased from 86.73+/-26.99 to 57.17+/-20.93 cm/s following hyperventilation. Diffuse correlation spectroscopy revealed a decrease in the cerebral blood flow index of 20.8% following hyperventilation at sea level, 30% following hyperventilation after 2 days at altitude and 36.4% following 7 days at altitude. Conclusion: Consistent with previous studies, baseline PSV initially increases at altitude and subsequently decreases towards baseline with acclimatization. Diffuse correlation spectroscopy demonstrated a consistent reduction in cerebral blood flow index following hyperventilation at all time points. There was a non-significant trend towards a more robust decrease in CBF with hyperventilation after increasing time at altitude. Acknowledgements: Xtreme Alps Investigators, American Alpine Club Research Grant. 2011.

CEREBRAL HEMODYNAMICS DURING SLEEP AT HIGH ALTITUDE. Samuel Lucas[1], Philip Ainslie[2], Kate Thomas[1], Kelly Shepherd[3], Andrew Dawson[3], Marianne Swart[3], Rebekah Lucas[1], Karen Peebles[1], Jui-Lin Fan[1], James Cotter[1], Rishi Basnyat[4], Keith Burgess[5]. [1]University of Otago, Dunedin, New Zealand, [2]University of Otago, Dunedin, New Zealand; University of British Columbia Okanagan, Kelowna, Canada, [3]Peninsula Sleep Laboratory, NSW, Australia, [4]Nepal International Clinic, Kathmandu, Nepal, [5]Peninsula Sleep Laboratory, NSW, Australia; University of Sydney, Sydney, NSW, Australia. *Email: sam.lucas@otago.ac.nz*. Introduction: The purpose of this study was: 1) To examine the pattern of cerebral blood flow (CBF) during sleep at high altitude; 2) to explore if the changes in CBF from wakefulness to NREM sleep would be related to the occurrence of central sleep apnea (CSA), and 3) whether such patterns or responses would be altered following partial acclimatization. Methods: We studied 12 volunteers at sea level, upon arrival to high altitude (5,050 m; days 2-4) and following partial acclimatization (at 12-15 days). Measurements included overnight polysomnography (n=12) with transcranial Doppler measurement (n=9) of middle cerebral artery blood flow velocity (MCAv) (during sleep [>2 hrs] and preceding waking

period). Upon arrival to high altitude, MCAv during wakefulness and stage 2 sleep was elevated (P<0.01) compared to sea level (+25±28% and +25±30%, respectively); these changes returned to sea-level values by 12-15 days. The decrease in MCAv from wakefulness to NREM sleep was similar (P>0.05) upon arrival and following partial acclimatization to high altitude compared to sea level (-10 and -9 cm/s vs. -11 cm/s). During partial acclimatization, CSA index increased from 76.9 ± 48.9 to 115.9 ± 20.2 events/hr over the two-week period (P=0.01). Development of CSA was mild-to-moderately related to the drop in MCAv (R2=0.14 and 0.26, at days 2-4 and 12-15 respectively). Sleeping at high altitude did not reduce total sleep time (370, 390 and 402 min; sea level, days 2-4 and days 12-15, respectively). However, the proportion total sleep time of stage 2 sleep was greater at high altitude compared to sea-level (due to less stage 3 and 4 sleep). REM sleep remained constant across each time point (15-17% of total sleep time). During REM sleep at days 2-4 (n=3), MCAv increased compared to the preceding sleep stage (+34%) and wakefulness (+13%); while after 12-15 days (n=3), REM sleep MCAv was higher only relative to preceding sleep stage (+15%). The CBF oscillations (MCAvmax – MCAvmin) measured during stage 2 sleep at high altitude were of a greater magnitude at days 2-4 (33 ± 9 vs. 23 ± 7 cm/s at days 12-15) and peaked 35% higher (96 ± 21 vs. 71 ± 23 cm/s), whereas time between peaks was ~1 s shorter at days 12-15 compared to early arrival at high altitude. Conclusion: In summary, the pattern of CBF is altered during sleep at high altitude compared to sea level and with partial acclimatization. Reductions in MCAv from wakefulness to NREM sleep - via either direct or indirect mechanisms – may play a contributory, albeit modest, role in the pathophysiology of CSA. Acknowledgements: This study was carried out within the framework of the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology as foreseen by the Memorandum of Understanding between Nepal and Italy, and thanks to contributions from the Italian National Research Council and the Italian Ministry of Foreign Affairs. This study was supported by the Otago Medical Research Foundation, SPARC New Zealand, The Peninsula Health Care p/l and Air Liquide p/l. 2011.

CEREBRAL HYPOXIA IS THE CONCLUSION OF SYNCOPE IN HARNESS (SUSPENSION TRAUMA)? Francesca Lanfranconi[1], Luca Pollastri[1], Manuela Bartesaghi[1], Valentina Scotti[1], Massimiliano Novarina[1], Cinzia Molteni[1], Hailey Vergani[1], Giuseppe Miserocchi[1]. [1]Dept Health Sciences, Laboratory of Clinical Physiology and Sport Medicine, Univ Milano-Bicocca, via Cadore 48, Monza, Italy. *EMAIL: francesca.lanfranconi@unimib.it* INTRODUCTION: Following a fall, the subject hanging on the harness and waiting for rescue may incur into the risk of a lethal multivisceral hypoxia distress due to orthostatic stasis named “suspension trauma”. To this condition are exposed all the workers involved in work at height. Purpose: evaluate by non-invasive methods the individual risk of developing suspension trauma. METHODS: Forty adults (mean±st.dev: age 39,1±8,2; body mass index 24,2±3,03; 85% males, 15% females) were enrolled: 16 were regular users of harness, 14 regularly involved in physical training but were not users of harness and 10 controls. Each subjects underwent: a) an incremental

exercise test on treadmill until exhaustion; b) a motionless suspension test until medical signs of orthostatic intolerance appeared or extreme subject discomfort was reported (suspension time recorded). Oxygen uptake, heart rate and blood pressure were measured, as well as brain oxygenation status by near-infrared spectroscopy. RESULTS: No difference in the mean suspension time was found in the 3 groups (average of all participants 28.7±11.4 min (range 8-56), coefficient of variation 40%). No correlation was found between the level of aerobic fitness and suspension time. Four persons revealed a risk of syncope during suspension in harness: a decrease in brain oxygenation was evident before the development of signs of pre-syncope although a not oscillatory pattern of cardiovascular response was noticed. CONCLUSION: The experience in use of harness and the aerobic fitness do not correlate with the suspension time and thus the onset of developing suspension trauma. The cerebral hypoxia is a co-factor and not the main cause of syncope. 2015.

CEREBRAL HYPOXIA ISCHEMIA IN NEONATES AND DETECTION OF SUBSEQUENT DESCENDING CORTICOSPINAL TRACT DEGENERATION BY MAGNETIC RESONANCE IMAGING. Sanju Lama, Min Qiao, Amy Ng, Delphi Barua, David R Kirk, Tadeusz Foniok, Ursula I Tuor. University of Calgary, National Research Council . *Email: slama@ucalgary.ca.* Introduction: The diagnosis of descending corticospinal tract (DCST) degeneration also termed Wallerian degeneration is potentially possible by magnetic resonance imaging (MRI). Following cerebral hypoxic-ischemic injury, the DCST which includes the motor fibers of the posterior limb of the internal capsule, cerebral peduncle, basis pontis and medullary pyramid have shown MRI signal intensity changes in recent pediatric studies. Whether or not this reflects associated axonal degeneration changes needs histopathological confirmation. Materials and Methods: Seven day old Wistar rat pups underwent unilateral common carotid occlusion followed by hypoxia (8% oxygen) to produce an ipsilateral middle cerebral artery territory infarct. T2 and Diffusion Weighted Images (DWI) were acquired at 24h and/or 48h post injury and the animals euthanized subsequently. Tissue changes were investigated by staining with Hematoxylin and Eosin (H/E for cellular injury) and SMI 31 (for axonal neurofilaments). Results: Image analysis demonstrated significant left to right differences in T2 and DWI intensities in the DCST regions examined at both the given time points. The changes were most significant at the level of cerebral peduncle and internal capsule ($p< 0.001$). H/E staining of the sections showed corresponding cell injury and death. SMI 31 staining of neurofilaments within axons showed loss of staining ipsilaterally. Conclusion: Central nervous system Wallerian degeneration occurs in response to substantial hypoxic-ischemic cortical injury and can be detected by MRI. Evidence for degeneration occurred along the DCST distal to the primary site of injury. This provides a model for detailed future investigation of MR imaging as a diagnostic tool for detecting Wallerian degeneration and the underlying tissue correlates. Acknowledgement: Heart and Stroke Foundation, Alberta and AHFMR/HBI Traineeship 2008. 2009.

CEREBRAL OUTPUT OF FREE RADICALS IN AMS; IMPLICATIONS FOR BARRIER FUNCTION AND OXIDATIVE METABOLISM. Damian M Bailey, Sarah Taudorf, Ronan M Berg, Carsten Lundby, Kevin A Evans, Philip E James, David A Hullin, Joe M McCord, Bente K Pedersen, Kirsten Moller. University of Glamorgan, University of Copenhagen, Cardiff University, Royal Glamorgan Hospital, University of Colorado Denver. *Email: dbailey1@glam.ac.uk.* Objective: The present study examined if AMS is associated with impaired cerebral oxidative metabolism subsequent to blood-brain barrier (BBB) disruption caused by a free radical-mediated reduction in trans-cerebral nitric oxide (NO) bioavailability. Methods: Ten males were examined in normoxia (N) and following 9h passive exposure to 12.9%O_2 (H). Global cerebral blood flow (CBF) was measured by the Kety-Schmidt technique with samples obtained from the radial artery and jugular venous bulb. Serum alkoxyl radicals (LO•) were detected via an electron paramagnetic resonance spin-trapping technique. Plasma nitrite (NO_2•) was assessed by ozone chemiluminescence and ELISA employed for plasma 3-nitrotyrosine (3-NT) and serum S100, detection. The metabolic rate of oxygen ($CMRO_2$) was calculated by the Fick principle and AMS scores assessed via questionnaires (LL, ESQ-C). Data were analyzed using paired samples t-tests and Pearson Product Moment Correlations. Results: Hypoxia increased the arterial inflow and net cerebral output of LOï [N: -73 (mean) ± 192 (SD) vs. H: -360 ± 253 arbitrary units/g/min, $P < 0.05$] and 3-NT (-3.1 ± 9.0 vs. -10.7 ± 18.2 nmol/g/min, $P < 0.05$). Hypoxia decreased the arterial inflow and blunted NO_2• uptake (126 ± 94 vs. 16 ± 47 nmol/g/min, $P < 0.05$) whereas S100, inflow increased without altering cerebral exchange (-16.9 ± 6.5 vs. -14.4 ± 8.9 ng/g/min, $P < 0.05$). $CMRO_2$ remained preserved (2.43 ± 0.54 vs.2.49 ± 0.34 µmol/g/min, $P > 0.05$) due to an increase in CBF and O_2 extraction. Relationships were confined exclusively to the increases in LO• output, NO_2• uptake, 3-NT inflow and AMS scores ($r = -0.50$ to 0.80, $P < 0.05$). Conclusions: These findings identify cerebral oxidative-nitrative stress as a novel risk factor for AMS though the underlying mechanism cannot be exclusively attributed to barrier disruption and thus by consequence, vasogenic edema or impaired oxidative metabolism. 2009.

CEREBRAL OXYGENATION IN KYRGYZ HIGHLANDERS WITH HIGH ALTITUDE PULMONARY HYPERTENSION. Michael Furian[1,2], Tsogyal D. Latshang[2], Sayaka Aeschbacher[2], Silvia Ulrich[2], Aizat K. Myrzaakmatova[3], Talant Sooronbaev[3], Almaz Aldashev[4], Konrad E. Bloch[2]. [1]Institute Human Movement Sciences and Sport, Federal Institute Technology, Zurich, Switzerland, [2]Pulmonary Division and Sleep Disorders Center, Univ Hospital of Zurich, Switzerland, [3]National Center for Cardiology and Internal Medicine, Bishkek, Kyrgyzstan, [4]Research Institute for Molecular Biology and Medicine, Bishkek, Kyrgyzstan. *EMAIL: konrad.bloch@usz.ch* INTRODUCTION: High altitude pulmonary hypertension (HAPH) is associated with sleep apnea and hypoxemia (Latshang 2012). In lowlanders with sleep apnea cerebral tissue oxygenation (CTO) and cerebrovascular autoregulation are impaired (Pizza et al 2010). We evaluated the hypothesis that highlanders with HAPH have a reduced CTO and cerebrovascu-

lar response to hyperoxia. METHODS: 17 highlanders with HAPH (mean±SD pulmonary artery pressure (mPAP) 35±3 mmHg) and 17 age-matched Controls (mPAP 24±4 mmHg, P<0.05 vs. HAPH) were studied in Aksay, Kyrgyzstan, at 3250 m. Cerebral near-infrared spectroscopy, finger pulse oximetry (SpO_2), end-tidal PCO_2, blood pressure and ECG were recorded while subjects rested quietly breathing room air (FiO_2 0.21) and oxygen (FiO_2 1.0) for 20 min each, in random order. RESULTS: In highlanders with HAPH on FiO_2 0.21, SpO_2 was 89±2%, CTO 68±3%. In Controls SpO_2 was 91±3% (P=0.011 vs. HAPH), CTO 67±4% (P=0.339). In highlanders with HAPH, breathing FiO_2 1.0 (compared to FiO_2 0.21) increased SpO_2 (+9±3%, P<0.001) and CTO (+5±2%, P<0.001) and reduced cerebral hemoglobin concentration (CHb), a surrogate of changes in cerebral blood volume reflecting cerebrovascular response (-1.4±1.6 relative units, P=0.005). In Controls, breathing FiO_2 1.0 increased SpO_2 (+7±2 %, P<0.001 vs. FiO_2 0.21, P=0.013 vs. HAPH) and CTO (+3±1%, P<0.001 vs. FiO_2 0.21, P=0.106 vs. HAPH), and decreased CHb (-1.4±2.1 ru, P=0.028, P=0.734 vs. HAPH). In regression analysis, younger age and higher CTO (FiO_2 0.21) were significant predictors of CHb changes induced by hyperoxia while blood pressure and end-tidal PCO_2 were not. CONCLUSION: In highlanders with HAPH arterial oxygen saturation is reduced. However, their cerebral oxygenation and the cerebrovascular response to alterations in FiO_2 are not impaired compared to highlanders without HAPH suggesting presence of compensatory mechanisms preventing cerebral hypoxia. Grant support: OPO Foundation and Zurich Lung League. 2015.

CEREBROVASCULAR AND VENTILATORY RESPONSES TO ACUTE ISOCAPNIC-HYPOXIA IN HEALTHY AGING AND LUNG DISEASE: EFFECT OF VITAMIN C AS A PRO-OXIDANT. Sara E. Hartmann[1], Xavier Waltz[1], Christine K. Kissel[1], Lian Szabo[1], Brandie L. Walker[1], Richard Leigh[1], Todd J. Anderson[1], Marc J. Poulin[1]. [1]University of Calgary, Alberta, Canada. *Email: shartma@ucalgary.ca.* Introduction: Acute hypoxia results in increased cerebral blood flow (CBF) and ventilation (VE). The extent to which these physiological responses are impacted with normal aging, or in patients with enhanced oxidative stress, such as in chronic obstructive pulmonary disease (COPD), is unclear. The purposes of the study were to: 1) investigate the effect of aging and COPD on the cerebrovascular and ventilatory responses to acute hypoxia, and 2) assess the effect of a vitamin C intervention on the regulation of CBF and VE. Methods: In 12 YOUNGER, 14 OLDER, and 12 COPD, we measured cerebral blood flow velocity in the middle cerebral artery (VP; index of CBF), and VE during two 5-minute periods of acute isocapnic-hypoxia (50 mmHg), under conditions of: 1) saline-sham; and 2) intravenous vitamin C. Antioxidants (vitamin C, superoxide dismutase [SOD], glutathione peroxidase, and catalase), oxidative stress (malondialdehyde [MDA] and advanced protein oxidation product) and nitric oxide metabolism (NOx) were measured in plasma. Results: The CBF and VE sensitivity to hypoxia were reduced by ~60% with aging (OLDER vs. YOUNGER; $P < 0.02$). COPD patients exhibited similar VP and VE responses to OLDER ($P > 0.05$). Following the intervention, Vitamin C, SOD, catalase, and MDA increased, while NOx decreased.

Vitamin C selectively reduced the cerebrovascular response in YOUNGER subjects by 43% (P = 0.023), but had no effect on VE in any group. Conclusion: Vitamin C exhibited pro-oxidant properties, contrary to our hypothesis. These findings suggest that the cerebral vessels in YOUNGER individuals are more susceptible to oxidants, perhaps due to a greater reliance on nitric oxide. Peripheral chemosensitivity was not altered with Vitamin C, suggesting that acute increases in oxidative stress do not alter the ventilatory response. Acknowledgements: Heart and Stroke Foundation of Canada (HSFC), Canadian Institutes for Health Research (CIHR) and Natural Sciences and Engineering Research Council of Canada (NSERC). 2015.

CHANGE IN LUNG MECHANICS AFTER A HIGH ALTITUDE RACE. Manuela Bartesaghi[1], Gaia Mandolesi[2], Gabriele Simone Grasso[1], Maria Spiridonova[2], Gianpaolo Mossuto[2], Giulio Sergio Roi[3], Lorenza Pratali[4], Giuseppe Miserocchi[1], Annalisa Cogo[2]. [1]Dept. of Health Sciences, Università di Milano Bicocca, Milano, [2]Centro Studi Biomedici Applicati allo Sport, Università di Ferrara, Ferrara, [3]Centro Studi Isokinetic, Bologna, [4]Istituto Fisiologia, CRN, Pisa. *EMAIL: manuela.ba@tiscali.it* INTRODUCTION: To assess changes in respiratory mechanics induced by exhausting exercise at altitude. METHODS: We studied 10 athletes (8M; age 33-42) who ran from Cervinia (2030m) up to Rifugio Oriondè (2804m). We evaluated respiratory resistance (Rrs) by Forced Oscillatory Technique (FOT) (Jeager Master System, Germany) at impulse frequencies 5 up to 20 Hz, spirometry (Spiropalm Cosmed, I) and lung echo counting b-lines (Vivid I GE). $\%O_2$ saturation ($SatO_2$) was continuously monitored by pulse-oxymeter integrated in the LifeShirt System (Vivometrics, Ventura CA) during the race. RESULTS: Subjects ran 13.4 kilometers at an average upward speed of 418m/h. $SatO_2$ decreased significantly with exercise from 95±1,4 % to 83,5± 4,3 %, (p=0.001). Rrs at 20 Hz was unchanged while Rrs at 5 Hz it increased significantly from 2.07 ± 0.42 to 2.29 ± 0.67 (p 0.04). The difference R5 - R20 increased significantly after the race from 0.26 ± 0.16 to 0.36 ± 0.18; (p 0.01). Spirometry was normal at rest. After exercise we found no significant reductions in FEV1/FVC and FEV1 but a significant decrease in vital capacity from 5.48 ± 0.87L to 5.24 ± 0.79 L (p 0.0007). B-lines increased from 3.5 ± 5 at rest to 12 ± 7 after exercise (p 0.02). Values are expressed as mean ± SD. Statistics with t test. CONCLUSION: The increase in frequency dependence of resistance after the race likely reflects a combined effect of decreased patency of small airways and/or interstitial lung edema, in accordance with the increase in B-lines and the decrease in vital capacity. 2015.

CHANGE IN PERFORMANCE AND OXIDATIVE STRESS AFTER "LIVING HIGH TRAINING LOW" IN HYPOBARIC VS NORMOBARIC HYPOXIA. Gregoire Millet[1], Tadej Debevec[2], Jonas Saugy[1], Laurent Schmitt[3], Roberto Cejuela[4], Pauline Mury[5], Raphael Faiss[1], Vincent Pialoux[5]. [1]ISSUL, University of Lausanne, Switzerland, [2]"Jozef Stefan" Institute, Ljubljana, Slovenia, [3]National School of Mountain Sports/National Ski-Nordic Centre, Prémanon, France, [4]Departmental Section of Physical Education and Sports, University of Alicante, Alicante, Spain, [5]Center of Research and Innovation on Sports, University

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Introduction: Recent studies reported differences in physiological responses including oxidative stress between short exposures to normobaric (NH) and hypobaric (HH) hypoxia. This study investigated whether changes in endurance performance and prooxidant/antioxidant balance responses differ between "Living High Training Low" (LHTL) conducted in NH or HH. Methods: Twenty–four well-trained triathletes completed 18 days of LHTL (High 2250m in HH vs NH – Low 1100 m). Plasma levels of oxidative stress and 3-km running test were measured before and after the LHTL. Results: Following LHTL, the concentration of advanced oxidation protein products (AOPP) decreased (-26%; $P<0.05$) and nitrotyrosine increased (+9%; $P<0.05$) in the HH group only. Both, ferric-reducing antioxidant power (FRAP) (+38%; $P<0.01$) and superoxide dismutase (SOD) (+43%; $P<0.01$) were higher following the LHTL in the HH group, while the glutathione peroxidase (GPX) was lower in both NH (-35%; $P<0.05$) and HH (-48%; $P<0.01$) group. Catalase activity was only increased in the NH group (+24%; $P<0.05$). No changes in malondialdehyde (MDA) were observed in either group. Following LHTL, NOx was lower (-10%; $P<0.05$) and uric acid was higher (+43%; $P<0.01$) in the HH group with no changes in the NH group. Performance was more improved in the HH than in NH group (3.3% vs 1.2%; $P<0.05$). Conclusion: LHTL performed in HH seems to induce higher oxidative stress levels and larger performance enhancement. Acknowledgements: Ministère des Sports, INSEP, France. BASPO, Switzerland. 2015.

CHANGES IN LUNG COMET SCORES AFTER BRIEF HIGH-INTENSITY EXERCISE AT SEA LEVEL AND HIGH ALTITUDE. Chandra Patel[1], Sophia Larson[2], Jonathan Uniat[3], Paresh Giri[4], Gary Foster[3], James Anholm[3]. [1]VA Loma Linda Healthcare System, Loma Linda Univ Medical Center, [2]Loma Linda School of Medicine, [3]VA Loma Linda Healthcare System, [4]Loma Linda Univ Medical Center. *EMAIL: cpatel@llu.edu* INTRODUCTION: There is controversy as to whether intense exercise causes pulmonary edema. Lung comets seen during chest ultrasonography represent extravascular lung water in thickened intralobular septa. The purpose of this study was to determine if lung comets increase following brief, high intensity exercise at sea level (SL) and altitude. METHODS: Eight trained cyclists were studied before and after maximal cycling exercise at altitudes of 370m and 3800m. After a standardized warm-up subjects completed an incremental VO_2max test. The VO_2max test consisted of a ramp cycling protocol with the workload increasing continuously at 30 watts/min until subjects could no longer maintain their pedal cadence. Cyclists were transported by automobile from SL to altitude over 5-6 hours. Exercise testing was conducted 2-6 hours after arrival at altitude. The lung was imaged at 28 intercostal points on the right and left side of each athlete's chest before exercise and 5 min after completion of exercise. All images were recorded and analyzed in random order by an ultrasonographer blinded to the exercise and altitude conditions. Lung comets identified at the 28 imaged sites were summed for each subject at each altitude in the pre- and post-exercise conditions. Pre- and post-exercise lung comet scores were compared by

analysis of variance. RESULTS: Before exercise at SL the lung comet score was 3.6 ± 3.7 (mean ± SD). Immediately post-exercise at SL the lung comet score was 3.7 ± 2.7. At altitude, the pre-exercise lung comet score was 5.6 ± 1.5 and the post-exercise score was 8.1 ± 3.4. Lung comets were significantly increased at altitude compared to SL, p = 0.03. There was a trend toward increased lung comets post-exercise at altitude (p = 0.06). CONCLUSION: These findings suggest rapid ascent to high altitude and possibly intense exercise at attitude are associated with increased lung water. 2015.

CHANGES IN SLEEP PATTERNS DURING EXTENDED STAY AT HIGH ALTITUDE. Gerald Dubowitz, Allison Mulcahy. University of California San Francisco, Alameda County Medical Center. *Email: dubowitz@anesthesia.ucsf.edu.* Objective: To study the incidence and duration of sleep disturbances and correlation with subjective sleep quality in healthy human volunteers during extended stays at high altitude Methods: 8 Subjects (4 males and 4 females) were studied using continuous nocturnal pulse oximetry to evaluate sleep disturbances. Sleep pulse oximetry was measured at baseline during sleep at sea level and then each night during ascent and regularly during a subsequent stay at 4250m. Sleep disturbance was defined as a nocturnal saturation drop of greater than 4% below the mean value for a period of greater than 10 seconds. Sleep quality was assessed by subjective questionnaire to determine sleep latency, sleep quality and restfulness the following day. Results: At 4250m, mean nocturnal saturation overnight was 86%. Mean sleep disturbance was 29 dips/hr (range 3-99 dips/hr). At sea level the mean sleep disturbance was 1 dip/hour. During ascent the maximum sleep disturbance was 112 dips/hr. All subjects reported disturbed sleep at 1 and 2 days after arrival at 4250m and none reported disturbed sleep at 22 days. Discussion: Subjective improvements in sleep quality were reported after just 1-2 days at altitude but this did not necessarily correlate with fewer sleep disturbances. Some individuals had little objective sleep disturbance but still reported poor sleep quality. Conversely, some subjects who claimed to sleep well still exhibited significant desaturations events even after 20 days at altitude. Conclusions: Desaturation events are not solely responsible for sleep disturbance during ascent and during a prolonged stay at high altitude. Nocturnal desaturation and sleep disturbances continue after a prolonged stay at altitude, even though a subjective improvement in sleep occurs. 2009.

CHANGES IN THE CONTROL OF RESPIRATION AT HIGH ALTITUDE; INFLUENCE OF CEREBRAL BLOOD FLOW REACTIVITY TO CO_2. Jui-Lin (Mickey) J Fan, Keith R Burgess, Kate N Thomas, Karen C Peebles, Rebekah A Lucas, James D Cotter, Samuel J Lucas, Philip N Ainslie. University of Otago. *Email: mickeyfanny@hotmail.com.* Upon ascent to high altitude (HA), there is a universal development of periodic breathing pattern. We tested the hypothesis that alterations in cerebrovascular and ventilatory responses to changes in partial pressure of arterial CO_2 ($PaCO_2$) could account for the periodic breathing observed at HA. To examine this hypothesis we used indomethacin (INDO; 100 mg), a pharmacological means to selectively reduce middle cerebral artery velocity (MCAv; an

index of cerebral blood flow) and MCAv-CO_2reactivity, in 12 participants at sea-level (SL) and at HA (5,050 m). At SL, 90 min following INDO, MCAv and MCAv-CO_2reactivity were reduced by 23 ± 16% and 47 ± 27%, respectively (P<0.01). This reduction in MCAv-CO_2reactivity was reflected in an enhanced ventilatory CO_2 (VE- CO_2) sensitivity (P=0.04). Prior to INDO at HA, MCAv-CO_2reactivity was reduced compared to pre-INDO SL baseline (-36 ± 69%, P=0.06), while VE-CO_2 sensitivity was elevated (+1.5 ± 2.4 L/min/mmHg, P=0.07). At HA, INDO reduced MCAv and MCAv-CO_2 reactivity by 22 ± 8% and 88 ± 62%, respectively (P<0.01). In contrast to SL, at HA there was a differential change in the slope of the VE-CO_2 sensitivity with INDO [+0.5 ± 0.7 L/min/mmHg (SL) v -2.1 ± 2.8 L/min/mmHg (HA); P=0.02]. Interestingly, enhanced periodic breathing patterns were recorded in 10 participants at HA following INDO. These results indicate that reductions in MCAv-CO_2 reactivity - presumably via changes in brain [H+] at the level of the central chemoreceptors - may have a differential influence on ventilatory CO_2 sensitivity at SL and HA, and play a key role in the pathogenesis of periodic breathing. This study was supported by the Otago Medical Research Foundation, Peninsula Health Care p/l, Air Liquide p/l and the Italian National Research Council who kindly provided use of the EV-K2-CNR research laboratory. 2009.

CHANGES OF 25-OH-VITAMIN D DURING OVERWINTERING AT THE GERMAN ANTARCTIC STATIONS NEUMAYER II AND III. Mathias Steinach[1], Eberhard Kohlberg[2], Martina Maggioni[1], Stefan Mendt[1], Oliver Opatz[1], Alexander Stahn[1], Josefine Tiedemann[1], Hanns-Christian Gunga[1]. [1]Center for Space Medicine and Extreme Environments, [2]Alfred-Wegener Institute for Polar and Marine Research. *Email: mathias.steinach@charite.de.* Introduction: Humans in Antarctica face different environmental challenges among them low ultra-violet radiation which is crucial for Vitamin-D production in humans. Therefore we assessed changes in 25-OH-Vitamin D concentration during 12 months of overwintering at the German Stations Neumayer II and III (2007–2012). We hypothesized that (1) 25-OH-Vitamin D would significantly decrease, (2) changes would be affected by age, gender, initial fat mass, initial 25-OH-Vitamin D concentration and station residence and (3) values would not differ from comparable studies in high latitudes. Methods: 25-OH-Vitamin D concentrations were determined before, after, and monthly during the campaigns from venous blood samples of n=43 participants (28 male, 15 female). Initial fat mass was determined via bio impedance analysis and body plethysmography. Data were analyzed for change over time, dependency on the independent parameters and after categorization for sufficiency (>50nmol/l), insufficiency (25-50nmol/l), and deficiency (<25nmol/l). Results were compared with results from comparable studies. Results: We found a significant decrease of 25-OH-Vitamin D with dependency from month. Age, gender, fat mass or station residence had no influence. Only initial 25-OH-Vitamin D concentrations significantly affected subsequent 25-OH-Vitamin D values. Conclusion: Overwinterings at the Antarctic German Research Stations Neumayer II and III are associated with a decrease in 25-OH-Vitamin D concentrations, unaffected by age, gender, initial fat mass, and station residence. Higher initial Vitamin D concentrations might protect from subsequent deficiencies. Residence at

the Neumayer Stations may lead to lower Vitamin D concentrations than found in other comparable high latitudes. Acknowledgements: We would like to express our gratitude to the overwinterers of the Neumayer Stations II and III of the investigated overwintering campaigns 2007-2012. We thank the "Alfred Wegener Institute for Polar and Marine Research", Bremerhaven Germany, its representatives and employees who made this study possible. We thank our medical-technical assistant Mrs. Himmelsbach-Wegner for analyzing the serum samples. We thank Ms. Henriette Gängel and Ms. Rebecca Prell for their help in literature research. 2015.

CHEMOREFLEX RESPONSES IN AYMARA HIGH ALTITUDE NATIVES VERSUS ACCLIMATIZED LOWLANDERS. Alex Vesely, Marat Slessarev, David Preiss, Alexandra Mardimae, Dahlia Balaban, Richard Greene, Joseph Fisher, James Duffin. University of British Columbia, University of Toronto, New Mexico Highlands University. *Email: alex.vesely@utoronto.ca.* Objective: Previous work has shown differences between lowlanders and various populations of high altitude natives (HAN), and between healthy subjects and those with chronic mountain sickness patients with respect to chemoreflex control of breathing. Study methods have primarily involved measurement of hypoxic ventilatory responses and CO_2 ventilatory responses using steady state methods; our aim was to use a modified rebreathing technique to further characterize differences between HAN and lowlanders. Methods: We studied 8 healthy Aymara native to La Paz, Bolivia, and 6 lowlanders at the end of their second week in La Paz (3600 m). Subjects performed two modified Read rebreathing tests each involving i) hyperventilation prior to rebreathing to allow identification of the chemoreflex threshold, and ii) $PETO_2$ kept constant via computer controlled feedback loop at either 150 mmHg (hyperoxic) or 50 mmHg (hypoxic) to enable respective measurement of a central chemoreflex threshold and sensitivity and a combined response that includes both central and peripheral components. End-tidal gases and respiratory flows were measured; data were analyzed using peak detection and curve fitting algorithms using Labview. Results: The initial slope (gain) of the ventilatory response was unchanged from the hyperoxic to hypoxic tests in both the lowlanders (2.8 /-0.2 to 2.8 /-0.5 L/min/mmHg) and Aymara (1.8 /- 0.2 to 2.0 /- 0.2 L/min/mmHg), however the threshold PCO_2s of the responses decreased (35.3 /-1.3 to 28.8 /-0.9, and 36.4 /-1.3 to 31.7 /-0.7 mmHg, respectively; both $p<0.001$). Conclusion: Aymara showed decreased hyperoxic (central) ventilatory sensitivity versus lowlanders ($p<0.05$); whereas studies of other HAN such as Sherpas and Peruvians have found similar or higher sensitivities. This may be due to differences between the physiologic effects of rebreathing vs. other methodologies, or to differences in the populations studied. 2009.

CHRONIC HYPOXIA INDUCES DIFFERENTIAL TIME-DEPENDENT AND MUSCLE-SPECIFIC OXIDATIVE CHANGES TO THE SKELETAL MUSCLE PROTEOME. Philip Lewis[1], David Sheehan[2], Ken D. O'Halloran[1]. [1]Dept Physiology, [2]Dept Biochemistry, Univ College Cork, Ireland. *EMAIL: k.ohalloran@ucc.ie* INTRODUCTION: Chronic hypoxia (CH) induces differential functional remodelling in respiratory and limb muscles. Respiratory muscle redox homeosta-

sis is altered by CH. Protein oxidation potentially contributes to skeletal muscle plasticity in hypoxia-related respiratory diseases. The objective of this study was to determine the type and extent of respiratory and limb muscle protein oxidation in a mouse model of CH. METHODS: We quantified changes in protein carbonyl groups and free thiol groups in respiratory and limb muscles from mice exposed to 1, 3 and 6 weeks of CH ($FiO_2 = 0.1$) or normoxia. Diaphragm, sternohyoid, extensor digitorum longus (EDL) and soleus muscle homogenates were incubated with carbonyl- or thiol-reactive fluorophores before gel electrophoresis and fluorescence scanning. RESULTS: Differential, time-dependent changes in protein oxidation were observed between the four muscle groups. After 6 weeks of hypoxia, there was a significant reduction in free thiols in the sternohyoid ($P<0.0001$, Student's unpaired t-test) and diaphragm ($P=0.0046$), whereas significant increases in thiol groups were seen in soleus ($P<0.0001$) and EDL ($P=0.0007$) muscles. After 1 week of CH, there was no change in diaphragm carbonyl groups, but a significant increase was observed after 3 weeks ($P=0.0109$) with a further increase after 6 weeks ($P<0.0001$). CONCLUSION: We conclude that CH exposure causes differential protein oxidation in respiratory and limb muscles in a time-dependent manner. We have detected protein specific changes in oxidation and expression using 2-D 'spot' proteomics. Mass spectrometry will reveal the identities of these proteins. ACKNOWLEDGEMENTS: Funded by the Health Research Board (Ireland) and the Strategic Research Fund, Univ College Cork. 2015.

CHRONIC INTERMITTENT HYPOXIA ALTERS CHEMOREFLEX CONTROL OF LUMBAR SYMPATHETIC NERVE ACTIVITY AND CAROTID BODY PROTEIN EXPRESSION. Noah J Marcus, Yulong Li, Cynthia B Bird, Burt Olson, Scott S Smith, Kristin I Sorenson, Harold D Schultz, Barbara J Morgan. University of Wisconsin, University of Nebraska Medical Center. *Email: nmarcus@wisc.edu.* In rats, exposure to chronic intermittent hypoxia (CIH) raises arterial pressure. CIH also increases carotid sinus nerve activity under normoxic conditions and results in an augmented response to hypoxia. The purpose of this study was to determine whether CIH augments chemoreflex control of lumbar sympathetic nerve activity (LSNA). In addition we measured expression of key regulatory proteins in the carotid bodies. Sprague Dawley rats were exposed to CIH. After 28 days, the rats were anesthetized, paralyzed, and mechanically ventilated. The lumbar sympathetic chain was exposed and the nerves were placed on bipolar platinum electrodes. We measured LSNA during six 20-second apneas. In a separate group of rats, carotid bodies were removed, flash frozen, and later analyzed via Western blot for angiotensin type 1 receptor (AT1R) and neuronal nitric oxide synthase (nNOS) protein expression. Baseline LSNA and LSNA during 20-sec apneas were higher in CIH rats vs. CON. Expression of AT1R protein was higher and nNOS protein lower in carotid bodies from CIH rats vs. CON. These results suggest that CIH results in augmented chemoreflex control of LSNA, and that this change in function is associated with changes in key regulatory proteins in the carotid body. These adaptations to CIH may be responsible in part for the arterial pressure elevations observed in this model. Funded by NIH #HL074072. 2009.

CHRONIC INTERMITTENT HYPOXIA ALTERS VENTILATORY RESPONSES TO ACUTE HYPOXIA IN RATS. Barbara Morgan[1], Russell Adrian[1], Zun-yi Wang[1], Melissa Bates[1], John Dopp[1]. [1]University of Wisconsin-Madison. *Email: morgan@ortho.wisc.edu.* Introduction: The stimulatory effects of chronic exposure to intermittent hypoxia (CIH) on carotid body sensory activity are well-documented in in vivo and ex vivo preparations; however, it is unclear whether these effects translate into sensitization of carotid chemoreflex control of ventilation in intact, conscious animals. Some previous investigators observed enhanced ventilatory responses to acute hypoxia following CIH, whereas other investigators reported blunted responses, and still others found no effect of CIH on hypoxic ventilatory responses. Species, strain, and age differences and/or disparate CIH paradigms may be responsible for these inconsistencies; nevertheless, many of these previous investigations lack precise and complete assessments of hypoxic chemosensitivity, i.e. most did not incorporate measures of the circulating carotid body stimulus level or account for the decrease in metabolic rate that occurs in small mammals acutely exposed to hypoxia. Therefore, our aim was to assess the effect of CIH exposure on hypoxia-induced chemoreceptor sensitivity by constructing stimulus-response relationships based on measures of arterial oxygen saturation (SpO2) and the ventilatory equivalent for VCO2 to account for the hypometabolism of acute hypoxia. Methods: Adult male Sprague-Dawley rats were exposed to intermittent hypoxia (nadir SpO_2, 75%; 15 events/hour; 10 hours/day) for 21 days. We assessed the following responses to acute hypoxia in conscious rats before and after CIH: ventilation (VE; barometric plethysmography), VO2 and VCO2 (analysis of expired air), and SpO_2 (pulse oximetry via neck collar). Results: CIH exposure increased the slopes of the VE/VCO2/SpO2 and VT/Ti/VCO2/SpO2 (an index of central respiratory "drive") relationships. We also observed increased VE/VCO2 and breathing frequency under normoxic conditions following CIH. Conclusion: Thus, our CIH paradigm produced an augmentation in chemoreflex control of ventilation that was evident in conscious rats. Acknowledgements: Funded by: NHLBI (UO1 HL 105365). 2015.

CHRONIC INTERMITTENT HYPOXIA INCREASES NADPH OXIDASE EXPRESSION IN RAT STERNOHYOID MUSCLE. Robert Williams[1], Vincent Healy[1], Ken D. O'Halloran[1]. [1]Dept Physiology, Univ College Cork, Ireland. *EMAIL: k.ohalloran@ucc.ie* INTRODUCTION: Chronic intermittent hypoxia (CIH) is a defining feature of obstructive sleep apnea syndrome and has been shown to impair rat respiratory muscle function, an effect blocked by chronic antioxidant supplementation. The superoxide generating enzyme - NADPH oxidase (NOX), is implicated in CIH-induced plasticity in sensory and motor nerves. The aim of this study was to investigate if NOX expression is increased in the sternohyoid (an upper airway dilator) muscle following CIH exposure. METHODS: Male Wistar rats (n=4) were exposed to alternating cycles of 90s hypoxia (5%O_2 at nadir) and 210s normoxia (i.e. 12 cycles per hour), 8 hours per day for 2 weeks. Control rats (n=4) were exposed to normoxia in parallel. The sternohyoid muscles were excised post mortem and the expression of NOX2 (catalytic component) and p22phox

(transmembrane protein associated with NOX2) subunits in protein extracts were measured by western blotting and densitometry. Data were compared using a Student's t-test and significance was taken at P<0.05. RESULTS: There was a statistically significant three-fold increase in NOX2 expression in the sternohyoid muscle from CIH rats compared to controls; however, p22phox expression was unaltered. CONCLUSION: Our results suggest that NOX-dependent oxidative stress may underlie rat upper airway dilator muscle dysfunction following CIH. ACKNOWLEDGEMENTS: Funded by Univ College Cork, Ireland. 2015.

CHRONIC INTERMITTENT HYPOXIA INDUCES SYSTEMIC HYPERTENSION THROUGH INCREASED CARDIAC OUTPUT, NOT INCREASED PERIPHERAL VASCULAR RESISTANCE. Eric F. Lucking[1], Ken D. O'Halloran[2], James F. X. Jones[1]. [1]1School of Medicine and Medical Science, Univ College Dublin, Dublin, Ireland, [2]2Dept Physiology, School of Medicine, Univ College Cork, Cork, Ireland. *EMAIL: eric.lucking@ucdconnect.ie* INTRODUCTION: Obstructive sleep apnoea (OSA) is associated with cardiovascular morbidity and recognised as an independent risk factor for hypertension. Sympathetic nervous activity is commonly increased in OSA patients and animal models exposed to chronic intermittent hypoxia (CIH) and thought to result in increased vasoconstrictor tone. Our previous results indicate that CIH-induced hypertension is not a result of decreased vascular conductance (VC) or hypersensitivity to lumbar sympathetic stimulation. We hypothesised that CIH induces systemic hypertension by increasing cardiac output and blood volume rather than by decreasing VC. METHODS: Age-matched adult male Wistar rats (335±4g) were exposed to CIH (n=8) consisting of 90s hypoxia (5%O_2 nadir)/210s normoxia cycles, or sham (n=8) treatment (normoxia), for 8h/day for 2 weeks. Under urethane anaesthesia, the cardiac output was determined using transthoracic echocardiography. Blood volume was determined by measuring the dilution of Evans blue dye in blood plasma samples 10mins after I.V. infusion. Data are presented as Mean ± S.E.M and were analysed by Student's t-test. RESULTS: CIH exposure significantly increased mean arterial pressure (99.8±2 vs. 90.6±3.8 mmHg; p=0.025) and haematocrit concentration (44.9±0.3 vs. 43.1±0.7 %; p=0.017). In addition, CIH exposure significantly increased cardiac output (25.8±2.6 vs. 19.3±1.7 ml/min/100g; p=0.027) but did not significantly alter femoral VC or total blood volume. CONCLUSION: Our findings show that CIH-induced hypertension is a result of increased cardiac output, not increased vascular resistance. We conclude that hypertension is a consequence of the adaptive response to CIH exposure whereby the cardiac output is increased, in combination with increased hematocrit, in an attempt to increase oxygen flow to the hypoxic tissues. ACKNOWLEDGEMENTS: Irish Research Council and Univ College Dublin. 2015.

COGNITIVE PERFORMANCE OF RECREATIONAL CLIMBERS ON ACONCAGUA MOUNTAIN, ARGENTINA. Scott A Marshall[1], Michael L Russell[2], Rodney Coldren[3], Kathryn R Marshall[4], Marleigh Erickson[3], Katherine Brazaitis[4], David Irwin[5], Steve Sharp[3], Louis M French[4]. [1]Neurology and Critical

Care, San Antonio Military Medical Center, Fort Sam Houston, Texas, [2]US Army Medical Command, Fort Sam Houston, Texas, [3]Uniformed Services Univ the Health Sciences, Bethesda, Maryland, [4]The Defense and Veterans Brain Injury Center, Rockville, Maryland, [5]Univ Colorado at Denver, Anschutz Medical Campus, Denver, Colorado. *EMAIL: scott.marshall@amedd.army.mil* INTRODUCTION: Cognitive dysfunction (CD) exists among climbers to terrestrial high altitude. CD is seen in an elevation-dependant fashion, and likely correlates with the rate of ascent. A portable computerized cognitive assessment tool (CAT) may describe CD at the conclusion of a gradual, multi-day field acclimatization from 2500 feet to 13,800 feet. METHODS: 139 recreational mountaineers were evaluated with a CAT, the Automated Neuropsychological Assessment Metrics (ANAM) prior to beginning their climb at 2,500 feet and again four days later at 13,800 feet. The CAT included go no go, match to sample, procedural reaction time, simple reaction time, Stroop, and running memory subtests. Cognitive performance and demographics of participants were analyzed using Fisher's Exact Test for dichotomous or categorical data, and a t-test or paired t-test for continuous data. The F statistic was used to calculate correlation of continuous variables. RESULTS: 83 participants arrived at base camp and were re-tested. Go no go, simple reaction time, and Stroop subtests all demonstrated statistically significant improvements at high altitude. There was no significant difference in mean match to sample or procedural reaction time results. No differences were discerned in demographics or cognitive scores between those who achieved base camp and those who did not. Increasing age correlated with changes in neurocognitive scores at higher altitude. Symptoms of AMS did not correlate significantly with neurocognitive performance. CONCLUSION: Neurocognitive functioning after a slow ascent to non-extreme attitude improved, perhaps secondary to adaptive cognitive acclimatization over a gradual ascent or practice effect. Neuropsychological evaluation of a quicker ascent may have captured cognitive decrement at altitude or the gradual ascent taken by our population may be protective for CD. ACKNOWLEDGEMENTS: Fundng provided by a grant from the Defense Advanced Research Projects Agency (DARPA). 2015.

COGNITIVE RESPONSES TO HIGH ALTITUDE EXPOSURE IN SEA LEVEL AND HIGH ALTITUDE RESIDENTS OF ECUADOR. John Davis[1], Nathan Garvin[1], David Moilanen[1], Jessica Thorington[1], Cory Schall[1], Dale Wagner[2]. [1]Alma College, Alma, MI, [2]Utah State University, Logan, UT. *Email: davisj@alma.edu*. Introduction: High altitude inhabitants have cardiovascular and respiratory adaptations that make them well adapted to the high altitude environment. However, there have been several reports of impaired cognitive function in high altitude residents at very high altitude and climbers that spend long period of time at the highest altitudes. This study evaluated the influence of altitude of residence on cognitive function upon acute exposure to very high altitude. Methods: One hundred and thirty-two subjects participated in the study after giving informed consent. Subjects were recruited and tested at the base refugio at Mount Chimborazo in Ecuador (15,900 ft). This is an ideal location because many Ecuadorians that reside at a variety of altitudes can easily and quickly drive to the refugio. Cognitive measurements

included a go-no go (listening and responding to a non-visual stimulus), a word test (thinking of a series of words that fit into a particular category), and a hand movement test (repeating a series of hand positions). All measurements were done upon arrival at the refugio. Subjects were then divided into three groups based on their altitude of residence: Low altitude residents - LOW (0-5,000 ft), moderate altitude residents - MOD (5,000- 10,000 ft) and high altitude residents - HIGH(>10,000 ft). Results: Overall, there were no significant differences (P >0.05) between LOW, MOD, and HIGH for the word test or the hand movement test. However, the go-no-go test was significantly lower (P<0.05) in the HIGH group (8.4 ± .52 correct responses) than the LOW(9.6±.18 correct responses) or the MOD (9.58 ±.17 correct responses) groups. Conclusion: High altitude residents appear to have some indication of impaired cognitive function suggesting that long-term exposure to hypoxia might result in a maladaptation to long-term high altitude exposure. 2011.

COMPARING STEADY-STATE AND TRANSIENT TESTS OF THE PERIPHERAL CHEMOREFLEX IN HUMANS. Jamie Pfoh[1], Maria Abrosimova[1], Michael Tymko[2], Lindsey Boulet[2], Glen Foster[2], Anthony Bain[2], Philip Ainslie[2], Craig Steinaback[3], Liliana Cardona[1], Trevor Day[1]. [1]Mount Royal University, [2]University of British Columbia Okanagan, [3]University of Alberta. *Email: tday@mtroyal.ca.* Introduction: Carotid chemoreceptors detect reductions in arterial PO2, eliciting a peripheral chemoreflex (PCR). Steady-state hypoxia tests have been used to isolate the PCR and assess the hypoxic ventilatory response (HVR). However, these tests require expensive and sophisticated equipment to control end-tidal gases and the responses may be confounded by simultaneous cardiovascular and cerebrovascular responses. Transient tests of the PCR have also been developed to quantify the HVR, but have not been widely accepted. We aimed to compare the HVR responses between steady-state and transient tests and investigate the hypothesis that the response magnitudes would be well-correlated, within-subjects. Methods: Twenty healthy adults (25yrs; BMI 24 kg/m2; 8 males) underwent a steady-state end-tidal forcing test (10-min isocapnic hypoxia; 45 Torr PO2) and five consecutive trials of a three-breath 100% N2 test. We compared and correlated the two responses using a paired t-test and Pearson r correlation (significance assumed at P<0.05). Results: The steady-state isocapnic hypoxia test elicited a larger peak HVR response (2.7±0.4 L/min/-% SaO2) than the transient 100% N2 test (1.04±0.2 L/min/-% SpO_2; P=0.02). Interestingly, the tests were not correlated, within-subjects (r=0.07, P=0.78). Conclusion: Because these tests elicited different response magnitudes and are not predictive of each other, we suggest that these tests are distinctly different in their chemostimuli and the utility of either test depends upon the context and experimental question. The transient PCR test may have broad utility in the lab, clinic or field due to its (a) ease of application and (b) stimulus and temporal domain specificity and (c) minimal cardiovascular and cerebrovascular effects. Acknowledgements: Alberta Innovates Health Solutions Summer Studentship, Faculty of Science and Technology Innovation Grant, MRU Internal Research Grant. 2015.

COMPOSITE WEIGHT LOSS ON MOUNT EVEREST. Maryam Khosravi, Denny Levett, Liesel Wandrag, Mike Grocott, Mike Stroud, For the Caudwell Xtreme Everest Research Group. UCL CASE, University College London, Southampton University,. *Email: zchaxn7@googlemail.com.* OBJECTIVE: Weight loss associated with exposure to hypobaric hypoxia at altitude is well-recognised, yet the underlying pathophysiology remains unclear. Studying the composition of such weight loss may suggest mechanisms. METHODS: We studied weight changes and body composition using bioelectrical impedance (BIA) in 23 healthy volunteers at sea level (SL), and after arrival at Everest Base Camp (5300m). Nine subjects, (4 females) subsequently remained at 5300m (BaseCamp group) whilst 14 (2 females) climbed higher to altitudes of up to 8848m (Climbers) over a study period of 70 days. RESULTS: BaseCamp group: Mean SL weight of 71.4 Kg (SD 9.74) and an overall mean weight loss of 4.72 kg (SD 1.94) from sea level to day 70 ($p = 0.16$); of which 1.93 kg (SD 1.76, 40%) was fat mass ($p = 0.71$), and 2.86 kg (SD 2.53, 60%) was lean mass ($p = 0.09$). Climbers: Mean SL weight of 81.4 kg (SD 13.1) and overall mean weight loss of 9.04 kg (SD 5.67) to day 70 ($p = 0.002$); of which 2.96 kg (SD 2.61, 33%) was fat mass ($p = 0.033$) and 6.14 kg (SD 3.89, 67%) was lean mass ($p = 0.002$). CONCLUSIONS: With prolonged exposure to high altitude, weight loss is greater in those exposed to higher elevations, yet if this were due to energy deficit alone, greater fat loss, which would yield more energy, might be expected. However, in both groups greater lean mass loss was observed, suggesting that there may be an alternative contributory process. By decreasing the high metabolic demands of lean tissue in a body under great physiological strain, loss of muscle mass may be a beneficial adaptive, rather than maladaptive, response. 2009.

COMPUTATIONAL MODEL OF TISSUE OXYGENATION IN RAT SKELETAL MUSCLE: APPLICATION OF A FUNCTIONAL MICROVASCULAR IMAGING SYSTEM. Graham M Fraser, Craig D Steinback, Gemma M Dias, Daniel Goldman, Christopher G Ellis. University of Western Ontario. *Email: gmfraser@uwo.ca.* Our objective was to identify the extent of tissue pO_2 decrease in skeletal muscle during hypoxia (HX). A dual wavelength functional microvascular imaging system was used to visualize capillary blood flow, measure red blood cell (RBC) oxygen saturations (ScO_2) and reconstruct the three-dimensional geometry of the capillary network. Custom analysis and vascular mapping software was used to provide vascular geometry, capillary hemodynamics and ScO_2 to a computational model of tissue oxygen transport. The constant consumption computer model was used to calculate the pO_2 in the tissue volume (60 x 268 x 240 μm) surrounding the microvascular network of interest. Hypoxia was induced by increasing the N2: O_2 ratio of inspired gases. The animal was mechanically ventilated at 74 breaths per minute to maintain isocapnia. Mean tissue pO_2 during hyperoxia (OX; SaO_2 100%) was 27.5 ± 6.7 torr (minimum regional $pO_2 = 14.6$ torr) with a mean measured capillary entrance SO_2 of 61.9 ± 9.6%. HX (SaO_2 74%) resulted in a decreased tissue pO_2 of 20.1 ± 5.9 ($p < 0.001$) (minimum regional $pO_2 = 7.0$ torr) with a mean measured capillary entrance saturation of 53.2 ± 14.7%. Mean capillary RBC supply rate during OX was 4.5 ±

3.7 vs. 5.1 ± 4.5 cells/second/capillary in HX. This demonstrates our capability to calculate the functional impact of HX on tissue oxygenation in vivo using discrete capillary network data. Supported by CIHR grant held by Dan Goldman and Christopher Ellis. 2009.

CONCURRENT USE OF VAS AND LAKE LOUISE SYMPTOM SCORES FOR ACUTE MOUNTAIN SICKNESS (AMS). Kayser, B., Aliverti, A., Pellegrino, R., Delacca, R., Quaranta M, Pompilio P, Miserocchi G, Cavalleri L, Ora Josuel, Biasco L, Milanese M, Fasano V, Pomidori L, Cogo, A. ISMMS, University of Geneva, Switzerland, Politecnico di Milano, Milano, Italy, Centro di Fisiopatologia Respiratoria, Azienda Ospedaliera S. Croce e Carle, Cuneo, Italy, Politecnico di Milano, Milano, Italy, Department of Experimental Medicine, University Bicocca-Milano, Monza, Italy., DICMI, University of Genova, Genova, Italy, Dipartimento di Medicina Clinica, University of Rome "La Sapienza", Rome, Italy, Department of Cardiology, University of Turin, Asti, Italy, Unita Operativa Pneumologia, S. Corona Hospital, Pietra Ligure, Italy, Institute of Respiratory Diseases, University of Milan, Italy, Dept of Clinical and Experimental Medicine, University of Ferrara, Italy. *Email: Bengt.Kayser@unige.ch.* Assessment of AMS depends on subjective appreciation of severity of symptoms. Presence and severity of AMS is therefore based on the quantification of reported subjective sensation. A popular questionnaire is the Lake Louise Symptom Scoring System (LLS) which uses categorical variables to rate intensity of AMS-related symptoms (headache, gastrointestinal distress, dizziness, fatigue, sleep quality) on 4 point ordinal scales, the sum of answers being the LLS self-score (LLSs, range 0-15). Recent data indicate a potential for the use of a visual analogue scale (VAS) to quantify AMS as a continuous variable (Wagner et al. HAMB, 8: 27-31, 2007). LLSs correlated to VAS (r=0.65), test-retest and inter-rater reliability were good. LLS sub-items (LLSi) were not separately investigated. We tested the hypothesis whether global and single item VAS and LLS scores scale linearly. We asked 14 un-acclimatized subjects (age 41[14], mean[SD] yr, height 176[3]kg, weight 75[9]kg) who spent 2 days at 3647m and 4 days at 4560m to fill out LLS questionnaires, with a VAS for each item as well as a VAS for the overall sensation of AMS, twice a day (n=172). Even though significantly correlated (r=0.84) the relationship between LLSs and global VAS was distorted showing a threshold effect for LLSs scores below 6 with most VAS scores on one side of the theoretical linear identity line of VAS vs. LLSs. Similar threshold effects were seen for the LLSi and single item VAS scores. These findings indicate non-linear scaling characteristics which render direct comparison of studies done with either VAS or LLS alone difficult. Support: Italian Alpine Club, Polytechnic Milan, University of Geneva, University of Ferrara. 2009.

CONDUIT ARTERY STRUCTURE AND FUNCTION IN LOWLANDERS AND SHERPAS AT HIGH ALTITUDE. Nia CS Lewis[1], Greg R duManoir[2], Samuel EJ Lucas[3], Apirna Singh Basnet[4], Philip N Ainslie[5]. [1]Centre for Heart, Lung and Vascular Health, Univ British Columbia, Kelowna, Canada, [2]Human Kinetics Diploma Program, Okanagan College, Penticton, Canada, [3]The Dept Physical

Education, Univ Otago, Dunedin, New Zealand, [4]Banner Good Samaritan Medical Centre, Arizona, USA, [5]Centre for Heart Lung and Vascular Health, Univ British Columbia, Kelowna, Canada. *EMAIL: nia.lewis@ubc.ca* INTRODUCTION: Research detailing the normal vascular adaptions to high altitude (HA) is minimal and confounded by pathology (e.g., chronic mountain sickness) and methodological issues. This study aimed to examine potential alterations in vascular function and structure in: 1) healthy lowland individuals during acute and prolonged exposure to HA; and 2) in HA natives (Sherpas) born and permanently residing at HA when compared to lowland individuals. METHODS: Using ultrasound and automated edge detection software, we assessed brachial artery endothelial function (independent- and dependent-flow mediated dilation [FMD]), carotid intima thickness (cIMT) along with arterial stiffness (central- and peripheral-pulse wave velocity [PWV]; applanation tonometry) in 12 normotensive lowlanders (aged 32±7y). Beat-to-beat measures of arterial blood pressure (Finometer) and heart rate (ECG) were also obtained. Measures were made at sea level (SL; 334 m) and following arrival at HA (5050 m) between days 3-4 and 12-14. The same measurements were also made in 12 Sherpas at 5050 m (aged 33±14y). RESULTS: Compared with SL, arrival to HA elevated lowlanders' heart rate (77 ± 4 vs. 56 ± 3 b/min; $P<0.001$) and central-PWV (6.55 ± 0.27 vs. 5.98 ± 0.20 m/s; $P=0.001$), and reduced their independent-FMD (6.8 ± 0.4 vs. 7.9 ± 0.4%; $P=0.004$) and dependent-FMD (14.5 ± 0.8 vs. 16.6 ± 0.9%; $P=0.006$). In contrast, blood pressure, cIMT and peripheral-PWV were unchanged with ascent to HA ($P\geq0.10$). All effects persisted at days 12-14 at HA. These changes in vascular function and structure at HA in lowlanders were comparable to Sherpas CONCLUSION: In conclusion, initial exposure to HA was associated with impairment in both endothelial and smooth muscle function as well as an increase in central arterial stiffness; however, these changes were neither exacerbated nor resolved with prolonged exposure (~2 weeks). Also, a lifetime of HA exposure does not intensify the impairments seen in vascular function and structure. ACKNOWLEDGEMENTS: Supported by NSERC and CRC. Research conducted under the memorandum between Nepal Health Research Council and EVK2-CNR. 2015.

CONTRIBUTION OF MEMBRANE PROGESTERONE RECEPTORS α AND β TO RESPIRATORY CONTROL. Ryma Boukari[1], Orlane Rossignol[1], François Marcouiller[1], Aida Bairam[1], Vincent Joseph[1]. [1]Université Laval / CRCHU de Québec. *Email: joseph.vincent@crsfa.ulaval.ca.* Introduction: Progesterone is a potent respiratory stimulant. In women, it protects against Chronic Mountain Sickness, and at sea level it reduces sleep apneas frequency. We showed that membrane progesterone receptors α and β (mPRα and mPRβ) are expressed in respiratory nuclei in the brainstem, but their roles on respiratory control are unknown. We tested the hypothesis that deletion of mPRα and β would affect ventilation, apneas during sleep, and ventilatory chemoreflex. Methods: Adult female mice were instrumented for infusion of small interfering RNA (siRNA) against mPRα or mPRβ (or control solution) in the fourth ventricle for 2 weeks. Ventilation (Ve) and CO_2 production (VCO2) were recorded (whole body plethysmography) during 4 h (0900-

1300) in normoxia, then in response to hypoxia (12% O2), hypercapnia (5% CO_2) or hypoxic-hypercapnia (5% CO_2 + 12% O_2), 10 min each. Ve and apnea frequency were determined during sleep (periods of regular breathing longer than 10 minutes), and Ve was determined during the early (1min) and late (5min) exposure to each test gas. Results: Compared to control, deletion of mPRα decreased Ve and Ve/VCO2 during sleep, did not affect apnea frequency, and decreased the ventilatory response during the early phase of hypercapnia. The deletion of mPRβ had no effect on Ve during sleep, but decreased VCO2, increased Ve/VCO2, increased apnea frequency and suppressed the ventilatory response during the early phase of hypoxia and hypoxic-hypercapnia. Specific deletions of mPRα and mPR β in respiratory nuclei of the brainstem have been verified by immunohistochemistry. Conclusion: We conclude that mPRα and β play distinct roles on respiratory control and ventilatory chemoreflex and likely mediate, at least in parts, the respiratory effects of progesterone. Acknowledgements: Funded by CIHR (MOP-102715). 2015.

CORRELATION OF THE SPECTRUM OF ACUTE ALTITUDE-ASSOCIATED NEUROLOGICAL DISORDERS WITH ULTRASOUND EVALUATION OF OPTIC NERVE SHEATH DIAMETER AND PAPILLEDEMA -- STANDARDIZATION, CONTROVERSIES AND A NOVEL TECHNIQUE. Melanie Watts, Allison Mulcahy, Sachita Shah, Jeffery H Gertsch, Buddha Basnyat. Alameda County Medical Center, University of California-San Diego School of Medicine, Departement of Neurosciences, Himalayan Rescue Association and Nepal International Clinic. *Email: melanie.r.watts@gmail.com.* Altitude medicine is dominated by a group of neurological syndromes which our group has termed the Spectrum of Acute Altitude-associated Neurological Disorders (SAAND). Among these disorders are High Altitude Headache, Acute Mountain Sickness, and High Altitude Cerebral Edema in ascending order of morbidity. With High Altitude Headache among the most common symptoms at altitude, and High Altitude Cerebral Edema the most deadly, it stands to reason that the establishment of a rapid, quantitative field test for the diagnosis of SAAND would advance the field of altitude medicine. Measurement of Optic Nerve Sheath Diameter (ONSD) and Papilledema by way of ultrasound is a new technique that has been applied to the diagnosis of elevated intracranial pressure in a diverse range of clinical syndromes. Insofar as SAAND pathophysiology has been postulated to include the phenomenon of raised intracranial pressure, ONSD measurements via ultrasound have been applied in the interest of establishing a sensitive, specific, and more objective diagnosis. However, following two months study of the techniques, from October to November 2008, at 4280 and 4928 meters in the Nepal Himalayas, questions and areas of controversy have been identified in the use of ultrasound for determination of ONSD and Papilledema in the study of altitude illness, including: · Standardization of training and interpretation · Recommendations on equipment and technical parameters · Standardization/optimization of ultrasound determination of the ONSD using the novel coronal axis technique (live demonstration to be provided) · Recommendations concerning research techniques for correlation of ONSD, ultrasonographic papilledema and observation with clinical tools such as the Lake

Louise Acute Mountain Sickness Questionnaire and pulse oximetry · Recommendations to address controversy in the literature and clinical questions most in need of research. 2009.

CT QUANTIFICATION OF AIRWAY WALL THICKNESS, LUMINAL AREA, AND LUNG WATER FRACTION AFTER EXPOSURE TO NORMOBARIC HYPOXIA WITH AND WITHOUT INHALED AMILORIDE. Steven Chase[1], Courtney Wheatley[1], Sarah Baker[1], Bryan Taylor[2], Robert Wentz[1], Manda Keller-Ross[1], Eric Snyder[3], Bruce Johnson[1]. [1]Mayo Clinic, [2]University of Exeter, [3]University of Minnesota. *Email: chase.steven@mayo.edu.* Introduction: There is a constant flux of fluid through the lungs with balance maintained by lymphatics (interstitial space) and epithelial sodium channels (ENaC) (alveolar space). Altitude exposure challenges fluid regulation mechanisms in the lungs. We sought to better understand the contribution of ENaC on lung fluid balance by exposing healthy adults to normobaric hypoxia with/without inhaled amiloride (ENaC blocker). We hypothesized that with amiloride, CT-determined airway wall thickness (AWT) would increase, airway luminal volume (ALV) would decline and lung water fraction (LWF) would increase. Methods: Subjects (Age=30±8, 55% female, n=21) were given either 1.5mg amiloride or saline before and 6hr into exposure to normobaric hypoxia (12.5% O2) for 12hr. A chest CT was taken before and after exposure. Apollo software (VIDA Diagnostics) allowed for segmentation of the airways. AWT and ALV were calculated for each airway branch (~8 generations). MATLAB was used to quantify the fraction of water in the lung parenchyma through a ratio of tissue, air, and blood. Measures were made pre/post hypoxia with/without amiloride. Results: Changes after exposure to hypoxia with/without amiloride for ALV (-2.22±11.1% vs 0.22±12.2%, p=0.25), AWT (-0.71±3.15% vs -1.00±2.24%, p=0.38), and LWF (-2.84±19.3% vs -4.49±23.3%, p=0.20) were similar. No correlation was found between ΔLWF and ΔAWT or ΔALV, however a relationship was found between ΔAWT and ΔALV (R2=+0.407). Two subjects with amiloride vs. 1 with saline demonstrated >20% increase in LWF. When subjects were separated into quartiles based on ΔLWF, minimal differences were observed for top and bottom quartiles for ΔAWT and ΔALV between amiloride and saline (p>0.2). Conclusion: Based on CT analysis of airway structure and LWF, inhaled amiloride had no observable effect on lung fluid regulation in response to normobaric hypoxia in healthy adults. This suggests insufficient blockade of ENaC, blockage of ENaC alone is insufficient to influence fluid regulation, or alveolar fluid flux does not play a large role with normobaric hypoxia. Acknowledgements: NIH HL71478 and HL108962. 2015.

CULTURED CORTICAL NEURONS OF DAURIAN GROUND SQUIRREL (CITELUS DAURICUS) TOLERATE OXYGEN-GLUCOSE DEPRIVATION. Juanjuan Zhao[1], Shan Gao[1], Junzhan Jing[1], Mingyue Zhu[1], Zhen Chai[1]. [1]Peking Univ, Beijing, China. *EMAIL: zhenchai@pku.edu.cn* INTRODUCTION: An abrupt interrupt of oxygen and glucose supply to brain tissue may lead to severe neuronal injury. A natural model for the investigation of tolerance to extreme nutrition reduc-

tion is the mammalian hibernator, who survives the torpor state without any neurological defect. METHODS: To determine whether hibernator neurons in primary culture are tolerant oxygen-glucose deprivation injury, if so, then to explore the underlying mechanism. RESULTS: Cultured cortical neurons from daurian ground squirrels (Citelus dauricus, abbreviated as GS, a mammalian hibernating species) survived better after 3h oxygen-glucose deprivation and the following glutamate toxicity, which led to calcium overload. The free calcium concentration in the cytosol ([Ca2+]i) after glutamate application was measured using fura-2 AM. Compared with rat neurons, GS neurons maintained much lower [Ca2+]i 3h, even 24h after glutamate application. Channels and transporters contributing to [Ca2+]i elevation and removal were then studied. Not surprisingly, the stimulated amplitude of [Ca2+]i elevation as well as the decay time constant in GS neurons was both significantly smaller. The roles of three main Ca2+ exclusion apparatuses (SERCA, NCX and mitochondria) were further considered. It turned out that higher activities of SERCA and NCX contributed to the faster velocity in GS neurons, showing a putative role of calcium extrusion in the difference of maintaining calcium homeostasis between GS and rat neurons. CONCLUSION: Cultured GS neurons were more tolerant to oxygen-glucose deprivation than rat neurons, which resulted from the capacity of GS neurons maintaining calcium homeostasis via its on/off reaction. ACKNOWLEDGEMENTS: This work was supported by the grants from Major State Basic Research Development Program of China (No. 2012CB518200 and 2006CB504100) and the grants from National Natural Science Foundation of China (No. 30730013). 2015.

DEAD SPACE MASK ELIMINATES CENTRAL APNEA AT ALTITUDE. David Patz[1], Michael Patz[2], Peter Hackett[3]. [1]St. Mary's Hospital, Grand Junction, Colorado, [2]Univ Washington School of Medicine, Dept Anesthesiology, [3]Univ Colorado School of Medicine, Dept Emergency Medicine. *EMAIL: davidsamuelpatz@hotmail.com* INTRODUCTION: Travelers to high altitude may have disturbed sleep due to periodic breathing with frequent central apneas. We tested whether a mask with added dead space could reduce the central apneas of altitude. METHODS: 16 subjects were recruited, age 18-35, residing at 4600 ft. (1400m). They each slept one night with full polysomnographic monitoring, including end tidal C02, in a normobaric hypoxia tent simulating 12,000 ft. (3658m) altitude. Those who had a central apnea index (CAI) > 20/hr. returned for a night in the tent for dead space titration during which they slept with increasing amounts of dead space, aiming for a CAI < 5/hr. or < 10% of baseline. Then each subject slept another night with the titrated amount of dead space. RESULTS: Of the 16 subjects, 5 had a central apnea index > 20/hr., mean 49.1, range 21.4 – 131.5/hr. In each of the 5, the dead space mask reduced the CAI by at least 88% to a mean of 3.1, range 0.9-7.1/hr., (p=0.04). Hypopnea index was unchanged. Three subjects required 500ccs or less. One subject required 750ccs, and one required 2.1L. Dead space did not appear to increase the C02 reserve. CONCLUSION: At 12,000 ft., central apneas can be effectively reduced with a dead space mask, but clinical utility will require further evaluation. ACKNOWLEDGEMENTS: Funding: Institute for Altitude Medicine. 2015.

DECREASED INCIDENCE OF ACUTE MOUNTAIN SICKNESS DUE TO IMPROVED KNOWLEDGE ABOUT HIGH ALTITUDE ILLNESS AT 4559M. Michele M Schoeb, Simon Brechbuhler, Marco Maggiorini. University of Zurich. *Email: schoebm@access.unizh.ch.* Objective: To assess and compare high altitude illness incidence at 4559m with historical data, and assess today mountaineers knowledge about the disease. Method: In July 2008 during 3 weeks, we interviewed and examined 209 unselected mountaineers staying overnight at the Margherita hut (4559m). Using a questionnaire we asked for their knowledge about acute mountain sickness (AMS) and personal experience. AMS was assessed using the Lake Louise score questionnaire and the environmental symptom questionnaire AMS-c (cerebral) sub-score. These results were then compared with those obtained using the same AMS scores in this location at the same period of the year in 1993 (n = 136). Results: The incidence of AMS at 4559m decreased using the Lake Louise score (≥5) and the AMS-c sub-score (≥0.7) from 39% and 40% in 1993 to 22% and 23% in 2008, respectively. The mean (SD) LL and AMS-c in 1993 was 4.03 (3.3) and 0.74 (0.84) and in 2008 3.15 (2.34) and 0.45 (0.54), respectively ($p < 0.001$). The average number of nights spent above 2500 in the 30 days preceding the ascent to 4559 was 2.1 (3.1) in 1993 and 5.3 (5.3) in 2008 ($p < 0.001$). In 2008 97% of the interviewed mountaineers know AMS and 93% that HAPE is caused by a wet lung. 58% know that AMS develops above 2500m and 70% are aware that either headache or insomnia are the most frequent AMS symptoms. To prevent AMS, 87% mentioned acclimatization and 58% opted for an ascent rate between 300-600m/day. Drug prophylaxis was considered as an option by 54%, acetazolamide was recommended by 88% of them. However, only 4% used acetazolamide in 2008. Conclusion: During the last 15 years AMS incidence at the Margherita hut is decreasing merely because of an increased awareness about high altitude illness and a better acclimatization in the 30 days preceding ascent. 2009.

DECREASED SERUM IRON LEVELS IN INDIVIDUALS WITH HAPE CORRELATE WITH HIGH HEPCIDIN EXPRESSION AT HIGH ALTITUDE (4559 M); CAUSE OR CONSEQUENCE OF EXAGGERATED HYPOXIC PULMONARY VASOCONSTRICTION? Heimo Mairbäurl[1], Martina Muckenthaler[2], Sandro Altamura[2], Marco Maggiorini[3], Peter Bärtsch[1]. [1]Medical Clinic VII, Sports Medicine, Univ Heidelberg, Germany, [2]Pediatric Oncology, Hematology &Immunology, UnivHospital Heidelberg, Germany, [3]Intensive Care Unit, Internal Medicine, Univ Hospital Zürich, Switzerland. *EMAIL: Heimo.Mairbaeurl@med.uni-heidelberg.de* INTRODUCTION: Elevating serum iron (Fe) decreases hypoxic pulmonary vasoconstriction {Smith et al., JAMA, 2009}. It is therefore possible that exaggerated hypoxic pulmonary vasoconstriction and high altitude pulmonary edema (HAPE) is related to low serum iron caused by insufficient iron reabsorption subsequent to altered hepcidin, a liver-expressed hormone that regulates iron homeostasis. METHODS: To test this hypothesis we measured FE and hepcidin in samples obtained at low (LA) and high altitude (HA; 4559 m) in a previous study {Maggiorini et al., AIM, 2006} from controls (CO) and HAPE susceptibles with HAPE in this study (HAPE). RESULTS: At 4559m tricuspid pres-

sure gradient (TI-DP) was higher in HAPE (day1: 52.9±8.2; day2: 45.4±15.1) than in CO (day1: 33.4±4.8; day2: 27.6±5.3). Fe (LA: CO: 13.5±5.7; HAPE: 16.9±8.5) was increased significantly on the first morning after arrival at high altitude (CO: 21.8±12.3; HAPE: 19.7±10.4) and returned to LA in CO (day2: 12.5±4.7), but decreased more in HAPE (8.0±2.3). Hepcidin (LA: CO: 4.3 ± 2.8; HAPE: 6.6 ± 2.9) was decreased significantly at HA in CO (day1: 2.1±0.7; day2: 0.8±0.3) but increased in HAPE (day1: 9.2±8.7; day2: 17.1±16.6). CONCLUSION: Decreased Fe with prolonged stay at HA is related to stimulated erythropoiesis as shown earlier {Mairbäurl et al., JAP, 1990}. It was more pronounced in HAPE, which goes hand in hand with increased hepcidin, probably due to inflammation. This indicates that individuals with HAPE may not be able to sufficiently replenish iron stores. Because TI-DP was increased already on HA-24h, when Fe was not decreased and hepcidin was normal, it appears unlikely that a lower Fe at HA-48h contributed to exaggerated pulmonary vasoconstriction in HAPE. Prevention of changes with dexamethasone and tadalafil (not shown) might indicate that altered iron metabolism is a consequence of HAPE rather than its cause. **2015.**

DEEP DIVERS: HYPOXIA TOLERANCE AT THE OTHER EXTREME. Jessica Meir. University of British Columbia, Vancouver, Canada. *Email: meir@zoology.ubc.ca*. Introduction: Continuous measurements of PO_2 in the blood while diving have recently revealed exceptional hypoxemic tolerance and highly efficient O2 utilization in the consummate avian and pinniped divers, the emperor penguin and elephant seal. PaO_2 values as low as 12-23 mmHg were documented in diving elephant seals, corresponding to routine SaO_2 of 8-26%, the lowest ever measured in freely diving seals. This is well below limits of other animals, and nearly equivalent to "critical PaO_2" (10 mmHg) of seals in forced submersions, defined by EEG marking the threshold of cerebral dysfunction. Methods: Characterization of O_2-hemoglobin dissociation curves revealed that emperor penguin hemoglobin has significantly higher affinity for O_2 compared to other birds. This allows more complete utilization of respiratory O_2 stores and increased blood O_2 content when PO_2 is low. Application of the dissociation curve to PO_2 profiles also indicates differing blood O_2 store management strategies. Results: In emperor penguins, arterial SO_2 remains near 100% for much of the dive, preserving high O_2 content for critical organs like the brain. Arterial SO_2 does not decrease significantly until final ascent, consistent with the decline in ambient pressure and decrease in air sac and arterial PO_2. These profiles demonstrate the significance of the respiratory O_2 store and high affinity hemoglobin of the emperor penguin. In contrast, although there is a transient rise and peak near 100% arterial SO_2 in the initial dive phase of elephant seals, these values decreased rapidly after this point. Conclusion: Venous SO_2 profiles demonstrate highly efficient and near complete utilization of the venous blood O_2 store in both species. These findings reflect differences in the magnitude of the respiratory O_2 store and maintenance of gas exchange during diving between this bird and mammal, and depict attributes that undoubtedly contribute to their extraordinary dives. Acknowledgements: Paul Ponganis. Funding: NSF grants OPP-0538594, IOS-0641801. 2011.

DELAYED SECOND CEREBRAL BLOOD FLOW RESPONSE TO SUSTAINED HYPERCAPNIC STIMULUS. Rosemary Regan, Marat Slessarev, Jay Han, Alexandra Mardimae, Dahlia Balaban, Stephanie Dorner, Cathie Kessler, James Duffin, Greg Wells, Joseph Fisher. University of Toronto. *Email: Rosemary.regan@utoronto.ca.* Middle cerebral artery blood velocity ('MCAV', a surrogate for cerebral blood flow,'CBF'), response time constants (τ-up) to increases in end-tidal PCO_2 ($PETCO_2$) have been measured as being between 8.8 and 92.6 s and many studies assume maximum values have been reached within 2-4 minutes. In these studies the rates of change of $PETCO_2$ were limited by lung gas wash-out times or $PETCO_2$ was poorly sustained. We used an improved stimulus to re-examine the CBF response to a pseudo-square wave sustained change in $PETCO_2$. A prospective end-tidal targeting system (RespirAct™, TRI, Toronto, Canada) was used to provide a standardized, repeatable, and stable isoxic ($PETO_2$ resting) square wave changes in $PETCO_2$ in 12 seated healthy subjects (2 F). After 5 min of normocapnia (control), $PETCO_2$ was raised by 10 mmHg for 10 min before returning to control. We monitored MCAV (ST3, Spencer Technologies), digital blood pressure (BP) (Nexfin, BMEYE) and minute ventilation (VE). In all subjects, $PETCO_2$ was within 2 mmHg of target value within 3 breaths. Increases in $PETCO_2$ were 9.56 ± 1.1 mmHg above control. The average SD of $PETCO_2$ per person for the hypercapnia stage was 0.56 mmHg. The τ-up was 11.5 ± 5.5 s. Six subjects reached their maximum MCAV within 3 τ-up and sustained it for the duration of the stimulus (Response A). The remaining subjects (Response B) exhibited a secondary continuous rise in MCAV for an additional 3.2-6.9 min (median=3.7min) before reaching a plateau. There were no differences in anthropomorphic or early response pattern between groups. We conclude 1) τ-up is indeed short as has previously been reported and 2) some subjects have a delayed secondary increase in MCAV response to a sustained, constant stimulus, perhaps due to vascular myogenic mechanisms. 2009.

DESTINATION GOKYO: ACCLIMATISATION AND RE-ACCLIMATISATION IN HIGH ALTITUDE TREKKERS. Meaghan J MacNutt, Paul B Laursen, Shiksha Kedia, Maniraj Neupane, Jhapindra Pokharel, Parash Parajuli, A. William Sheel. University of British Columbia, Mountain Medicine Society of Nepal. *Email: mjmacnutt@hotmail.com.* We conducted a field study to determine whether the process of hypoxic acclimatisation is facilitated by a recent sustained exposure to high altitude. Six low altitude natives (4M, 2F; 27 ± 5 years) were monitored throughout two identical 10d treks to high altitude (5360m) in the Solu Khumbu region of Nepal. The initial acclimatisation (IA) and re-acclimatisation (RA) treks were separated by a 10d de-acclimatisation period at 1300m. Participants completed questionnaires daily and physiological function was monitored extensively throughout each trek. There were reductions in trekking time (12%, $p<0.01$), trekking intensity (20%, $p=0.04$), AMS scores (51%, $p=0.01$) and acetazolamide consumption (79%, $p=0.05$) in RA compared to IA. The haematocrit response was repeatable between treks but [haemoglobin] increased more in RA than IA (15 vs 9% above baseline, $p=0.01$). There were no differences in minute ventilation, heart rate, blood pressure, oxyhaemoglobin saturation or blood [lactate] at rest, during

exercise, or during recovery from exercise between IA and RA. We show no evidence for improved cardiorespiratory acclimatisation in RA compared to IA, however participants were able to achieve the same degree of acclimatisation on the second trek with much less reliance on acetazolamide. The magnitude of the haematological response was greater in RA, but this may not fully explain why trekkers felt better and found the trekking easier the second time. Psychological factors and fitness gains might also contribute to these functional improvements. Although these findings support previous anecdotal evidence that recent experience at altitude facilitates subsequent acclimatisation, we have little physiological data to explain why this is the case. However, had acetazolamide consumption remained constant between treks, improvements in physiological acclimatisation during RA may have become evident. Acknowledgements NSERC, Heart and Stroke Foundation of Canada. 2009.

DETERMINATION OF THE COMET TAIL ARTIFACT BY CHEST SONOGRAPHY IS USEFUL IN THE DIAGNOSIS OF HIGH ALTITUDE PULMONARY EDEMA. Christoph Dehnert, Thomas Boehm, Michael Streit, Stephanie Kiencke, Stefanie Zegel, Peter Bartsch, Marco Maggiorini. Medical Clinic, University Hospital Heidelberg, Kantonspital Chur, University Hospital Zurich, University Hospital Basel, Medical Clinic. *Email: christoph.dehnert@med.uni-heidelberg.de.* If x-ray is available chest x-ray is the gold standard in the diagnostic of high altitude pulmonary edema (HAPE). Recently an ultrasound phenomenon called "comet tail artifact" has been suggested for diagnosis and monitoring of HAPE. Comet tails are caused by multiple reflections of the ultrasound beam in chest sonography if pulmonary edema is present. PURPOSE: To evaluate the comet tail artifact for diagnosis of HAPE after rapid ascent to 4559m in healthy individuals and compare it to chest x-ray. METHODS: 28 healthy individuals ascended to 4559m within 24h and stayed there for 3 to 5 days. Chest sonography was performed daily in upright sitting position. Each lung was scanned in 4 vertical lines from the front and the back. The number of comet tails in each intercostal space was determined simultaneously by three examiners. The total number of all comet tails is the comet tail-score (CTS) at each examination. Chest x-rays were taken on days 2 to 4 or if HAPE was suspected based on clinical findings. RESULTS: In 4 subjects clinically or radiographically assured HAPE developed. Due to technical reasons chest-x-ray could not be performed in two of them but HAPE was unequivocally diagnosed on clinical basis. These 4 subjects had CTS from 23 to 43, mean 31 ± 9. Maximum CTS were reached when HAPE occurred. Subjects without HAPE had CTS from 1 to 17, mean 5 ± 4, but only two had scores above 10. One of these two (CTS 17) was suspected to have HAPE from clinical basis, but the x-ray showed no clear signs of edema. CONCLUSION: Determining CTS by chest sonography seems to be a useful tool for the diagnosis of HAPE. Although the threshold values for CTS need to be established in a larger sample, these data suggest that HAPE is unlikely if CTS does not exceed 10 and it is very likely if CTS is above 20. 2009.

DEXAMETHASONE BLOCKS THE SYSTEMIC INFLAMMATION OF ALVEOLAR HYPOXIA AT SEVERAL SITES IN THE INFLAMMATORY CASCADE. Norberto Gonzalez[1], Jie Chao[1], Paula Donham[1], Ichiro Kuwahira[2]. [1]University of Kansas Medical Center, Kansas City, KS USA, [2]Tokai University Tokyo Hospital Tokyo, Japan. *Email: ngonzale@kumc.edu*. Introduction: Alveolar hypoxia induces a rapid and widespread systemic inflammation in rats and mice. The inflammation is not initiated by the low systemic PO2 but by the release of Monocyte Chemoattractant Protein-1 (MCP-1) from alveolar macrophages (AMO) activated by hypoxia. Circulating MCP-1 activates perivascular mast cells (MC), which release renin to generate Angiotensin II (Ang II), one of the mediators of the inflammation. Dexamethasone (Dexa) is an anti-inflammatory glucocorticoid effective in altitude illnesses. We have shown that Dexa blocks the systemic inflammation of acute alveolar hypoxia. Methods: These experiments were directed to determine the effects of Dexa (0.1 mg/kg, 24 h before experiment) at three sites in the cascade: AMO, MC, and the leukocyte/endothelial interface. Intravital microscopy of the cerebral and mesentery microcirculation of rats was combined with primary cultures of AMO and MC. Results: AMO: Dexa blocked the respiratory burst and the release of MCP-1 from AMO cultures that follows the reduction of medium O2 from 15 to 5%. MC: Dexa produced MC depletion. This results from Dexa-induced inactivation of Stem Cell Factor, a cytokine needed for MC development. Dexa also prevented the MC degranulation normally induced by MCP-1. Leukocyte/endothelial interface: Dexa prevented the leukocyte recruitment and increased vascular permeability in mesentery and brain induced by Ang II, a mediator originated downstream of MC during alveolar hypoxia. Conclusion: Dexa prevents the systemic inflammation of alveolar hypoxia by acting at three key sites in the cascade: AMO, MC, and the leukocyte/endothelial interface. Some of these effects may contribute to its beneficial effects in altitude illnesses. Acknowledgements: Supported by NIH HL 39443 and AHA 0815652G. 2011.

DEXAMETHASONE DECREASES PULMONARY ARTERY PRESSURE AND IMPROVES GAS EXCHANGE WHILE USED FOR THE TREATMENT OF AMS INDEPENDENTLY OF HAPE SUSCEPTIBILITY. Marco Maggiorini[1], Beat Kaufmann[2], Thomas Böhm[3], Stephanie Kiencke[4], Bart De Boek[5], Katija Auinger[1], Michèle Schöb[1], Christoph Siebenmann[1], Stefanie Zügel[6], Yvonne Nussbaumer[7], Konrad Bloch[7], Thomas Lutz[8], Christoph Dehnert[9]. [1]Medical ICU and ZIHP University Hospital Zurich, [2]Cardiology, University Hospital Basel, [3]Radiology, Kantonspital Chur, [4]Cardiology, Spital Bruderholz, Basel, [5]Cardiology, University Hospital Bern, [6]Cardiology, University of Heidelberg, [7]Pneumology and ZIHP University Hospital Zurich, [8]Institute of Veterinary Physiology AND ZIHP, Vetsuisse Faculty University of Zurich, [9]Sports Medicine, University of Heidelberg. *Email: klinmax@usz.uzh.ch*. Introduction: Dexamethasone has been show to be effective for the prevention of high altitude pulmonary edema (HAPE) in susceptible persons. However, it is unknown whether it prevents HAPE when taken for the treatment of acute mountain sickness (AMS). Therefore we investigated the effects

of dexamethasone when taken after the first night at 4559m in HAPE susceptible and resistant mountaineers. Methods: Seventeen HAPE resistant (HAPEr) and 8 HAPE susceptible (HAPEs) mountaineers were investigated after the first night at 4559m. All HAPEs and 6 HAPEr were treated with dexamethasone before the second night and treatment was continued until descent 63 hours later. The AMS score (Lake Louise) was assessed before and daily after the start of treatment. Systolic pulmonary artery pressure (SPpa) and hemoglobin O_2 saturation (HbO_2) were measured before and 40 hours after treatment. A chest radiography was taken before and 60 hours after treatment, unless signs of HAPE were observed before. Results: None of the HAPEr persons developed HAPE, whereas one of the HAPEs did so 16 hours after treatment start. The Lake Louise (LL) AMS score decreased after treatment by 1 point in HAPEr persons without dexamethasone (controls) ($p = 0.64$), by 3 points in HAPEr receiving dexamethasone (HAPE-r-dex) ($p = 0.03$) and by 2.5 points in the HAPEs group ($p = 0.04$). SPpa was before treatment 45 ± 7 mmHg in controls, 44 ± 7 mmHg in HAPEr-dex and 56 ± 12 mmHg in HAPEs ($p = 0.03$). Treatment decreased SPpa by 4 mmHg in HAPEr-dex ($p = 0.03$) and 8 mmHg in HAPEs ($p = 0.01$), whereas in controls SPpa did not change. HbO2 increased by 3% in controls, by 8% and 7% in HAPEr-dex and HAPEs, respectively ($p = 0.02$). Conclusion: The results of our study suggest that independent of mountaineers HAPE susceptibility dexamethasone taken for AMS treatment decreases SPpa and improves gas exchange. In HAPE susceptible persons dexamethasone is likely to decrease HAPE incidence, which confirms our previous results. Acknowledgements: Center of Integrative Physiology (ZIHP) University of Zurich, Harmann-Müller Stiftung, Fondazione Crivelli for funding. 2011.

DEXAMETHASONE DECREASES PULMONARY ARTERY PRESSURE AND IMPROVES GAS EXCHANGE WHILE USED FOR THE TREATMENT OF AMS INDEPENDENTLY OF HAPE SUSCEPTIBILITY. Marco Maggiorini[1], Beat Kaufmann[2], Thomas Böhm[3], Stephanie Kiencke[4], Bart De Boek[5], Katija Auinger[1], Michèle Schöb[1], Christoph Siebenmann[1], Stefanie Zügel[6], Yvonne Nussbaumer[7], Konrad Bloch[7], Thomas Lutz[8], Christoph Dehnert[9]. [1]Medical ICU and ZIHP University Hospital Zurich, [2]Cardiology, University Hospital Basel, [3]Radiology, Kantonspital Chur, [4]Cardiology, Spital Bruderholz, Basel, [5]Cardiology, University Hospital Bern, [6]Cardiology, University of Heidelberg, [7]Pneumology and ZIHP University Hospital Zurich, [8]Institute of Veterinary Physiology AND ZIHP, Vetsuisse Faculty University of Zurich, [9]Sports Medicine, University of Heidelberg. *Email: klinmax@usz.uzh.ch*. Introduction: Dexamethasone has been show to be effective for the prevention of high altitude pulmonary edema (HAPE) in susceptible persons. However, it is unknown whether it prevents HAPE when taken for the treatment of acute mountain sickness (AMS). Therefore we investigated the effects of dexamethasone when taken after the first night at 4559m in HAPE susceptible and resistant mountaineers. Methods: Seventeen HAPE resistant (HAPEr) and 8 HAPE susceptible (HAPEs) mountaineers were investigated after the first night at 4559m. All HAPEs and 6 HAPEr were treated with dexamethasone before the second night and treatment was continued until descent 63 hours later. The AMS score

(Lake Louise) was assessed before and daily after the start of treatment. Systolic pulmonary artery pressure (SPpa) and hemoglobin O_2 saturation (HbO_2) were measured before and 40 hours after treatment. A chest radiography was taken before and 60 hours after treatment, unless signs of HAPE were observed before. Results: None of the HAPEr persons developed HAPE, whereas one of the HAPEs did so 16 hours after treatment start. The Lake Louise (LL) AMS score decreased after treatment by 1 point in HAPEr persons without dexamethasone (controls) ($p = 0.64$), by 3 points in HAPEr receiving dexamethasone (HAPE-r-dex) ($p = 0.03$) and by 2.5 points in the HAPEs group ($p = 0.04$). SPpa was before treatment 45 ± 7 mmHg in controls, 44 ± 7 mmHg in HAPEr-dex and 56 ± 12 mmHg in HAPEs ($p = 0.03$). Treatment decreased SPpa by 4 mmHg in HAPEr-dex ($p = 0.03$) and 8 mmHg in HAPEs ($p = 0.01$), whereas in controls SPpa did not change. HbO2 increased by 3% in controls, by 8% and 7% in HAPEr-dex and HAPEs, respectively ($p = 0.02$). Conclusion: The results of our study suggest that independently of mountaineers HAPE susceptibility dexamethasone taken for AMS treatment decreases SPpa and improves gas exchange. In HAPE susceptible persons dexamethasone is likely to decrease HAPE incidence, which confirms our previous results. Acknowledgements: Center of Integrative Physiology (ZIHP) University of Zurich, Harmann-Müller Stiftung, Fondazione Crivelli for funding. 2011.

DEXAMETHASONE DECREASES SYSTEMIC INFLAMMATORY AND STRESS RESPONSE AND FAVORS VASODILATION IN HIGH ALTITUDE PULMONARY EDEMA SUSCEPTIBLES AT 4559M. Marco Maggiorini, Michael Streit, Christoph Siebenmann, Stefanie Zegel, Heimo Mairbaeurl, Christoph Dehnert, Thomas Bohm, Konrad E Bloch, Yvonne Nussbaumer-Ochsner. University Hospital Zurich, University of Heidelberg, Kantonsspital. *Email: klinmax@usz.uzh.ch.* Objective: To investigate the mechanisms of dexamthasone (DEX) in preventing high altitude pulmonary edema (HAPE) we measured makers of systemic inflammation and stress, endothelin-1 (ET1) and markers of hormones with hemodynamic properties prior and after ascent and stay at 4559m. Methods: The following markers of inflammation, c-reactive protein (CRP) and monocytes chemoattractant protein-1 (MCP-1), of stress, cortisol (C), prolactin (PR) and carboxy-terminal provasopressin (copeptin), of vasoactive hormones N-terminal pro-B-type natriuretic peptide (NTproBNP), midregional proatrial natriuretic peptide (MR-proANP), midregional pro-adrenomedullin (MR-proADM) and ET1 were measured in 16 HAPE susceptible mountaineers. 10 of them were randomized to receive DEX (2x8mg) starting 24 hours before ascent to 4559m and for 5 days at high altitude (HA), early prophylaxis (EP), and 6 to receive placebo until the HA day 2 and thereafter, starting before the 2nd night, DEX (2x8mg), late prophylaxis (LP). Blood samples were taken at low altitude (490m) and at HA day 2 and 4. HAPE was diagnosed by chest radiography. Results: 2 participants of the LP group developed HAPE, one of them in the 2nd and the other in 4th night at HA. In subjects on placebo ascent to HA increased CRP, PR, copeptin, NTproBNP and ET1 ($p<0.05$). DEX EP suppressed the increase of CRP, PR and copeptin ($p<0.05$) but not of NTproBNP and ET1. DEX EP decreased C and MCP-1 ($p<0.05$) and increased

MR-proADM and MR-proANP ($p<0.05$). At HA day 2 MCP-1 was higher and MR-proANP lower in the LP group ($p<0.05$). DEX LP caused an increase in MR-proANP ($p<0.05$) a decrease of C, MCP-1 and copeptin ($p<0.05$), but did not change CRP, PR, NTproBNP and MR-proADM. Conclusion: Our results suggest that in HAPE-s DEX decrease HA associated systemic inflammatory and stress response, and improves markers of hormones with vasodilatory and natriuretic effects. This may explain the favorable effect of early DEX prophylaxis in HAPE-s person. 2009.

DEXAMETHASONE PROPHYLAXIS IMPROVES MAXIMAL OXYGEN UPTAKE OF HIGH ALTITUDE PULMONARY EDEMA SUSCEPTIBLE MOUNTAINEERS AFTER RAPID ASCENT TO 4559M. Christoph C Siebenmann, Konrad E Bloch, Yvonne Nussbaumer-Ochsner, Nicole Sch¸pfer, Marco Maggiorini. Institute of Human Movement Sciences and Sport ETH Zurich, University Hospital Zurich. *Email: csiebenm@ethz.ch.* Background: A prophylaxis with dexamethasone (dex) prevents high altitude pulmonary edema (HAPE). However, the influence on maximal oxygen uptake (VO_2max) is unknown. We investigated if a prophylaxis with dex would decrease the high altitude related decline in VO_2max in HAPE-susceptible mountaineers at 4559m. Methods: 16 HAPE-susceptible subjects performed VO_2max tests at 490m and after the first and the third night at 4559m. They were randomized to receive dex 2x8mg/d as an early (Dex-E) or late (Dex-L) prophylaxis. The Dex-E group started drug intake 24 hours before the ascent and continued until the descent. The Dex-L group started with placebo, but switched to dex before the second night at 4559m. Results: Ascent to 4559m decreased maximal work capacity by 30% ($p<0.001$) and maximal heart rate by 9% ($p<0.001$), but did not change maximal ventilatory capacity in both groups ($p > 0.1$ Dex-E vs. Dex-L). The second day at 4559m, compared to 490m, VO_2max declined by 40% in the Dex-L and 29% in the Dex-F group ($p=0.037$). The fourth day, the decline was 38% in the Dex-L and 33% in the Dex-E group ($p=0.284$). Arterial oxygen saturation (SpO_2max) during maximal exercise on the second day was 62% in the Dex-L and 79% in the Dex-E group ($p=0.002$). The fourth day, it was 78% in both groups. Conclusion: Our results indicate that early, but not late prophylaxis with dex attenuates the high altitude associated drop in VO_2max and SpO_2max in HAPE-susceptible mountaineers after rapid ascent to 4559m. 2009.

DEXAMETHASONE TAKEN AFTER ACUTE EXPOSURE TO 4559 DOES NOT PREVENT HIGH ALTITUDE PULMONARY EDEMA. Marco Maggiorini, Michael Streit, Christoph Siebenmann, Nicole Schopfer, Christoph Dehnert, Thomas Bohm, Konrad E Bloch, Yvonne Nussbaumer-Ochsner. University Hospital Zurich, University Heidelberg, Kantonsspital. *Email: klinmax@usz.uzh.ch.* Objective: To investigate whether dexamethasone (DEX) taken after exposure to high altitude prevents high altitude pulmonary edema (HAPE) during stay at 4559m for 4 consecutive nights. Methods: 20 HAPE susceptible mountaineers were in a double-blind study randomized to receive either placebo ($n = 9$) or DEX (2x8mg) (n

= 11) 24 hours prior to ascent to high altitude (HA). At 4559m before the 2nd night persons on placebo were switched to DEX (2x8mg), late prophylaxis group (LP), whereas others continued on DEX, early prophylaxis group (EP). The primary endpoint of the study was HAPE during ascent and stay at 4559m. Acute mountain sickness (AMS), vital signs, arterial blood gas and blood glucose were measured at HA day 2 and 4. Results: In the EP group all but one reached 4559m and none developed HAPE, whereas only 6 of the 9 in the LP reached 4559m, 2 of them developed HAPE on HA day 2 and 4 ($p = 0.04$ LP vs. EP). Dropouts during ascent were due to AMS in both groups ($p = 0.20$). During stay at 4559m, AMS-c (cerebral) subscore was ≥ 0.7 in 2 of 6 in the LP and in 1 of 10 in the EP group ($p = 0.24$). At HA day 2, compared to EP, participants in the LP group had (mean ± SD) higher AMC-c score (0.48±0.4 vs. 0.18 ± 16; $p = 0.04$) and heart rate (85±11 vs. 67±16 bpm; $p = 0.03$), and a lower PaO_2 (41 6 vs. 48±5 mmHg; $p = 0.04$). In the LP group the switch to DEX led, compared to HA day 2, to a decrease in AMS-c (-0.44, $p = 0.06$) and heart rate (-14, $p = 0.03$), and to an increase in PaO_2 (9 mmHg, $p = 0.03$), the difference between the LP and EP group at HA day 4 being not significant. Blood glucose, blood pressure and body temperature were not different between EP and LP at any time point. Conclusion: Our results confirm that in HAPE susceptible persons DEX taken 24 hours prior ascent prevents HAPE, however, taken 24 hours after arrival to 4559m it did not, this despite a decrease in AMS score and an improvement of arterial oxygenation. 2009.

DEXAMETHASONE TREATMENT FOR AMS IMPROVES HEART-LUNG INTERACTIONS DURING MODERATE EXERCISE IRRESPECTIVELY OF HIGH ALTITUDE PULMONARY EDEMA SUSCEPTIBILITY. Katja Auinger[1], Christoph Siebenmann[1], Beat Kaufmann[2], Stephanie Kiencke[3], Bart De Boek[4], Christoph Dehnert[5], Michèle Schöb[1], Yvonne Nussbaumer[6], Oliver Goetze[7], Thomas Lutz[8], Marco Maggiorini[1]. [1]Medical ICU and ZIHP University Hospital Zurich, [2]Cardiology, University Basel, [3]Cardiology, Spital Bruderholz, Basel, [4]Cardiology, University Hospital Bern, [5]Sports Medicine University Heidelberg, [6]Pneumology, University Hospital Zurich, [7]Gastroenterology University Hospital Zurich, [8]Institute of Veterinary Physiology and ZIHP, Univeristy of Zurich. *Email: klinmax@usz.uzh.ch*. Introduction: Dexamethasone prophylaxis has been shown to improve exercise performance in high altitude pulmonary edema susceptible (HAPEs) persons. Whether dexamethasone treatment for acute mountain sickness (AMS) affects exercise performance at high altitude has not been determined. We investigated the effect of dexamethasone on heart-lung interactions during moderate exercise in HAPEs and non-HAPE susceptible (nHAPEs) during stay at 4559m. Methods: Pulmonary hemodynamics and arterial hemoglobin oxygen saturation (HbO_2) were measured at rest and at 30% of VO_2max in 17 nHAPEs and 8 HAPEs mountaineers after ascent to 4559m within 24 hours and during stay for 4 consecutive nights. Six nHAPEs (nHAPEs-dex) and all HAPEs (HAPEs-dex) received dexamethasone 8mg bid. Measurements were performed before and 40 hours after dexamethasone treatment start. We measured cardiac index (CI) using the Lithium indicator technique and assessed pulmonary artery pressure by measuring the

tricuspid valve regurgitation jet velocity (dpTI). Oxygen delivery (DO_2) was then calculated using standard formula. Results: Before dexamethasone dpTI at rest was 35 mmHg, 37 mmHg and 47 mmHg (p=0.03) and HbO2 76%, 76% and 73% (ns) in nHAPEs (control), nHAPEs-dex and HAPEs-dex, respectively. Dexamethasone treatment decreased resting dpTi by 8 mmHg in nHAPEs-dex and by 10 mmHg in HAPEs, whereas dpTI did not change in controls ($p < 0.01$). Similar behavior of dpTI was observed at 30% of VO_2max. HbO_2 was approximately 5% higher in dexamethasone treated persons at rest and during exercise ($p < 0.01$). During exercise DO_2 increased by 107 ml/min/m2 in nHAPEs-dex and by 105 ml/min/m2 in HAPEs, whereas it remain unchanged in controls ($p < 0.001$), the difference between groups being significant ($p < 0.05$). Conclusion: Treatment of AMS with dexamethasone improves pulmonary hemodynamics and arterial oxygenation resulting in a higher oxygen delivery to the tissues during moderate exercise at 4559m in both nHAPEs and HAPEs persons. Acknowledgements: Center of Integrative Physiology (ZIHP) University of Zurich, Harmann-Müller Stiftung, Fondazione Crivelli for funding. 2011.

DIETARY FACTORS ASSOCIATED WITH ACUTE MOUNTAIN SICKNESS. Stephan Sanders, Jamie H MacDonald, Samuel J Oliver, Medical Expeditions. Yale University and Medical Expeditions, Bangor University. *Email: stephansanders@hotmail.com.* In hypoxia, high carbohydrate and fluid intake have been hypothesised to improve oxygen saturation and reduce acute mountain sickness (AMS). Paradoxically, dietary intervention studies have failed to reduce AMS and fluid retention may worsen symptomology. Therefore the objective of this study was to investigate diet and AMS. Forty two participants completed a 21 day trek in the Himalaya. Energy and fluid intake was manipulated by randomising participants to a carbohydrate energy drink supplement (CHO) or placebo (PLB). AMS was recorded by Lake Louise questionnaire. Dietary intake and urine production recorded in daily diaries were used to calculate macronutrient intake and fluid balance. Incidence of AMS was analysed by chi-square ($\chi\leq$). Other variables were analysed by two way analysis of variance (F) and bivariate correlation (r). Statistical significance was accepted at $p < 0.05$. Despite increasing carbohydrate and fluid intake, allocation to CHO had no influence on AMS incidence ($\chi\leq = 2.5$, $p = 0.11$). Furthermore, median splitting of participants into high vs. low total dietary carbohydrate intake or high vs. low total dietary fluid intake had no influence on AMS incidence. Comparing those with AMS to those without revealed carbohydrate intake was comparable ($F = 1.8$, $p = 0.19$), as was fluid intake ($F = 0.0$, $p = 0.88$). Neither carbohydrate ($r = -0.02$, $p = 0.96$) nor fluid intake was correlated with AMS severity ($r = -0.24$, $p = 0.54$). However, those with AMS had greater fluid retention (35 ± 4 vs. 22 ± 2 ml/kg/day: $F = 7.56$, $p = 0.01$) due to lower urine output. Increased carbohydrate and fluid intake was achieved with a practical and palatable dietary intervention but did not reduce AMS severity. However, AMS incidence was associated with increased fluid retention. This study was supported by Science in Sport and the Ministry of Defence (Army). 2009.

DIFFERENCES IN ALVEOLAR DIFFUSIVE PROPERTIES DURING WORK AND IN HYPOXIA. Manuela Bartesaghi[1], Luca Pollastri[1], Valentina Scotti[1], Gaia Mandolesi[2], Annalisa Cogo[2], Francesca Lanfranconi[1], Giuseppe Miserocchi[1]. [1]Dept Health Sciences, Laboratory Clinical Physiology and Sport Medicine, Univ Milano-Bicocca, Italy, [2]Biomedical Sport Studies Center, Univ Ferrara, Dept Experimental Medicine, Ferrara. *EMAIL: manuela.ba@tiscali.it* INTRODUCTION: Evaluation of inter-individual differences in lung diffusive properties caused by exercise and hypoxia. METHODS: We studied 22 subjects (M 17) aged 35 ±7 years. Experiments were done in normoxia (SL, PIO_2 157 mmHg) and at altitude (HA, 3269m, PIO_2 107 mmHg). Capillary blood volume (Vc) was determined at total lung capacity by having subjects inspire gas mixtures with CO and CH4 at 20, 40 and 60% O_2 at SL and only 40 and 60% O_2 at HA. We also measured respiratory reactance at 5 Hz (X5) with forced oscillations technique (FOT). Subjects were studied in a "resting" condition and during a steady state sub-threshold exercise on a cycle ergometer. RESULTS: Subjects were grouped as follows: G1 (N=11) had lower control values of Vc at rest both at SL and in HA (129.1 ± 57.7ml and 123.5 ± 49.7ml, respectively) and showed a significant increase of Vc during exercise both at SL and at HA (193.8 ± 65.7ml , p 0.02, and 223.3 ± 86.9 ml, p 0.05) . Conversely, G2 (N=11) had greater control values of Vc at SL and HA at rest (210.5 ± 75ml and 152.2 ± 54.8ml), and during exercise showed a decrease of Vc at SL (178.6 ± 58.5ml) and no significant change in HA (180 ± 65.2ml). X5 decreased in both groups in HA, the change being significantly greater in G2, suggesting the development of interstitial lung edema. CONCLUSION: The opposite control on Vc deserves an interpretation relating to the change perturbation in lung fluid balance. 2015.

DIFFERENCES IN CEREBRAL BLOOD FLOW RESPONSES TO CO_2 AND O_2 IN HIMALAYAN AND ANDEAN HIGLANDERS AS INDICATORS OF HUMAN ENVIRONMENTAL ADAPTATION. Marat Slessarev, Alexandra Mardimae, Dahlia Balaban, David Preiss, Alex Vesely, Otto Appenzeller, Shoji Ito, Eitan Prisman, Richard Greene, James Duffin, Joseph A Fisher. University of Toronto, University of British Columbia, NMHEMCRF, New Mexico Highlands University, University of Toronto. *Email: marat.slessarev@utoronto.ca.* Modern humans emerged from central Africa to colonize the highlands of Asia and the Americas about 40,000 and 15,000 years ago respectively. The greater duration of hypoxic stress may have resulted in an adaptation in the Himalayans that may account for their lower incidence of chronic mountain sickness. We compared cerebral blood flow (CBF) responses to CO_2 and O_2 in Himalayans and in Andeans as indicators of the extent of adaptation to life at high altitude. We compared middle cerebral artery blood velocity (MCAV) (as an index of CBF) in response to a Duffin(1) hypoxic (50 mmHg) and hyperoxic (150 mmHg) rebreathing tests in 14 male residents (age: 41.8 ± 2.3 years) of Ladakh India (altitude 4550m) and 8 residents (6 male, age: 38.1 ± 8.6 years) of La Paz, Bolivia (altitude 3850 m). The slope of MCAV-CO_2 relationship in Himalayans was greater than in Andeans under both hyperoxic (3.67±1.27 vs 1.85±0.91, p=0.010) and hypoxic (4.36±2.16 vs 2.93±0.75,

p=0.038) conditions. PO_2 had no effect on the slope of the MCAV-CO_2 relationship in either population (p=0.232 H, p=0.160 A). The hypoxic MCAV-CO_2 relationship was shifted to the left of the hyperoxic relationship only in Andeans ($PETCO_2$ at baseline CBF: 39.9±17.3 mmHg during hyperoxia vs 30.2±1.4 mmHg during hypoxia, p=0.021). We conclude that CBF in Himalayans is more sensitive to CO_2 than in Andeans irrespective of O_2 tensions. Hypoxia has no effect on CBF-CO_2 relationship in Himalayans, while in Andeans hypoxia results in a leftward shift of MCAV-CO_2 relationship. Higher sensitivity of CBF to CO_2 in Himalayans, and their lack of CBF response to hypoxia may be adaptive processes to life at high altitude. (1) Duffin. JAP 1998;406:15. 2009.

DIFFERENCES IN OXYGEN DESATURATION, GLUCOSE CONCENTRATIONS AND METABOLISM, DURING EXERCISE PERFORMED IN HYPOXIA AFTER A HIGH-CARBOHYDRATE OR A HIGH-PROTEIN BREAKFAST. Aurélien Pichon[1], Keyne Charlot[1], Jean-Paul Richalet[1], Didier Chapelot[1]. [1]Université Paris 13, laboratoire des 'Réponses cellulaires et fonctionnelles à l'hypoxie', Bobigny, France. *Email: aurelien.pichon@univ-paris13.fr*. Introduction: At rest, hypoxia-induced oxygen desaturation has been shown to be reduced by prior ingestion of a carbohydrate (CHO) solution. It is not known whether this effect would occur during exercise or after a realistic meal, and if differences in metabolism, glucose profiles, sympatho-vagal balance are associated. Moreover, there are arguments to hypothesize that this difference would be enhanced if compared to a high-protein (PRO) meal. Methods: Subjects were 11 young male subjects who consumed either a high-CHO (70% CHO, 12% protein) or a high-protein (35% CHO, 48% protein) breakfast matched in energy (2340 kJ) and in weight (375 g). Thirty min later, they were exposed consecutively to: 15 min of normoxia (NX), 15 min of hypoxia (HX; FIO_2 = 13.5%) and two 15 min exercise periods (60% VO_2max) in hypoxia (HXEX1 and HXEX2). Interstitial glucose concentrations, respiratory exchange ratio (RER), oxygen saturation (SaO2), and heart rate variability (HRV) were investigated continuously during the whole exposure. Lactate was measured at the end of HXEX1 and 5 min after HXEX2. Results: The magnitude of decrease in SaO_2 was smaller in high-CHO than in high-PRO only in HXEX1 (75.9 ± 1.8 vs 78.4 ± 1.6 %, P = 0.01) whereas glucose concentrations were higher in NX and HX but not during exercise periods due to a greater decrease in high-CHO than in high-PRO (-1.62 ± 0.72 vs -0.51 ± 0.38 mmol.L-1, P < 0.01). RER and CHO oxidation were higher whereas fat oxidation was lower in high-CHO than in high-PRO over all periods. Lactate concentrations were higher in high-CHO than in high-PRO at the end of HXEX1 but not 5 min after HXEX2. Energy expenditure and HRV indices were not different between conditions. Conclusion: A high-CHO breakfast induces a lower reduction in SaO_2 and a sustained lower fat oxidation in the response to exercise in hypoxia than a high-protein breakfast. This may have some consequences in the nutritional recommendations of individuals who have to perform short exercises at high-altitude. 2011.

DIFFERENCES IN OXYGEN SENSITIVITY BETWEEN SPRAGUE-DAWLEY RAT STRAINS, A COMPARATIVE STUDY. Louise Ostergaard[1], Martha Tissot van Patot[2], Alain Rudiger[3], Max Gassmann[1], Marco Maggiorini[3]. [1]Institute of Veterinary Physiology, Vetsuisse Faculty, and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland, [2]Department of Anesthesiology, University of Colorado Denver Health Sciences Center, Denver, Colorado; USA, [3]Medical Intensive Care Unit of the Department of Internal Medicine, University Hospital Zurich, Zurich, Switzerland. *Email: ostergaard@vetphys.uzh.ch*. Introduction: In humans there is a large degree of individual variability in "oxygen sensitivity". In acute hypoxia, a condition that is highly dependable on the individual susceptibility is high altitude pulmonary edema (HAPE). HAPE can develop after rapid ascent to an altitude above 2500 meters and is characterized by high permeability pulmonary edema with alveolar accumulation of a protein rich edema fluid. This study is designed to characterize different rat strains as animal models of HAPE and furthermore, to elucidate strain-specific differences in the response to changes in oxygen tension by verifying the phenotype of the so-called Madison and Hilltop strains. Methods: Three different Sprague-Dawley (SD) rat strains were used: A widely distributed SD strain from Harlan, Germany, Hilltop SD from Hilltop, Pennsylvania, USA and Madison SD from Harlan, Indiana, USA. The rats were exposed to oxygen tensions of 10% for either acute time periods (up to 48 hours) or chronic (21 days). Endpoint measurements include, but are not limited to measurements of pulmonary leak, histology, blood gas analysis, BALF constituents and hemodynamics. Results: We found that the Madison SD present with a pulmonary capillary leak after 24 hr exposure to 10% oxygen, whereas this was not the case in the SD rats from Germany and the Hilltop SD. There were no changes in the composition of the blood gases, but differences in hemoglobin content and O_2 transport capacity was found. Furthermore, preliminary data indicate that HIF-1 is activated in Hilltop, but not Madison SD rats in response to hypoxia, which also correlates with higher erythropoietin content in Hilltop SD. Conclusion: We conclude that great differences in oxygen sensitivity exist among different strains of SD rats, which is obviously important when interpreting data concerning oxygen sensitivity and adaptation. We now plan to fully characterize the two SD stains, Hilltop and Madison, in order to obtain more knowledge on the general response to hypoxia and why some individuals are more susceptible than others. 2011.

DIFFERENCES IN THE TIME COURSE OF SYMPATHETIC ACTIVATION BY HYPOXIA AND HYPERCAPNIA. Craig D Steinback, J Kevin Shoemaker. The University of Western Ontario. *Email: cdsteinb@uwo.ca.* We tested the hypothesis that isocapnic hypoxia (HX) and iso-oxic hypercapnia (HC) elicit differences in muscle sympathetic nerve activity (MSNA) in humans. Nine participants (27 ± 4 yr; mean ± SD) underwent 5 min of HX (end-tidal PO_2, $PETO_2$ = 45 Torr; end-tidal PCO_2, $PETCO_2$ = 1 Torr above resting values) and 5 min of HC ($PETCO_2$ 8 Torr

from baseline). Ventilation (VE), heart rate (HR; ECG), mean arterial pressure (MAP; photoplethysmography) and MSNA (microneurography) were measured on a breath-by-breath and beat-by-beat basis. The time courses of the VE response and sympathetic activation were determined using 1 minute moving averages with 30 second overlaps. HX caused a larger average VE response (13.6 ± 6.4 L/min) compared to HC (10.3 ± 4.8 L/min; P=0.003), though ventilation became significantly elevated from baseline within 30 sec during both HX and HC. MSNA burst amplitude was increased during both HX (0.21 ± 0.07 vs. 0.27 ± 0.11 mV, P=0.02) and HC (0.18 ± 0.5 mV vs. 0.22 ± 0.9 mV, P=0.004) while HX, but not HC, also caused an increase in sympathetic burst frequency (21 ± 9 vs. 28 ± 7 bursts/min, P = 0.03). However, when burst frequency was normalized to HR, burst probability (burst/100 heart beats) remained unchanged during both HX and HC. Time course analysis indicated MSNA burst amplitude became significantly increased from baseline by 30 sec during HX and by 90 sec during HC. These data suggest differential processing in the regulation of MSNA burst amplitude, which is dissociated from ventilatory drive, at the onset of HX versus HC. This is the first study to examine and document differences in the on-transient sympathetic responses to HX and HC. This study was approved by the local ethics board and funded by HSFC and NSERC. 2009.

DIFFERENTIAL STIFFENING OF PERIPHERAL AND CENTRAL ARTERIES DURING ACUTE HYPOXIA. Margie H Davenport[1], Craig D Steinback[1], Billie-Jean Martin[1], Todd J Anderson[1], John V Tyberg[1], Marc J Poulin[1]. [1]University of Calgary, Calgary, Canada. *Email: mdavenpo@ucalgary.ca*. Introduction: Chronic intermittent hypoxia is thought to be a major factor leading to increased arterial stiffness in obstructive sleep apnea (OSA). However, the specific effects of acute hypoxia on arterial stiffness remain poorly understood. The purpose of this study was to determine the effects of acute hypoxia on elastic and muscular artery stiffness. Methods: Continuous arterial blood pressure (ABP) waveforms (photoplethysmography (Finometer) and applanation tonometry (Millar Instruments)) and heart rate (HR; 3-lead ECG) were collected in 10 subjects (4 female, 26±6yrs, mean±SD) at baseline (end-tidal PCO_2 = +1 Torr above resting values; end-tidal PO2 = 88 Torr) and during acute isocapnic hypoxia (HX; end-tidal PCO_2 = +1 Torr above resting values; end-tidal PO_2 = 45 Torr). The ECG r-wave and diastolic foot of ABP were used to calculate segment-specific pulse-wave velocities (PWV) for central elastic (heart-femoral PWV; cePWV), peripheral elastic (heart-carotid PWV; pePWV), and peripheral muscular (heart-finger PWV; pmPWV) arterial segments. Results: Acute HX increased mean ABP (86±10 to 94±10 mmHg, P<0.05) and HR (69±17 to 81±15 bpm, P<0.001) as well as cePWV (4.0±0.5 to 4.2±0.5 m/s, P<0.01) and pmPWV (5.8±0.9 to 6.2±0.9 m/s, P<0.01), but not pePWV. The increase in pmPWV was also positively correlated with the change in mean ABP (r2 = 0.871, P<0.001). These data suggest 1) an increased central elastic arterial stiffness, 2) differential stiffening between peripheral elastic and muscular arteries, and 3) a strong association between peripheral muscular artery stiffness and mean ABP during HX. Conclusion: These data may have important implications for understanding the

mechanisms involved in the development of cardiovascular diseases such as atherosclerosis and hypertension in patients with OSA. Acknowledgements: This study was supported by the Canadian Institutes for Health Research and the Heart & Stroke Foundation of Canada. 2011.

DNMT3A REGULATES HIF-2ALPHA GENE ACTIVATION. Gabriel Lachance[1], Chet E. Holterman[1], Aleksandra Franovic[1], Stephen Lee[1]. [1]University of Ottawa, Ontario, Canada. *Email: ga_lac@hotmail.com*. Introduction: Attainment of growth autonomy is the first and debatably most important hallmark of human cancer. Hypoxia-Inducible Factor-2alpha(HIF-2alpha) plays a central role in autonomous proliferation of genetically diverse cancers by activating the epidermal growth factor receptor proliferation axis. HIF-2alpha gene is usually repressed in epithelial cells but activated during the initial step of transformation Methods: HIF-2alpha gene expression in Human Renal Cell Carcinoma (RCC) biopsy samples was compared to normal adjacent tissue by RT-PCR. Human renal primary epithelial cells were then used as a model to study mechanism responsible for HIF-2alpha gene repression using Methylation Sensitive Restriction digest PCR (MSRPCR)and bisulfite sequencing. shRNA-based gene silencing and exogenous stable expression were used to confirm functional relevance of DNMT3a-dependant HIF-2alpha gene methylation in RCC. Results: Here, we demonstrate that HIF-2alpha gene is epigenetically regulated by DNA methyl transferase 3a (DNMT3a). HIF-2alpha gene is methylated and silenced by DNMT3a in normal tissues and primary culture cells. In contrast, cancer cells exhibit loss of DNMT3a function and HIF-2alpha derepression. Exogenous expression of DNMT3a in DNMT3a-deficient cancer cells methylates and inactivates HIF-2alpha gene abolishing their ability to engage in autonomous proliferation. Conclusion: These results provide new insights into mechanisms of epigenetic reprogramming in cancer and highlight the role of DNMT3a in the earliest step of oncogenesis. Acknowledgements: This work was supported by the National Cancer Institute of Canada. S.L. is a recipient of the National Cancer Institute of Canada Harold E. Johns Award. 2011.

DOES IMPROVING VO_2MAX BY 15% IN A FEMALE RUNNER EXACERBATE EXERCISE-INDUCED ARTERIAL HYPOXEMIA? Dominelli Paolo[1], Foster Glen[1], Dominelli Giulio[2], Henderson William[3], Koehle Michael[4], McKenzie Donald[4], Sheel William[1]. [1]School of Kinesiology, Univ British Columbia, Vancouver, Canada, [2]Dept Medicine, Univ British Columbia, Vancouver, Canada, [3]Program of Critical Care Medicine, Univ British Columbia, Vancouver, Canada, [4]Allan McGavin Sport Medicine Centre, Dept Family Practice, Univ British Columbia, Canada. *EMAIL: p.dominelli@alumni.ubc.ca* INTRODUCTION: Exercise-induced arterial hypoxemia (EIAH) is a phenomenon whereby arterial oxygen tension (PaO_2) and oxyhemoglobin saturation (SaO_2) fall below resting values during exercise. There is an inverse relationship between maximal oxygen consumption (VO_{2max}) and end-exercise PaO_2. However, this observation hasn't been studied after rigorous training using arterial blood. We assessed pulmonary gas exchange during treadmill running in a female athlete before and after extensive training. We hypoth-

esized that EIAH would worsen after the subject considerably improved their VO_{2max}. METHODS: A healthy 26-year-old female middle distance runner was tested twice in a 5-month span. Testing involved resting pulmonary function followed by a maximal treadmill exercise test. Before exercise, the subject was instrumented with an arterial catheter, esophageal balloon catheter and an esophageal thermistor, with the latter used to correct arterial blood gases. RESULTS: Resting pulmonary function was unchanged with training, but weight decreased by 1 kg for the 2nd test. Lung mechanics were similar throughout the exercise tests and no mechanical ventilatory constraints were noted. Maximal oxygen consumption improved in both absolute (3.4 vs. 4.0 l•min^{-1}) and relative terms (59 vs. 70 ml•kg-1•min^{-1}). However, at maximal exercise there was little difference in the alveolar-to-arterial oxygen gradient (A-aDO_2) (28 vs. 29 mmHg) and PaO_2 (85 vs. 89 mmHg). Furthermore, the subject's pattern of change in PaO_2 and A-aDO_2 throughout exercise was similar. End-exercise SaO_2 was slightly lower during the 2nd test (93.8 vs. 92.8%), but this was due to a lower pH (7.24 vs. 7.17). CONCLUSION: Contrary to our hypothesis, EIAH did not worsen in our subject despite a substantial increase in VO_{2max}. Findings from this case indicate that EIAH may be inherent and not due to training-induced increases in VO_{2max}. ACKNOWLEDGEMENTS: Natural Science and Engineering Research Council of Canada. 2015.

EARLY LIFE HYPOXIA IS ASSOCIATED WITH INCREASED SYSTEMIC ARTERIAL PRESSURE THAT PERSISTS TO LATE MATURITY. Matthew McIntosh[1], Bryan Ross[1], Charles V. Rohlicek[1]. [1]McGill Univ. *EMAIL: charles.rohlicek@mcgill.ca* INTRODUCTION: We have previously shown that hypoxia in early life is associated with an increase in systolic blood pressure in 2 month old male rats (Ross 2010). The aim of this study was to investigate whether the effects of chronic hypoxia for the first ten days of life on systemic arterial blood pressure in awake unrestrained adult rats persists into later adulthood and late maturity. METHODS: Experiments were conducted on adult male Sprague Dawley rats aged 2, 3 and 6 months. An experimental group (n=29) was raised in hypoxia (FiO_2 = 0.12) for the first ten days of life and subsequently raised in normoxia (Neonatal Hypoxia). A second group (n=32) was reared in normoxia without any prior exposure to hypoxia (Control). At 2, 3 and 6 months rats from each group were instrumented with an intravascular telemetric blood pressure transmitter monitoring abdominal aortic pressure (DSI). After 1 week of recovery, arterial pressure was recorded continuously for 48 hours in these unrestricted, awake animals. The average systolic and diastolic pressures were determined over two 12 hour night time (active) periods. Two-tailed, unpaired t-tests were performed ($P<0.05$). All data are presented as mean ± SEM. RESULTS: Systolic arterial pressures were significantly higher ($P<0.05$) in neonatally hypoxic rats at all ages (Control vs Neonatally Hypoxic: systolic pressure 2, 3 & 6 months: 124±3 vs. 131±2, 134±2 vs. 140±2, 137±2 vs.146±2 mmHg). There was no difference in diastolic pressure at any age. The relative difference between the two groups did not vary with age. Two month old neonatally hypoxic males weighed significantly less than controls (365±9 vs. 418±9 g, $P<0.05$). However, there was

no difference in weight between the two groups at 3 and 6 months of age. CONCLUSION: Our results indicate that neonatal exposure to hypoxia in the rat is associated with increases in systolic arterial pressure that persist throughout adulthood to late maturity. This raises the question of long-term cardiovascular risk factors incurred by hypoxemia in early life. ACKNOWLEDGEMENTS: Heart and Stroke Foundation of Quebec & McGill Univ Health Centre Research Institute. 2015.

EARLY OXYGEN DESATURATION IS RELATED TO AMS DEVELOPMENT DURING ACUTE EXPOSURE TO HIGH ALTITUDE (HA). Luca Pomidori[1], Gaia Mandolesi[1], Paolo Ghinelli[1], Giovanni Avancini[1], Annalisa Cogo[1]. [1]Biomedical Sport Studies Center, University of Ferrara, Italy. *Email: cga@unife.it*. Introduction: Early desaturation during acute HA exposure (hypobaric chamber) has been reported to be significantly related to AMS development. We aimed to study the hypoxic profile and the AMS development during the ascent from Alagna (1200m) to Capanna Regina Margherita (4559m) with an overnight stay in Rifugio Gnifetti (3647m). Methods: Forty-four (8F) subjects (mean age 37.4±12.7) were recruited at the cable care station in Alagna, equipped with a 24-h data memory pulse oximeter (Pulsox-3Si, Minolta, Osaka, Japan) and asked to fill the Lake Louise questionnaire. Results: Only data from Alagna to Rifugio Gnifetti and the subsequent night are reported. Fifteen subjects (34%) showed a LL score ≥3 (AMS+). In Alagna, SpO_2 data were similar (94.5%±1.6 vs 95%±1.4 n.s.) but during HA exposure AMS+ group had a significantly lower SpO_2: at Punta Indren (3275m) after a 30-45 minutes cable car ascent (84%±4.5 vs 86.8%±3.7 p <0.049; in Rifugio Gnifetti during 3 hours rest after the arrival (84%±2.1 vs 86.1±1.9 p<0.01) and during the subsequent night (76.7%±3.8 vs 79.1%±3.4 p 0.049). There was no significant difference during the 2 hours exercise to reach Rifugio Gnifetti (81.3%±2.9 vs 82.1%±2.5 n.s.). No difference was found in the lower peak SpO_2 during the night but the AMS+ group always spent more time with a lower SpO_2: percentage of total time spent with SpO_2 <85% at rest in Rifugio Gnifetti was 56%±20.6 vs 34%±18 p 0.017; percentage of total time spent with SpO_2<75% during the night was 35%±27 vs 18%±19 p 0.036. Conclusion: We conclude that in subjects who develop subsequent AMS, hypoxemia is always more pronounced and prolonged than in healthy subjects; the new finding is that the higher desaturation occurs very early at the beginning of altitude exposure. 2011.

EARLY TIME COURSE OF ACUTE MOUNTAIN SICKNESS DURING PASSIVE EXPOSURE IN NORMOBARIC HYPOXIA. Kai Schommer[1], Benjamin Spahr[1], Peter Bärtsch[1], Marek Sykora[2]. [1]Department of Sports Medicine, Medical Clinic, University Hospital Heidelberg, Germany, [2]Department of Neurology, University Hospital Heidelberg, Germany. *Email: kai.schommer@gmx.de*. Introduction: Symptoms of acute mountain sickness (AMS) typically develop after an exposure time of 6-8 hours, usually increase during an overnight exposure and are associated with increased sympathetic activation (SA). In previous studies with normobaric hypoxia we noticed that some subjects get sick early with symptoms

attributable to vagal stimulation (VS) such as nausea or vomiting and dizziness and recover from these symptoms during a prolonged stay in hypoxia. We propose that these subjects have an early vagal reaction and do not suffer from the classical AMS. Methods: We tested this hypothesis by measuring baroreflex sensitivity (BRS) which is increased with VS and decreased with SA. In a study on 30 subjects (age 26.3y, BMI 22.3kg/m2) we measured BRS and AMS scores before (Heidelberg, 100m) and after 1, 6 and 16 hours (after overnight) in acute normobaric hypoxia (FiO_2=12%; 4500m) and one week after the hypoxic exposure (100m). AMS was defined as a Lake Louise Score ≥5 and an AMS-C score ≥0.70. Results: After 16h, 50% had AMS and 50% had not. 33% had AMS after 6h, but not after 16h (AMS+-). 30% had AMS after 6 and 16h (AMS++), and 20% had AMS only after 16h (AMS-+) while 17% never had AMS. We compared subjects with an unusual time course of AMS (AMS+-) with subjects who showed a classical time course (AMS-+ or AMS++). BRS decreased in all subjects in hypoxia, but BRS was less decreased in AMS +- compared to AMS-+ and AMS++ (ANOVA, p for group 0.03, time <0.001). However, AMS+- had a more severe headache after 6h (mean 3.4±1.1 on a 5 point scale) compared to AMS-+ and AMS ++ (2.5±0.99) while "vagal" symptoms were similar. Conclusion: Our data confirm the increase in SA in AMS and they show that those who suffer only from early symptoms have higher VS without typical vagal symptoms. Our data demonstrate that there may be considerable difference in identifying subjects with AMS between studies that run only during day time vs. those which involve an overnight exposure. 2011.

ECOLOGICALLY VALID EXERCISE PERFORMANCE AT HIGH ALTITUDE. Justin S Lawley, Samuel J Oliver, Jamie H Macdonald, Medical Expeditions. Bangor University, Bangor University, Bangor University, Medical Expeditions. *Email: peu426@bangor.ac.uk.* At high altitude, exercise performance during sub-maximal and ecologically valid tests is poorly understood. The objective of this study was to investigate exercise economy and mountaineering performance. Forty two participants completed a 21 day expedition in the Himalaya. Mountaineering specific economy and performance were assessed in three ways at 20 & 5050m: a step test with two four minute steady state stages of three and seven metabolic equivalents representing flat and uphill walking; a bike test of similar duration/intensity investigating non-weight bearing exercise and cardio-respiratory parameters; and at 5050m only a preloaded 400m 1: 4 gradient time trial rucksack carry. Effects of carbohydrate and fluid intake on the above were investigated via carbohydrate drink supplementation. Data were analysed by analysis of variance (F) and t-test (t). Statistical significance was accepted at $p < 0.05$. During sub-maximal exercise at 5050m, cardiac output was maintained at sea level values by increased heart rate, except at seven METs when cardiac output was reduced (12.8 ± 3.0 vs. 9.7 ± 1.7 $L \cdot min^{-1}$, p = 0.04). Consequently, blood lactate and rating of perceived exertion (RPE) were increased. At rest and at three METS, the elevated heart rate required to maintain cardiac output was alleviated in those allocated to carbohydrate (F = 3.76, p = 0.009) and those with high fluid intake (F = 3.71, p = 0.046). Furthermore, in participants allocated to carbohydrate, time to complete the ruck-

sack carry was reduced (990 ± 109 vs. 1194 ± 259s, t = 2.280, p = 0.04) and RPE was lower during the preload (10.9 ± 2.0 vs. 12.9 ± 1.0, t = 3.09, p = 0.01). At high altitude, whole body oxygen delivery and economy are reduced, even in submaximal exercise. Cardiovascular strain may be alleviated and mountaineering performance optimised with high carbohydrate and fluid intake. This study was supported by Science in Sport and the Ministry of Defence (Army). 2009.

EFFECT OF ACE AND B2-ADRENERGIC RECEPTOR GENE POLYMORPHISM ON CARDIAC OUTPUT IN NORMAL HUMANS. Kim Zillo Rokamp[1], Martin Gartmann[2], Anna Sletgaard[1], Nikolaj Nordsborg[2], Jonatan Myrup[1], Niels Vidiendal Olsen[1], Niels H. Secher[1], Henning Bay Nielsen[1]. [1]Department of Anaesthesia, Copenhagen University Hospital (Rigshospitalet), [2]Department of Exercise and Sport Sciences, University of Copenhagen, Denmark. *Email: nvolsen@sund.ku.dk.* Introduction: Several single nucleotide polymorphisms (SNPs) have been identified in the human b2-adrenergic receptor gene (ADRB2). The G46A SNP causing a Gly16Arg amino acid substitution has been associated with enhanced b2-mediated vasodilation but the effect of haplotypes within ADRB2 on cardiac output has not been studied. Methods: By the pairwise tagging principle five SNPs (G46A, C79G, C491T, C523A, G1053C) were genotyped in 140 normal subjects aged 34 ± 12 years. Haplotypes were estimated using the PHASE software. In addition, the I/D polymorphism in the ACE gene was genotyped. Cardiovascular variables were determined by the Model flow method. Results: We identified four common ADRB2 haplotypes: GGCCG (42.8 ± 0.2 %), ACCCG (33.0 ± 0.4 %), ACCCC (9.8 ± 0.4 %) and GCCAC (9.6 ± 0.2 %), seen in 95 % of the subjects. Other haplotypes each had frequencies below 2 %. By an additive model for all genotypes in ADRB2, an analysis of variance showed an effect of the Gly16Arg SNP on resting values of cardiac output (Gly/Gly: 6.4 ± 1.2 L/min; Gly/Arg: 5.9 ± 1.1 L/min; Arg/Arg: 5.8 ± 0.9 L/min; P = 0.027). Compared with Arg16Arg homozygotes (N = 24), Gly16Gly homozygotes (N = 43) presented a mean increase in cardiac output of 0.66 L/min (95 % CI: 0.13 - 1.18 L/min; P = 0.016). None of the other ADRB2 genotypes affected cardiac output (overall P = 0.25). Cardiac output was not related to haplotype pairs (P = 0.11) or to the ACE I/D polymorphism (P = 0.87). ANOVA showed no interaction between ADRB2 and I/D polymorphisms (P = 0.24) and in Gly/Gly subjects presence of the I/I genotype (N = 14) did not increase cardiac output compared with the D/D genotype (N = 11; P = 0.26). Conclusion: In conclusion, the ADRB2 Gly16Arg polymorphism by itself produces higher values of resting cardiac output in Gly/Gly homozygotes compared with Gly/Arg heterozygotes and Arg/Arg homozygotes. Presence of the ACE I/I genotype does not enhance the effect of the ADRB2 Gly/Gly-genotype. Acknowledgements: The study was supported by grants from the Scientific Board of Copenhagen University Hospital (Rigshospitalet) 2011.

EFFECT OF ALTITUDE OF RESIDENCE ON THE CARDIOVASCULAR RESPONSES TO DYNAMIC AND ISOMETRIC HAND-GRIP EXERCISE AT 4900 METERS. John Davis[1], Andrew Grant[1], Marta Perez[1]. [1]Alma College, Alma,

Michigan. *EMAIL: davisj@alma.edu* INTRODUCTION: Previous studies have documented the beneficial physiological adaptations to living at high altitude. However, few studies have examined exercise responses for residents from a variety of altitudes at very high altitude. The purpose of this study was to examine the influence of altitude of residence on cardiovascular responses during dynamic and isometric exercise at high altitude. METHODS: Thirty subjects were tested at the Carroll Hut refugio at Mount Chimborazo in Ecuador located at 4900 meters. This is an optimal location because many Ecuadorians tourists who reside at a variety of altitudes can easily drive to the refugio. All measurements were taken immediately upon arrival. Subjects were divided into three groups based on their altitude of residence: LOW (0-1500 m), MOD (1500-3000 m), and HIGH (>3000 m). A maximum voluntary contraction (MVC) using a hand-grip dynamometer was performed for each subject. They then performed 30 contractions on the dynamometer at 50% MVC. Following the dynamic contractions, they performed an isometric contraction at 50% of MVC until they reached 25% of MVC. Heart rate, oxygen saturation, and systolic and diastolic blood pressure were measured before and during the dynamic and isometric exercise. RESULTS: Heart rates at rest and during both dynamic and isometric exercise were significantly higher in LOW (105±8.2, 112.7±9.5, 112±12.8 bpm) when compared to both MOD (84.5±16.2, 91.7±12.6, 91.3±11.8) and HIGH (78.9±17.6, 80.0±10.3, 84.3±11.9). Also, oxygen saturation was significantly lower at rest and during dynamic exercise in LOW (72.3±15.1, 71.4±10.3%) compared to MOD (78.7±5.5, 81.1±4.1) and HIGH (82.4±3.7, 85.0±3.9). Although changes were observed in systolic and diastolic blood pressure at rest and during dynamic and isometric exercise, these values were not statistically significant between groups ($p>0.05$). CONCLUSION: Altitude of residence had an important influence on resting cardiovascular responses at very high altitude. However, the additive effect of the exercise was similar in all three groups. 2015.

EFFECT OF CYCLOOXYGENASE INHIBITION ON THE SYSTEMIC PRESSOR RESPONSE TO INTERMITTENT HYPOXIA IN HEALTHY HUMANS. Christina Yang[1], Andrew E. Beaudin[2,8], Matiram Pun[2,8], Craig D. Steinback[2,8], Lea Bond[6,9], Andrea De Souza[6,9], Katherine E. Wynne-Edwards[6,9], Todd J. Anderson[3,7,8], Sofia B. Ahmed[4,7,8], Patrick J. Hanly[4,6,8] and Marc J. Poulin[2,5,6,7,8,10]. [1]Faculty of Science; Departments of [2]Physiology & Pharmacology, [3]Cardiac Sciences, [4]Medicine, and [5]Clinical Neurosciences; [6]Hotchkiss Brain Institute and [7]Libin Cardiovascular Institute Alberta; Faculties of [8]Medicine, [9]Veterinary Medicine and [10]Kinesiology; Univ Calgary, Calgary Alberta, Canada. *EMAIL: chyang@ucalgary.ca* INTRODUCTION: Intermittent hypoxia (IH) resulting from repetitive airway collapse during sleep is believed to be the primary pathway leading to an increased risk of hypertension in patients with obstructive sleep apnea; however, the mechanisms are incompletely understood. It is postulated IH-induced alterations in vasoactive prostanoids may play a potential role. METHODS: Using a double blind, crossover, randomized, placebo-controlled design, this study investigated the role of prostanoids (with the non-selective cyclooxygenase (COX) inhibitor indomethacin) on IH-induced alterations in blood pres-

sure (BP). On two experimental days separated by a minimum 4-day washout period, 12 males (26.2±5.0 y, BMI 24.9±2.5 kg/m2) underwent an acute isocapnic-hypoxia challenge before and after 6 hours of IH during wakefulness that simulated an oxygen desaturation index of 30 events/hour. Four days prior to each experimental day, subjects ingested (p.o. t.i.d.) either 50mg indomethacin (INDO) or a 100mg lactose placebo (PLBO). RESULTS: Compared to PLBO, INDO significantly increased pre-IH resting mean arterial BP (MAP; 83±8 to 90±8 mmHg; P<0.01), systolic BP (SBP; 117±13 to 123±11 mmHg; p=0.01) and diastolic BP (DBP; 66±9 to 73±9 mmHg; p<0.01). Subsequently, regardless of condition (INDO, PLBO), IH significantly increased MAP, SBP and DBP (P<0.05), but the increases in MAP, SBP, and DBP were not different between INDO and PLBO (p=0.62). Finally, the DBP response to isocapnic hypoxia was increased after IH (p=0.04); the gain for the INDO and PLBO responses increased from 0.3±0.2 to 0.4±0.2 mmHg/% desaturation after IH. There was no difference in the DBP responses between INDO and PLBO (p=0.25). CONCLUSION: In conclusion, non-specific inhibition of COX increased resting mean, systolic and diastolic BP, but did not influence the systemic pressor response to IH nor enhance the DBP response to isocapnic-hypoxia, suggesting that there may be other mechanisms involved. ACKNOWLEDGEMENTS: Funded by NSERC, AI-HS, CIHR, and HSFC. 2015.

EFFECT OF END-TIDAL PCO_2 ON CEREBRAL OXYGEN DELIVERY DURING PROGRESSIVE HYPOXIA. Joseph A Fisher, Alexandra Mardimae, Marat Slessarev, David Preiss, Dahlia Balaban, Alex Vesely, Richard Greene, James Duffin. University of Toronto, University of British Columbia, New Mexico Highlands University. *Email: joe.fisher@utoronto.ca.* With progressive poikilocapnic hypoxia, cerebral blood flow (CBF) is initially maintained and then appears to increase progressively above a threshold. Hypoxia-induced hyperventilation reduces $PaCO_2$ and thereby CBF. Hypocapnia also shifts the oxyhemoglobin dissociation curve (ODC) to the left, increasing hemoglobin (Hb) O_2 saturation (SaO_2). We studied the independent effects of each of these factors on brain O_2 delivery (DO_2=CBFx[Hb]xSaO_2) in vivo. We used a specialized gas blender (RespirAct™, TRI, Toronto, Canada) and a sequential rebreathing circuit to prospectively target end-tidal PCO_2 ($PetCO_2$) and PO_2 ($PetO_2$) in 5 healthy lowlanders (2 females) within 3 days of arriving at 3600m. In 3 separate trials we maintained $PETCO_2$ at resting levels, 10 mmHg below resting, or 10 mmHg above resting, during which $PETO_2$ was targeted at 100, 70, 60, 50, and 40 mmHg in steps lasting 2 min. We monitored middle cerebral artery velocity (MCAV) as a surrogate of CBF, and pulse O_2 saturation (SpO_2). At PO_2 of 100, as expected, MCAV, and thus DO_2, were a function of $PetCO_2$. During normocapnic progressive hypoxia, changes in MCAV exactly mirrored those in SpO_2. Indeed, the calculated DO_2 remained constant indicating their reciprocal magnitudes. During hypercapnia and hypocapnia DO_2 retained its initial levels relative to normocapnia but converged with its normocapnic level at $PetO_2$ ~30 mmHg (extrapolated). We conclude: (1) what may appear to be a threshold change in CBF during hypoxia can be explained as an exponential rise in CBF to maintain DO_2 in the face of the exponential decline in SaO_2

as $PetO_2$ approaches the steep portion of the ODC; (2) hypocapnia decreases DO_2 and DO_2 does not attain normocapnic levels until hypoxia is near the limits of physiologic tolerance. 2009.

EFFECT OF ENDURANCE TRAINING IN HYPOXIA ON CYCLING PERFORMANCE AND LACTATE METABOLISM. Virgile Lecoultre, Luc Tappy, Philippe Schneiter, Yves Schutz. University of Lausanne. *Email: virgile.lecoultre@unil.ch.* The hypothesis of the present study was that endurance cyclists performing 3 training sessions per week during 4 weeks in normobaric hypoxia (HYP) (~3000 m) would enhance more endurance performance and lactate turnover than a control group (NOR) following the same training program in normoxia. Before and after the training period, endurance performance was assessed during VO_2max tests and a 40-km time-trial (TT) performed in normoxia. A VO_2max test in hypoxia was also carried out. Lactate metabolic clearance (MCR) and endogenous production (EP) rates and glucose turnover were measured by means of stable isotope tracer infusion. At baseline, endurance performance was correlated with lactate MCR ($r=0.52$, $p<0.05$). After training TT performance was significantly improved in NOR and HYP. VO_2max was significantly ($p<0.05$) increased in NOR only. In hypoxia, a slight increase ($p<0.05$) of maximal aerobic power and an increased ventilatory response during exercise ($p<0.001$) were found in HYP only. Mean lactate MCR and EP were unchanged. In both groups glucose turnover and FFA concentration were decreased ($p<0.05$). 3 athletes involved in HYP exhibited overtraining symptoms. These results indicate that lactate clearance may be a key metabolic component of endurance performance. In addition, our results suggest that the "Live Low Train High" protocol (LLTH) has no further effect on sea-level endurance performance than does normoxic training and that LLTH may not improve performance at altitude to a great extent. These results raise questions about effectiveness of a LLTH protocol for performance enhancements and the athletes health with regard to overtraining. This study was funded by the "Sport and Movement" grant, Macolin, Switzerland. 2009.

EFFECT OF FAST ASCENT ON HEART RATE VARIABILITY AFTER 2-DAY REST. Outi M. Villet[1], Heikki M. Karinen[2,4], Juha E. Peltonen[3,4], Heikki O. Tikkanen[3,4]. [1]Heart and Lung Center, Helsinki Univ Central Hospital, Finland, [2]Unit for Occupational Health, Dept Health Sciences, Univ Tampere, Finland; Dept Sports and Exercise Medicine, Institute Clinical Medicine, Univ Helsinki, Finland, [3]Dept Sports and Exercise Medicine, Institute Clinical Medicine, Univ Helsinki, Finland, [4]Foundation for Sports and Exercise Medicine, Helsinki, Finland. *EMAIL: outivillet@gmail.com* INTRODUCTION: General recommendation for safe daily ascent rate is 300 m per day at altitudes higher than 2500-3000m. However, faster rates are commonly used in high mountains increasing the risk for development of acute mountain sickness (AMS). Accordingly a method to predict individual's likelihood for AMS before the faster ascent would be useful. We evaluated the effect of fast ascent (400-1400m/day) on heart rate variability (HRV) in 41 healthy and fit lowlanders during high altitude expeditions. We compared the baseline (after 2-day

stay at same altitude without AMS symptoms, altitude range 3000-5600 m) and post ascent HRV parameters between subjects with (n=13) or without (n=28) AMS after ascent. METHODS: HRV measurements were performed in the morning after 7-8 hrs rest by Suunto T6 heart rate monitors in supine position. Lake Louise scoring (LLS) was used for AMS diagnosis. LLS≥3 indicated AMS. Autonomic cardiac function was assessed for 2 minutes by analysis of R-R intervals (RRI). Determined parameters included SDNN (standard deviation of RRI) and low and high frequency powers (LF and HF) among others. RESULTS: A day before the ascent SDNN2min and lnHF were significantly lower in subjects, who developed AMS after the ascent (SDNN2min 46 ± 29, lnHF 5.1 ± 1.2) than in those without AMS (SDNN2min 61 ± 30, lnHF 6.5 ± 0.9). Moreover, after the ascent Δ lnLF and Δ LF/HF were negative and significantly lower among subjects, who developed AMS (Δ lnLF -0.7 ± 0.8, Δ LF/HF -1.8 ± 3.4) than in those without AMS (Δ lnLF 0.3 ± 0.9, Δ LF/HF 1.7 ± 2.5). CONCLUSION: Low SDNN2min and lnHF values before the ascent seemed to be associated to poor acclimatization. These HRV parameters might provide a beneficial method to assess individual's risk for development of AMS after fast ascent. ACKNOWLEDGEMENTS: Partially funded by Finnish Ministry of Education. 2015.

EFFECT OF HIGH ALTITUDE EXPOSURE AND ACETAZOLAMIDE ON OPTIC NERVE SHEATH DIAMETER AND HIGH ALTITUDE HEADACHE: A DOUBLE BLIND RANDOMIZED PLACEBO CONTROLLED TRIAL. Justin Lawley[1], Jamie Macdonald[1], Samuel Oliver[1]. [1]School of Sport, Health and Exercise Sciences, Bangor, Wales. *Email: pepa16@bangor.ac.uk*. Introduction: The objective was to determine whether intracranial pressure, determined by measurement of optic nerve sheath diameter (ONSD), is causally related to high altitude headache. Methods: Following sea level measurements at 3 and 12 hours (SL), twenty three subjects were passively transported to 3777m (HA) via cable car, whereby, headache (HAH), ONSD, oxygen saturation (O_2sat) and urine production were determined at 3, 12, 24 and 36 hours. After 12 hours exposure to HA, subjects were classified as either High Altitude Headache resistant (HAH-r; n=11, VAS=0±1) or High Altitude Headache susceptible (HAH-s; n=12, VAS=19±14). Acetazolamide (250mg) or placebo were then prescribed stratified for initial headache severity and gender. Mean values were compared via analysis of variance and relationships between variables were analysed by generalized estimation equations. Results: Acetazolamide had no effect on HAH (P=0.74), ONSD (P=0.98) or O_2sat (P=0.18), but did increase urine production (P=0.00). When investigating HAH susceptibility, O_2 sats decreased in HAH-s more than HAH-r (85±3 versus 88±2%, P=0.03). However, ONSD increased similarly in both groups and remained significantly elevated above SL values for the entire study period (SL, 3h 5.3±0.5 & 12h 5.3±0.5 versus HA 3h 5.4±0.6, 12h 5.6±0.5, 24h 5.7±0.4 & 36h 5.6±0.5mm, P=0.00), despite acclimatisation resolving headache to sea level values (SL, 3h 2±5 & 12h 0±2 versus HA 3h 9±13, 12h 10±14, 24h 8±12 & 36h 1±4mm, P=0.03). Furthermore, a multi-level association was observed between HAH and O_2 sat (B=-1.39, P=0.00) but not HAH and ONSD (B=0.59, P=0.57). Conclusion: Optic nerve sheath diameter was not

associated with headache during 36 hours of exposure to high altitude. Acknowledgements: This research was supported by: North Wales NHS small research grant, BCM specials, Compagnie du Mont Blanc and SonoSite. 2011.

EFFECT OF HIGH ALTITUDE EXPOSURE ON SOLUBLE UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR PLASMA CONCENTRATION. Matthias P Hilty[1], Stefanie Zuegel[2], Michelle Schoeb[1], Katja Auinger[1], Christoph Dehnert[2], Marco Maggiorini[1]. [1]Medical Intensive Care Unit, Univ Hospital of Zurich, [2]Institute for Sports Medicine, Rupert-Karls Univ Heidelberg. *EMAIL: matthias@hilty.info* INTRODUCTION: Soluble urokinase-type plasminogen activator receptor plasma concentration (suPAR) has been studied as prognostic parameter in various infectious diseases including sepsis. To better understand mechanisms influencing suPAR, we studied the effect of acute high altitude (HA) exposure. METHODS: 31 healthy persons ascended to 4559m within 24h. At baseline, on HA days 2 (HA2) and 4, Lake Louise Score for acute mountain sickness (AMS=LLSAMS≥5) was assessed and arterial blood samples taken to measure blood gases, c-reactive protein (CRP), interleukin-6 (IL6) and suPAR. On HA2, participants were divided into group 1 including participants without AMS, and group 2 including participants with AMS or previous history of high-altitude pulmonary edema (HAPE) defining HAPE susceptibility. Group 2 was treated with dexamethasone 8mg bid until descent. RESULTS: 10 participants with AMS and 10 HAPE susceptibles were treated with dexamethasone (group 2). 11 were included in group 1. Acute exposure to HA increased suPAR from 1.9±0.44 to 2.27±0.51ng/ml ($p<0.001$), CRP from 0.7±0.46 to 3.57±4.63mg/l ($p<0.001$) and IL6 from 0.9±0.8 to 2.7±2.0ng/l ($p<0.001$). Increase in suPAR did not differ between groups 1 and 2. Stay at 4559m for 4 consecutive days did not change plasma levels of suPAR, CRP and IL6. The intake of dexamethasone decreased suPAR ($p=0.04$) and IL6 ($p=0.006$), but not CRP. The ascent associated decrease in PaO_2 was significantly correlated with IL6 ($r=0.478$, $p=0.001$), but not suPAR ($r=0.266$, $p=0.078$); the increase in IL6 was not correlated with suPAR ($r=0.226$, $p=0.135$). Baseline suPAR was significantly higher in HAPE susceptibles than the others ($p=0.042$). CONCLUSION: HA exposure leads to a significant increase in suPAR. The missing correlations between suPAR and IL6 and suPAR and PaO_2 suggest a different mechanism than an inflammation triggered increase in suPAR or a direct effect of hypoxia. Acclimatization does not affect suPAR but dexamethasone intake does. Baseline suPAR may predict susceptibility to HAPE. 2015.

EFFECT OF HYPOXIA SEVERITY ON QUADRICEPS FATIGABILITY DURING EXHAUSTIVE INTERMITTENT CYCLING. Olivier GIRARD[1], Martin BUCHHEIT[2], Ryan CHRISTIAN[1], Sébastien RACINAIS[1]. [1]Aspetar - Qatar Orthopaedic and Sports Medicine Hospital, Doha - Qatar, [2]Aspire- Academy for Sport Excellence, Doha - Qatar. *EMAIL: olivier.girard@aspetar.com* INTRODUCTION: This study examined the effects of hypoxia severity on quadriceps fatigability during exhaustive intermittent cycling. METHODS: Fifteen well-trained cyclists performed an intermittent cycling exercise until exhaustion at

supra-maximal intensity - 15 s at 30% of the anaerobic power reserve (609±23 W) with a fixed pedaling frequency of 110 rpm, interspersed with 45 s of passive rest - in normoxia (simulated altitude/end-exercise arterial O_2 saturation = 0 m/96%), moderate (2200 m/90%) and severe hypoxia (4200 m/79%). Neuromuscular tests including electrical femoral nerve and transcranial magnetic stimulations during brief (5-s) and sustained (30-s) maximal isometric voluntary contractions (MVC) of the knee extensors were performed at baseline and 7 min post-exhaustion. RESULTS: Exercise performance differed (P<0.001) among the three conditions of oxygenation (39 ± 8, 22 ± 3 and 13 ± 2 sprint repetitions in normoxia, moderate and severe hypoxia, respectively). At exhaustion, the reduction in peak twitch amplitude (P<0.001) in response to supra-maximal, unpotentiated single (-53.2%) and potentiated paired (-33.1%) electrical stimuli was identical across conditions. Compared with baseline, strength loss during brief MVC was similar at exhaustion in normoxia and moderate hypoxia (P<0.01), while a smaller (P=0.136) force decline occurred with severe hypoxia. When contraction was prolonged, exercise-induced decreases in force were consistent across conditions (P<0.01). This was accompanied by lower (P<0.05) end-exercise voluntary activation values obtained from both motor nerve and motor cortex stimulations during brief and sustained MVCs, with no hypoxia severity effect. CONCLUSION: Despite earlier exercise cessation with increasing hypoxia severity, end-exercise reductions in quadriceps twitch force were similar across conditions. These results indicate the existence of a critical threshold of peripheral fatigue with intermittent cycling to exhaustion, independently of the hypoxia level. Another novel finding was that a suboptimal output from the motor cortex may also contribute to exhaustion. 2015.

EFFECT OF INTERMITTENT HYPOXIA ON ERYTHROPOIETIN, SOLUBLE ERYTHROPOIETIN RECEPTORS AND VENTILATION IN HEALTHY HUMANS. Julien V Brugniaux, Vincent Pialoux, Glen E Foster, Cailean T Duggan, Michael Eliasziw, Patrick J Hanly, Marc J Poulin. University of Calgary. *Email: jbrugniaux@free.fr.* Erythropoietin (Epo) has recently been demonstrated to stimulate ventilatory acclimatization to hypoxia. It is also known from studies in mice that the regulation of soluble Epo receptors (sEpoR) plays a central role in the stimulation of ventilation via Epo. We hypothesized that sEpoR would be downregulated by exposure to intermittent hypoxia (IH), thereby allowing Epo concentration to rise. A secondary objective was to determine the strength of the association between these hematological adaptations and alterations in the acute hypoxic ventilatory response (AHVR). Nine healthy male subjects were exposed to 6h of IH [2min normoxia (peak end-tidal PO_2 ($PETO_2$) = 88.0 Torr) and 2min hypoxia (nadir $PETO_2$ = 45.0 Torr)] for four consecutive days (Days 1-4), preceded by two normoxic control days (4 days apart; Baseline), and followed by one recovery day (4 days after IH; Day 8). Epo and sEpoR concentrations and AHVR were measured on Baseline, Days 1, 2, 4 and 8. We observed a nadir in sEpoR on Day 2 (-70% vs. Baseline; p<0.01), concomitant with the peak in Epo secretion (+50% vs. Baseline; p<0.01). Following exposure to IH, tidal volume (VT) increased while breathing frequency remained unchanged, leading to an increase in overall ventilation (VE) as

measured during the AHVR test. Similarly, AHVR increased progressively along with IH (p=0.008). There was a negative correlation between Epo and sEpoR (r=-0.261, p=0.05), and between sEpoR and VT (r=-0.331, p=0.02). Epo was positively correlated with VE (r=0.458, p=0.001) and AHVR (r=0.475, p=0.001). We conclude that sEpoR is downregulated upon exposure to IH and modulates the Epo response to such an exposure. Furthermore, the alterations in AHVR and breathing pattern following IH seem partially mediated by the increase in Epo. Funding: AHFMR, HSFC, and Hotchkiss Brain Institute. 2009.

EFFECT OF NEUROGLOBIN DEFICIENCY ON GENE EXPRESSION IN THE MOUSE RETINA. Christian Ansgar Hundahl[1], Sten Ilmjärv[2], Hendrik Luuk[2]. [1]Centre of Excellence for Translational Medicine, Univ Tartu, Estonia, [2]Dept Physiology, Univ Tartu, Estonia. *EMAIL: c.hundahl@gmail.com* INTRODUCTION: The retina has the highest oxygen consumption per weight unit of all tissues making oxygen delivery of paramount importance in retinal physiology. Neuroglobin (Ngb), a neuronal expressed heme-globin, has been shown to be highly expressed in the mouse retina and it has been proposed as a key player in retinal oxygen homeostasis. We used Ngb knock out mice to test the effect of Ngb deficiency on gene expression in dark- and light-adapted retinas. METHODS: Large-scale gene expression profiling was performed on retinas from Ngb knock out and wild type mice kept in either darkness or exposed to 90 min or 300 min of light using Mouse Exon 1.0 ST array (Affymetrix). RESULTS: Functional annotation enrichment analysis indicated that in the dark-adapted retina Ngb deficiency resulted in the up-regulation of genes primarily involved in intracellular transport and down-regulated genes primarily involved in pyrimidine metabolism. The interaction of genotype and light resulted in differential expression of genes primarily involved in intracellular transport and Rac protein signaling. CONCLUSION: The present results do not support the notion that Ngb is of major importance for retinal oxygen homeostasis since Ngb deficiency did not affect pathways involved in oxidative metabolism. In addition, Ngb does not seem to be involved in light mediated signaling in the retina. Further studies are needed to clarify the role of Ngb in intracellular transport and Rac protein signaling. ACKNOWLEDGEMENTS: Financial support was given by Lundbeck Foundation, the NOVO-Nordisk Foundation, The Foundation for Providing Medical Research and the Estonian Ministry of Science and Education (SF0180125s08). 2015.

EFFECT OF $PETCO_2$ ON HYPOXIC CEREBRAL VASODILATATION IN LOWLANDERS AT 3600M. Alexandra Mardimae, Marat Slessarev, David Preiss, Dahlia Balaban, Alex Vesely, Jay S Han, Gustavo Zubieta-Calleja, Luis Zubieta-Calleja, Richard Greene, Joseph A Fisher. University of Toronto, University of British Columbia, New Mexico Highlands University. *Email: mardimae@gmail.com.* Acute hypoxia, such as that experienced upon arrival at altitude, increases cerebral blood flow (CBF). However, the overall effect on CBF depends on the level of CO_2, for hypocapnia causes vasoconstriction. Previous studies have identified thresholds for hypoxic vasodilatation during normocapnia and hypercapnia, how-

ever the effects of concurrent sustained hypocapnia on this threshold are unknown. Our aim was to observe the effect of hypocapnia on hypoxic vasodilatation in a group of lowlanders during acute acclimatization at altitude. A specialized gas blender (RespirActTM) and a sequential rebreathing circuit were used to prospectively target end-tidal PCO_2 ($PETCO_2$) and PO_2 ($PETO_2$) in 5 healthy lowlanders (2 females) within 3 days of arriving at 3600m. In 3 separate trials $PETCO_2$ was maintained at resting levels (normocapnia), 10 mmHg below resting (hypocapnia), and 10 mmHg above resting (hypercapnia), during which $PETO_2$ was targeted at 100, 70, 60, 50, and 40 mmHg in steps lasting 2 minutes. Middle cerebral artery velocity (MCAV) was measured continuously, and O_2 saturation (SpO_2) was recorded at the end of each $PETO_2$ step. Our results suggest that the hypoxic vasodilatory threshold occurs at a lower $PETO_2$ during hypocapnia than during normocapnia (median $PETO_2$ threshold = 55.5 versus 71 mmHg, respectively). Furthermore, at $PETO_2$ levels below this threshold, the time constant of change in MCAV per change in $PETO_2$ was shorter during hypocapnia than in normocapnia (median exponential tau = 9.3 versus 14.6, respectively). These observations suggest that the hypoxic vasodilatory threshold is blunted in the face of hypocapnia, which may indicate that the vasoconstrictive effects of CO_2 predominate over attempts by the cerebral vasculature to increase O_2 delivery during progressive hypoxia at PO_2 above this threshold. Below this threshold, the effects of progressive arterial desaturation appear to compensate for the decreased O_2 delivery through increased vasodilatation. 2009.

EFFECT OF REDUCED BAROMETRIC PRESSURE ON DETECTION OF INTRAPULMONARY SHUNT AND MEASURES OF PULMONARY GAS EXCHANGE EFFICIENCY IN NORMOXIA AND HYPOXIA. Frank Petrassi[1], James Davis[1], Kara Beasley[1], Oghenero Evero[2], Joel Futral[1], Andrew Subudhi[2], David Polaner[2], Holger Eltzschig[2], Robert Roach[2], Andrew Lovering[1]. [1]University of Oregon, Eugene, USA, [2]University of Colorado Anshutz Medical Campus, Aurora, USA. *Email: lovering@uoregon.edu.* Introduction: Studies conducted at sea level and high altitude (>5000m) indicate that blood flow through intrapulmonary arteriovenous anastomoses (QIPAVA) at rest and during exercise is reduced at high altitude as detected by transthoracic saline contrast echocardiography (TTSCE). It remains unknown whether the observed reduction in QIPAVA was an artifact of an effect of reduced barometric pressure (PB) on TTSCE. We hypothesized that if QIPAVA is a source of shunt and is reduced during exercise in hypobaria compared to normobaria, then the alveolar to arterial oxygen difference (AaDO2) also would be reduced in hypobaria. Methods: We compared QIPAVA and AaDO2 in healthy humans, without patent foramen ovale, during submaximal exercise in normobaric (PB = 625 mm Hg, Aurora, CO) and hypobaric (PB = 406 mm Hg) conditions using a hypobaric chamber. Subjects (n=7) exercised at 70, 100, 130, 160 W, or until exhaustion, in hypobaric hypoxia (HH), fraction of inspired oxygen (FIO2) = 0.209 and normobaric hypoxia (NH), FIO2 = 0.132. Subjects (n=8) exercised at 100, 130, 160, and 190 W or until exhaustion in hypobaric normoxia (HN), FIO2 = 0.336 and normobaric normoxia (NN), FIO2 = 0.209. Results: Bubble scores determined using TTSCE were higher in NN and NH compared to HN and HH, respectively,

suggesting greater QIPAVA in normobaria. However, the AaDO2 was similar in comparable conditions, specifically HH to NH and HN to NN. Conclusion: Because recent work by our group has demonstrated that QIPAVA results in an increased AaDO2, these data suggest that TTSCE may not be a valid technique for detecting QIPAVA at altitudes above 5000m. Acknowledgements: Funded in part by University of Oregon Office of Research, Innovation and Graduate Education and the Altitude Research Center Foundation; K Minkel, A Yousif, SH Wang & B Tawfik. 2015.

EFFECT OF SIMVASTATIN ON HYPOXIC PULMONARY VASOCONSTRICTION (HPV) IN HUMANS. Klaus D Torp, Jason D Archibald, Angela A Joseph, Joseph A Kisslo, William D White, Richard E Moon. Mayo Clinic, Duke University Medical Center. *Email: torp.klaus@mayo.edu.* HPV may benefit patients with regional lung pathology, helping to maintain arterial oxygenation by matching ventilation with perfusion. But in global hypoxic environments such as acute altitude exposure, HPV may cause generalized pulmonary vasoconstriction and pulmonary hypertension, which may result in high altitude pulmonary edema (HAPE). The mechanism of HPV is incompletely understood, but may include hypoxia-induced down-regulation of endothelial nitric oxide synthase (ecNOS). Hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors cause up-regulation of ecNOS activity. Simvastatin (Zocor) has been shown to completely reverse hypoxic down-regulation of ecNOS in vitro in human endothelial cells, suggesting that it could attenuate HPV. The aim of this study was to determine in vivo, whether administration of an HMG-CoA reductase inhibitor in clinical doses can block the acute pulmonary vasoconstrictive response to hypoxia. Methods: 6 normal volunteers underwent echocardiographic systolic pulmonary artery pressure (SPAP) measurements at rest, while breathing room air, or a hypoxic gas (FIO_2 = 0.11). Measurements were obtained after each 15-minute interval of normoxia, then hypoxia, followed again by normoxia. EKG and SpO_2 were continuously monitored. After 3 days of oral simvastatin (40 mg twice daily) this protocol was repeated. Repeated measures 2-way ANOVA was used for statistical analysis. Results: (Mean ± standard deviation with values following simvastatin administration in parentheses): SpO_2 decreased from 98.3 ±1.4 (98.7±1.0%) during normoxia to 64.3±5.6% (67.3±5.0%) after hypoxia and back to 98.7±1.5% (99±1.3%) after normoxia. The SPAP increased from 18.0±2.8mmHg (18.8±2.9mmHg) to 33.0±8.1mmHg (34.5±6.5mmHg) and back to 19.2±3.1mmHg (18.0±3.5mmHg) respectively. Conclusion: Short-term administration of simvastatin in clinically relevant doses does not seem to alter resting hypoxic pulmonary vasoconstriction. No outside funding was obtained for this study. 2009.

EFFECTS OF ACUTE AND CHRONIC EXPOSURE TO ALTITUDE ON FOREARM BLOOD FLOW AND REACTIVE HYPEREMIA. John Davis[1], Jessica Thorington[1], Steve Lux[1], Sophia Hrutkay[1]. [1]Alma College. *Email: davisj@alma.edu*. Introduction: Several studies have looked at reactive hyperemia acutely; however, few studies have examined the effect of long-term altitude exposure on reactive hyperemia. The purpose of this study was to determine the effect of moder-

ate altitude exposure on forearm blood flow (FBF) and reactive hyperemia (RH). Methods: Nineteen healthy subjects (ages 19-57) underwent a reactive hyperemia test at sea level (SL1), upon acute exposure to 3400m (ALT1), after 14 days at 3400m (ALT2), and upon return to sea level (SL2). After five minutes of baseline FBF readings, a sphygmomanometer was inflated to 250mmHg for five minutes to occlude blood flow into and out of the lower arm. Upon release of the occlusion, readings were taken every ten seconds for fifteen minutes. During the test, subjects were seated and asked to remain still. FBF was measured using venous occlusion plethysmography with a mercury-filled strain-gauge. The five minutes of baseline readings were averaged to give a baseline FBF value for each subject. Time taken for blood flow to return to baseline was measured after occlusion. RH was represented as the area under the curve (AUC) of the blood flow following the release of occlusion. Results: Baseline FBF was highest at ALT1 (3.17±1.17 mL blood/dL tissue/min, $p<0.05$); after return to sea level (SL2) it was lower than both ATL1 and ALT2 (1.43±.45 mL blood/dL/min, $p<0.05$). AUC was found to be at its greatest during acute altitude exposure, (ALT1 higher than both SL1 and SL2, 2470.5±890, $p< 0.05$). The time to return to baseline after occlusion was significantly longer at ALT2 (138.9±90 seconds) in comparison to returning to SL2 (83.7±41.4 seconds, $p<0.05$). Conclusion: Blood flow and reactive hyperemia are increased by hypoxia at moderate altitude. The blood flow and hyperemic responses appear to be highest upon acute altitude exposure. 2011.

EFFECTS OF ACUTE LEG ISCHEMIA DURING CYCLING ON O_2 AND CO_2 STORES. Burke A Gurney, Jack Loeppky. University of New Mexico. *Email: bgurney@salud.unm.edu.* Introduction: Superimposing ischemia on exercising limbs provokes a "metaboreflex" increasing ventilation (VE) and systemic blood pressure, presumably from elevated H in the limbs. The increased VE and ischemia result in body O_2 and CO_2 store changes that have not been adequately described. We estimated changes in whole body O_2 and CO_2 stores during and after steady state exercise with leg ischemia induced by leg cuff inflation. Methods: Six physically fit subjects performed 75W steady state exercise for 15 min on a cycle ergometer. After 5 min, cuffs on both upper and lower legs were inflated to 140 mmHg. Cuffs were deflated after 10 min and exercise continued for another 5 min. Results: VO_2 and VCO_2 significantly increased during the first 30 s after inflation and significantly decreased between 60 and 90 sec and then rose linearly until deflation. VO_2 and VCO_2 significantly increased further after cuff release, peaking between 30-60 s, then returning to near baseline exercise levels. Discussion: Model-estimated changes in total O_2 and CO_2 stores were compared with time-integrated store changes from VO_2 and VCO_2. During 5 min after cuff deflation VO_2 and VCO_2 exceeded the model-estimated change in stores by 273 and 697 ml, respectively, reflecting the O_2 cost repayment of the anaerobic work component and lactate buffering to neutralize circulating metabolites resulting from the preceding ischemia. These events represent a respiratory alkalosis during ischemia, followed by a metabolic acidosis after cuff release when metabolites from the anaerobic work return to the central circulation. The O_2 store

changes depend mainly on perfusion through lung and tissue, while CO_2 store changes are primarily determined by VE, venous blood redistribution and $HCO3^-$ buffering of lactate. These factors are important to consider if ischemic training is undertaken for rehabilitation. 2009.

EFFECTS OF CHANGES IN END-TIDAL PO2 AND PCO2 ON NEURAL RESPONSES DURING SUSTAINED ATTENTION. Thomas Bullock[1], Andrew Beaudin[2], Brad Goodyear[3], Barry Giesbrecht[1], Marc Poulin[2]. [1]Department of Psychological and Brain Sciences and Institute for Collaborative Biotechnologies, University of California, Santa Barbara, CA, USA, [2]Department of Physiology & Pharmacology and Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary Alberta, Canada, [3]Department of Radiology and Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary Alberta, Canada. *Email: bullock@psych.ucsb.edu.* Introduction: Cardiovascular diseases that perturb oxygen supply to the brain are linked with impairments in a variety of cognitive functions. Understanding the link between cerebrovascular function and cognition requires approaches that can assess neurovascular coupling, including both vascular responses and the neural mechanisms associated with cognition. Methods: To investigate this link, 7 participants performed a sustained attention task under different perturbations in the end-tidal (i.e., arterial) PO_2 ($PETO_2$) and PCO_2 ($PETCO_2$) while we simultaneously recorded electroencephalographic (EEG) activity from 19 scalp electrodes and cerebral blood flow velocity through middle and posterior cerebral arteries (MCAV/ PCAV, respectively) using transcranial Doppler ultrasound. The sustained attention task included a resting phase and a visual target detection phase requiring participants to monitor a sequence of colored circles and detect infrequent long-duration circles (targets). Gas challenges included air ($PETO_2$/$PETCO_2$ uncontrolled), isocapnia-hypoxia ($PETCO_2$ = 1 Torr above resting values; $PETO_2$ = 50 Torr), hypercapnia-euoxia ($PETCO_2$ = 8 Torr above resting values; $PETO_2$ = 88 Torr) and hypocapnia-euoxia ($PETCO_2$ = 12 Torr below resting values; $PETO_2$ = 88 Torr). Results: EEG time-frequency decomposition was used to assess fluctuations in stimulus-related neural activity in different frequency bands relative to baseline. Theta activity [4-8 Hz] increased between ~100 - 300 ms post visual stimulus onset in the air and hypoxia conditions relative to hypercapnia (p=0.036, p=0.024, respectively) and hypocapnia (p=0.039, p=0.059, respectively). A preliminary analysis revealed increases in percent change in PCAV were associated with reduced occipital EEG power in the lower alpha range [8-10Hz] during the target detection phase relative to rest in the air and hypercapnia conditions (r=0.76, p=0.046; r=0.88, p=0.010, respectively). Conclusion: Because theta and alpha power are often linked with executive control and suppression of visual processing, these preliminary data suggest that transient changes in arterial PO_2 and PCO_2 levels modulate activity of these important cognitive functions. Acknowledgements: This research was supported by an AIHS Visiting Scientist award (Giesbrecht); the Institute for Collaborative Biotechnologies through contract W911NF-09-0001 from the U.S. Army Research Office, and NSERC. 2015.

EFFECTS OF HIGH ALTITUDE ASCENT ON AMBULATORY BLOOD PRESSURE IN A HYPERTENSIVE KIDNEY TRANSPLANT RECIPIENT. Benoit Phelan[1], Luke Mather[2], Nirijam Regmi[3], Jennifer Starling[4], David Twillman[4], Matthew McElwee[5], Purshotam Paudel[6], Buddha Basnyat[7], Linda Keyes[4]. [1]Dalhousie University, [2]University of Washington, [3]Mountain Medicine Society of Nepal, [4]University of Colorado, [5]Case Western University, [6]Tribhuwan University, [7]Nepal International Clinic. *Email:* benoit.phelan@gmail.com Introduction: High altitude may increase blood pressure (BP) and the kidney plays an important role in acclimatization. We report a case of a climber with a kidney transplant and hypertension (HTN) ascending to 4310m. Methods: P.G.R, a 55 yo man, received a kidney transplant in 2002 and had since climbed to over 6500m. At the time of our encounter in October 2014 he weighed 66kg, had a BMI of 23 and a self reported BP of 130/70 mmHg. Medications were losartan, doxazosin, mycophenolate mofetil, tacrolimus, deflazacort and allopurinol. ABP was measured as part of a prospective observational cohort study on BP, HTN and high altitude. Using the Welch-Allyn ABPM 6100 heart rate (HR), systolic BP (sBP), and diastolic BP (dBP) were collected every 30 min between 07:00 and 22:00, and hourly between 22:00 and 07:00. MAP was calculated (cMAP) using Welch-Allyn software. 24h-ABP was measured for a total of 53 hrs during ascent from 2640m to 4310m. Results: P.G.R.'s overall SBP, DBP, cMAP and HR ranged from 88-165, 47-101, 61-122 and 59-131, respectfully. Means were 130±17.3, 75±12.4, 93±14.4 and 83±16.7. The percentage of overall, awake and asleep BP values above AHA normal cut-offs were 41.9%, 21.6% and 94.7% respectfully. Nocturnal BP did not show normal dipping, but increased by 13.7%. BP was similar across altitudes. There was no difference between sleeping BP at 3440m and 3770m. He had no AMS at 3400m or 4400m and subsequently climbed to over 5000m without problems. Conclusions: Altitude had minimal effect on BP and HR in this individual with a kidney transplant on antihypertensive medications. Despite elevated nocturnal BP, the patient was asymptomatic and demonstrated no adverse short-term effects. This case builds on previous reports that individuals with kidney transplants may safely travel to high altitude. Further studies are required to assess the clinical significance of elevated nocturnal BP at high altitude. Acknowledgements: Wilderness Medical Society, Nepal International Clinic. 2015.

EFFECTS OF HIGH ALTITUDE RELATED HYPOXIA AND INHALED ILOPROST ON TRICUSPID ANNULAR PLANE SYSTOLIC EXCURSION. Edith E Kortekaas, Bhavini Jaiswal, Katja Ruh, Gary P Foster, James D Anholm, Medical Expeditions. University Medical Centre Utrecht, Loma Linda University, VA Loma Linda Healthcare System. *Email: e.kortekaas@umcutrecht.nl.* Background: Altitude hypoxia has minimal effect on left ventricular function; however, hypoxia and subsequent hypoxic pulmonary vasoconstriction may be associated with more profound changes in right ventricular (RV) function. Tricuspid Annular Systolic Excursion (TAPSE) is a sensitive echo-derived measure of RV dysfunction and is a useful prognostic index in patients with pulmonary arterial hypertension. The effects of prolonged high altitude (HA) hypoxia on RV function using TAPSE have

not been studied. Iloprost, a prostacyclin analogue used in the treatment of pulmonary hypertension, may alter the impact of hypoxia on RV function. Objectives: To determine the effects of high altitude related hypoxia and inhaled iloprost on the TAPSE index of RV function. Methods: This study was a double blind, randomized, placebo-controlled cross-over trial of 7 healthy volunteers (aged 24 ± 4 years old) who were participants of the Medex 2008 Expedition to Dhaulagiri, Nepal. TAPSE echocardiogram measurements were performed before and after inhaled iloprost and placebo, both at sea level and high altitude at 5050m. Results: SpO_2 was 98% ± 1% (mean ± SD) at SL and 84% ± 5% at HA, $p<0.01$. TAPSE was 25.68 ± 2.70 mm at SL and 21.45 ± 2.52 mm at HA, $p=0.02$. Iloprost had no significant effect on TAPSE measurements. Conclusions: In healthy volunteers, HA related hypoxia alters TAPSE measurements of RV systolic function. Acknowledgements: All studies were carried out with Medical Expeditions, a charitable group that promotes high altitude research and education, http: //www.medex.org.uk. 2009.

EFFECTS OF HIGH-ALTITUDE PERIODIC BREATHING ON ARTERIAL OXYHAEMOGLOBIN SATURATION AND HEART RATE. Giuseppe Insalaco[1], Salvatore Romano[1], Adriana Salvaggio[1], Luca Pomidori[2], Gaia Mandolesi[2], Paolo Ghinelli[2], Annalisa Cogo[2]. [1]National Research Council of Italy, Institute of Biomedicine and Molecular Immunology, "A. Monroy", Sleep Laboratory, Palermo, Italy, [2]Biomedical Sport Studies Center, University of Ferrara, Italy. *Email: cga@unife.it*. Introduction: We aimed to investigate the effect of periodic breathing (PB) at high altitude on arterial oxygen saturation (SaO_2) and heart rate (HR). Methods: Nine élite climbers, males, age 24-52 underwent overnight cardio-respiratory monitoring (Lifeshirt System Vyasis) at sea level and at Everest North Base Camp (5180m),during the 1st (BC1) and the 10th (BC2) night. Between the two measurements all subjects climbed up to 7000m and spent at least 2 nights at 6100m with no use of oxygen and no symptoms of AMS. Their breathing pattern, SaO_2 and HR were analysed. Results: At sea level no respiratory disturbances were observed. PB was commonplace in all subjects at HA. PB cycle duration significantly increased ($p<0.0001$) from BC1 (21.7±1.9 s) to BC2 (26.7±2.1 s). From the BC1 to the BC2 mean SaO_2 increased significantly during wakefulness (77%±3 vs 82%± 3;$p<0.001$). The mean higher SaO_2 levels with PB of all subjects were 75.3±3.6% at BC1 and 82.4±2.9% at BC2 ($p<0.0008$). The mean lower SaO_2 levels with PB of all subjects were 68.2±4.0% at BC1 and 74.5±4.3% at BC2 ($p<0.01$). HR fluctuates with ventilation, with an increase whenever ventilation is resumed or increased after a respiratory pause or hypoventilation. HR reached a peak before the end of the ventilatory phase and then decreased suddenly in most cases. The mean higher HR during PB was 72.4±8.8 at BC1 and 63.3±6.0 at BC2 ($p<0.0002$) and the mean lower HR was 53.6±7.5 at BC1 and 43.6±7.3 at BC2 ($p<0.0001$), without any significant change in amplitude. Conclusion: The data demonstrate the role of PB in determining HR fluctuations. With acclimatization (BC1 versus BC2), a decrease in HR, without any change in amplitude in sleeping HR behavior, and an improvement in SaO_2 during sleep has been shown. Acknowledgements: Ev-K2-CNR 2011.

EFFECTS OF HYPOXIA AND REPRODUCTIVE FACTORS ON RISK OF BREAST CANCER. Parisa Parsa. Child and Mother Research center, Hamadan University of Medical and Health Sciences. *Email: pparsa2003@yahoo.com.* Introduction: Breast cancer is the leading women's cancer worldwide. However, there are geographical considerable differences with high rates of disease in North America and North Europe and relatively low rates in Africa and Asia. Methods: This systematic review article examines the effects of hypoxia and other reproductive factors on risk of breast cancer: early menarche, nulliparity or late age at first birth, late menopause, as well as hormonal factors. Results: According to the research hypoxia and other reproductive factors could have significant effects on risk of breast cancer. Conclusion: Knowing risk factors of breast cancer could significantly contribute to an improved prevention of this cancer. Furthermore, this review aimed to highlight potentially controversial conditions in the Asian countries compared to other parts of the world which could in the future improve early prevention of breast cancer in Asian women. 2011.

EFFECTS OF INTERMITTENT HYPOXIA ON ARTERIAL BLOOD PRESSURE AND CEREBRAL BLOOD FLOW IN HEALTHY HUMANS: ROLE OF CYCLOOXYGENASES. Matiram Pun[1], Sofia B. Ahmed[2], Andrew E. Beaudin[1], Patrick J. Hanly[3], Marc J. Poulin[4]. [1]Departments of Physiology and Pharmacology, University of Calgary, [2]Department of Medicine and the Libin Cardiovascular Institute of Alberta, University of Calgary, [3]Department of Medicine and Hotchkiss Brain Institute, University of Calgary, [4]Departments of Physiology and Pharmacology, Clinical Neurosciences, Hotchkiss Brain Institute and the Libin Cardiovascular Institute of Alberta, University of Calgary. *Email: mpun@ucalgary.ca*. Introduction: Chronic intermittent hypoxia (IH) is associated with an increased risk of hypertension, cardiovascular (CV) and cerebrovascular disease, though the mechanisms remain elusive. Previous studies suggest a role for prostaglandins, and commonly used non-steroidal anti-inflammatory drugs such as cyclooxygenase (COX) inhibitors increase both blood pressure (BP) and CV risks. Methods: We have designed a double blind, randomized, placebo-controlled crossover study to assess the role of COX in IH-induced alterations in the regulation of BP and cerebral blood flow (CBF). Healthy volunteers will ingest either Indomethacin (non-selective COX inhibitor; 150mg in three divided doses) or Celecoxib (COX-2 inhibitor; 400mg in two divided doses with a visually matching placebo in between) or placebo (three times daily) for four days preceding and during the experimental protocol. Participants will be exposed to 6-hrs of isocapnic IH (end-tidal PO_2 ($PETO_2$) cycling between 88 and 45 Torr every 60s) between morning and afternoon acute tests, consisting of six cycles of isocapnic IH (end-tidal PCO_2, $PETCO_2$=+1 Torr above resting; $PETO_2$ cycling between 88 and 45 Torr every 90s), followed by 5-min of isocapnic hyperoxia ($PETO_2$=300 Torr) and 5-min of hyperoxic-hypercapnia ($PETCO_2$=+9 Torr). Results: BP (finger photoplethysmography) and CBF (transcranial Doppler ultrasound) will be measured continuously. Urine (vasoactive prostaglandins), venous (oxidative stress markers) and finger capillary

blood will be collected before each acute test. We hypothesize that BP will increase and CBF responsiveness will decrease following IH exposure to a greater degree with COX inhibitors compared to placebo with the greatest changes observed post-celecoxib ingestion. Conclusion: Our investigation of the effect of COX inhibitors on the vascular response to IH will improve our understanding of the role of prostaglandins in this relationship and the potential long term CV consequences of these medications. 2011.

EFFECTS OF METHAZOLAMIDE ON HYPOXIC PULMONARY VASOCONSTRICTION (HPV) IN CONSCIOUS DOGS. Philipp P Pickerodt, Roland C Francis, Axel Erbs, Willehad Boemke, Claudia Hoehne, Erik R Swenson. University of Washington, Harvard MGH, , Charite Universitaetsmedizin, Universitaet Leipzig. *Email: philipp.pickerodt@charite.de.* Objective: The carbonic anhydrase (CA) inhibitor acetazolamide (ACZ) reduces HPV in isolated lungs, intact animals and in humans. The efficacy against HPV is not due to CA inhibition, since selective inhibition of either intracellular or extracellular CA by other potent but structurally different CA-inhibiting sulfonamides does not reduce HPV. As compared to ACZ, methazolamide (MTZ), possesses one additional methyl-group on the thiadiazole ring of the compound. We hypothesized that MTZ should still effectively reduce HPV in conscious spontaneously breathing dogs. Methods: Six female Beagle dogs were kept under standardized environmental conditions. Each dog was studied twice in randomized order. Protocol 1: MTZ (3 mg/kg Bolus, followed by 3 mg/kg/h continuously). Protocol 2: Controls, without MTZ. During all experiments dogs breathed spontaneously via a ventilator circuit. First hour: Normoxia (FIO_2=0.21); followed by two hours of hypoxia (FIO_2=0.1). Arterial oxygen tension (PaO_2), mean pulmonary artery pressure (MPAP), pulmonary vascular resistance (PVR) were determined at the end of each experimental hour. Data are given as means ± SEM; $p< 0.05$ (GLM ANOVA). Results: During hypoxia PaO_2 was 36 ± 1 mmHg in Controls and 38 ±1 mmHg with MTZ. In Controls, MPAP increased from 12 ± 1 to 19 ± 1 mmHg and PVR increased from 256 ± 31 to 451 ± 42 dyn·s·cm^{-5}. With MTZ, MPAP was 11 ± 1 mmHg and PVR 244 ± 20 dyn·s·cm^{-5} during normoxia and 14 ± 1 mmHg and 324 ± 33 dyn·s·cm^{-5} during hypoxia. Conclusion: MTZ reduces HPV but it does not prevent HPV completely in this animal model. As compared to ACZ, the additional methyl-group in MTZ may slightly impair its capability to act on ACZ-sensitive cellular receptors or channels. 2009.

EFFECTS OF MODERATE ALTITUDE ON OXYGEN DEBT, OXYGEN DEFICIT, AND THE ONSET OF BLOOD LACTATE DURING EXERCISE. John Davis[1], Bailey Gooding[1], Jaycee Cole[1], Danielle Hicks[1], Morgan Clark[1]. [1]Alma College, Alma, Michigan. *EMAIL: davisj@alma.edu* INTRODUCTION: It has been previously reported that lactate metabolism is altered by exposure to altitude. To date, no studies have looked at the relationship between the onset of blood lactate (OBLA) and oxygen deficit and debt after acclimatization to altitude. METHODS: Six subjects (26 ± 13.3 yrs) underwent graded-exercise tests to exhaustion on a

cycle ergometer at sea level (SL1), upon acute exposure to 3400 m (ALT1), two weeks following acclimatization at 3400 m (ALT2), and upon return to sea level (SL2). Workloads were increased every two minutes following a two-minute warmup until volitional fatigue. For each test, maximal oxygen consumption, maximum blood lactate levels (MBL), OBLA and the oxygen deficit were measured, ultimately to compare the oxygen deficit to the oxygen debt. Breath-by-breath oxygen consumption values were measured before each exercise, during each workload, and after the exercise to calculate the oxygen deficit and debt. Venous blood lactate measurements were taken at each workload and for 6 minutes after the exercise. RESULTS: At ALT1 time point, VO_2max (-4.7 ml/kg/min) and MBL (-1.3 mmol) decreased significantly in comparison to SL1 ($P<0.05$). VO_2max returned to SL1 values by ALT 2, but MBL remained depressed through ALT2. At the same time, OBLA was not different between SL1 and ALT1 or ALT2 ($P>0.05$). The O_2 deficit and debt were both decreased from SL1 to ALT1 (Deficit = 18.7± to 15.1 ± 4.3 liters, Debt = 3.5 to 3.0 liters ± .7 liters) and returned to SL1 values by SL2 (Debt = 19.4 ± 10.2 liters, Deficit = 3.43 ± .9 liters). CONCLUSION: These data suggest that while OBLA was not affected by either acute or chronic exposure. Furthermore, the O_2 debt and deficit were depressed at moderate altitude as was VO_2max. Taken together, it appears as if moderate altitude does not influence the rate of lactate accumulation at low exercise levels, but does during maximal exercise. In contrast, O_2 adjustments to graded exercise and maximum aerobic capacity were both influenced by moderate altitude exposure. 2015.

EFFECTS OF MODERATE ALTITUDE TRAINING ON THE 1,500-M RACE PERFORMANCE OF ELITE JUNIOR SPEED SKATERS. Taketerru Maegawa[1], Hidenobu Kobai[2], Natsumi Suzuki[2], Toshiyuki Homma[2], Toshiharu Yokozawa[2]. [1]Dept Movement Sciences, Japan Women's College of Physical Education, Tokyo, Japan, [2]Dept Sports Sciences, Japan Institute Sports Sciences, Tokyo, Japan. *EMAIL: maegawa.taketeru@jwcpe.ac.jp* INTRODUCTION: The purpose of this study was to evaluate the effects of moderate altitude training on the 1,500-m race performance of elite junior speed skaters. METHODS: The subjects in this study were five junior speed skaters (2 boys and 3 girls) from the junior national skating team of Japan (age range, 15–18 years). They have participated in international competitions such as the Speed Skating Junior World Cup. The subjects had to live at altitudes of 1,100 m, wherein they underwent training for two weeks. This altitude training was performed during the tapering phase. On the first and the ninth day after the end of altitude training (A1 and A9, respectively), they performed the 1,500-m race on an indoor 400-m speed skating track. A1 was the official time trial, and A9 was the Japan Single Distance Speed Skating Championship. The blood lactate concentration (BLa) was measured after both the races. RESULTS: At A1, four of the five skaters broke their best individual record (BR) accomplished at the same track earlier. The average velocity in the 1,500-m race improved from 12.43 ± 0.84 m/s to 12.54 ± 0.78 m/s ($p = 0.057$). Moreover, at A9, all skaters broke their BR further, and the average velocity increased to 12.70 ± 0.79 m/s ($p < 0.001$ vs. BR, $p < 0.01$ vs. A1). The maximal BLa at A9 was significantly higher than that at

their BR ($p < 0.05$) and at A1 ($p < 0.01$). The average velocity and maximal BLa were significantly correlated in both boys and girls. CONCLUSION: In conclusion, the exposure to moderate hypobaric hypoxia during the regular tapering program of well-trained junior speed skaters produced worthwhile gains in the 1,500-m race performance, possibly through enhanced glycolysis. 2015.

EFFECTS OF NORMOBARIC HYPOXIA ON DYNAMIC EQUILIBRIUM. Dale Wagner[1], John Davis[2], Skyler Saunders[1], Brady Robertson[1]. [1]Utah State University, Logan, UT, USA, [2]Alma College, Alma, MI, USA. *Email: dale.wagner@usu.edu.* Introduction: Dizziness or lightheadedness, a common symptom following rapid ascent to high altitude, could result in diminished equilibrium. However, the effect of hypoxia on balance has not been well studied. The purpose of this study was to compare the effects of varying levels of nomobaric hypoxia on equilibrium before and after exercise. Methods: Following a familiarization trial, 12 males (27.3 ± 7.1 y) completed 3 sessions in random order on a NeuroCom SMART Balance Master: a sham trial at the ambient altitude of 1400 m (LOW) and simulated altitudes of 3000 m (MID) and 5000 m (HIGH) created by a hypoxic generator. The NeuroCom provided a sensory organization test (SOT) of equilibrium, and a motor control test (MCT) to assess the appropriate motor response. Composite scores were generated for both SOT and MCT. Additional information on somatosensory, visual, and vestibular responses was obtained. Each session consisted of 20 min of rest followed by the NeuroCom test, then 10 min of exercise (5 min walking at 3 mph and 5 min running at 6 mph) followed by a second NeuroCom test, all while connected to the hypoxic generator. Mean differences were identified with a two-way (pre/post exercise and altitude condition), repeated-measures ANOVA. Results: The composite SOT score was significantly lower ($p < 0.001$) during the HIGH condition (73.4 ± 12.0) compared to the LOW (80.8 ± 7.0) and MID (84.1 ± 5.0) altitudes. The inability to ignore inaccurate visual cues in a situation of visual conflict was the most common SOT error during the HIGH trials. MCT was not affected by altitude ($p = 0.16$) nor exercise ($p = 0.34$). Conclusion: Moderate hypoxia does not affect balance, but severe hypoxia significantly reduces equilibrium. Furthermore, it appears that the alterations in equilibrium are primarily from impairments in visual function. 2015.

EFFECTS OF SUSTAINED HIGH-ALTITUDE HYPOXIA AND AMS ON CEREBRAL DIFFUSION AND EDEMA. John Hunt[1], Rebecca Theilmann[1], Billy Hsu[1], Ethan Li[1], Zachary Smith[1], Miriam Scadeng[1], David Dubowitz[1]. [1]University of California San Diego, USA. *Email: dubowitz@ucsd.edu*. Introduction: There is overlap of symptoms between cerebral edema and uncomplicated acute mountain sickness (AMS) suggesting a common etiology. Previous hypoxic chamber studies at sea-level (normobaric hypoxia) produced a mixed picture of cytotoxic and vasogenic edema, but simulated acute high-altitude (hypobaric hypoxia) showed vasogenic edema. Objective: To investigate the impact of sustained hypobaric hypoxia on cerebral water using 3T MRI measures of diffusion following 2- and 7-days at high altitude. Methods: 18 subjects were recruited and grouped by Lake Louise

Scores (LLS) during the first 24hrs at altitude into; no AMS (LLS <=4, n=8) or clear symptoms of AMS (LLS >=8, n=8). Subjects with intermediate values were not included in further analysis (n=2). 16 subjects were studied (30.4 +/- 9 years, F=8, M=8). Apparent Diffusions Coefficient (ADC) measurements were made at normoxia, and while hypoxic following sustained hypoxia at White Mountain Research Station (3,800m altitude, mean SaO_2 85.4% at 2-days, 87.9% at 7-days). ROIs were selected in gray matter, white matter, Basal Ganglia (Caudate nucleus), and Corpus Callosum (Splenium). (Data reported as mean +/- s.d.) Results: There was a significant increase in ADC after 7-days hypoxia (vasogenic edema) in Basal Ganglia (+17.0 x10-6 mm2/s, $p<0.05$) and in Splenium (+27.2 x10-6 mm2/s, $p<0.05$). Other ROI did not show significant changes with duration of hypoxia or presence/absence of AMS. Conclusion: High altitude hypoxia results in vasogenic edema of Basal Ganglia and Splenium. No cytotoxic edema (decreased ADC) was seen for any subject, even with significant AMS. These findings differ from prior sea-level chamber studies. Normobaric and hypobaric hypoxia appear to have different effects on brain water. Our results indicate barometric pressure and hydration may be important covariables in the cerebral response to hypoxia. Acknowledgements: Supported by: NIH R01 NS053934 (DD). 2011.

EFFECTS OF VARYING LEVELS OF NORMOBARIC HYPOXIA ON MUSCLE OXYGENATION DURING LEG EXERCISE. John Davis[1], Dale Wagner[2], Michael Rees[2]. [1]Alma College, Alma, Michigan, [2]Utah State University, Logan, Utah. *Email: davisj@alma.edu.* Introduction: Previous studies have looked at muscle tissue oxygenation under a wide range of conditions including temperature extremes and in various disease states. The purpose of this study was to examine the muscle tissue saturation response under varying normobaric hypoxic conditions during different intensities of aerobic exercise (walking and running). Methods: Twelve healthy, fit participants (27.3 ± 7.1 yrs , 87.5 ± 10.2 kgs) were recruited and completed testing randomly in three different conditions simulating varying levels of hypoxia: 3000 m (MID), 5000 m (HIGH) and while breathing ambient air (LOW). A Higher Peak Mag10® hypoxic generator was used to produce normobaric hypoxia. During the HIGH, MID, and LOW conditions subjects walked for 5 min at 3 mph and then ran for 5 min at 6 mph on a motorized treadmill. Near-infrared spectroscopy (Portamon, Artinis Inc.) was used to measure oxygenated hemoglobin, deoxygenated hemoglobin, total hemoglobin, and tissue saturation index (TSI) throughout the exercise. The Portamon was placed over the vastus lateralis and secured. TSI is used as an index of muscle tissue oxygenation. In this study, the slope of the decline in TSI was determined and used to quantify the muscle oxygenation response. Results: Overall, TSI decreased at a greater rate for running than walking in all three conditions. The decline in TSI was greatest ($p<0.05$) in the HIGH running condition (-0.023 ± .006) compared to the MID (-.018± .004) and LOW conditions (-.007± .002). There were no differences in TSI slope for the three walking conditions. Conclusion: These data suggest that muscle oxygenation declined at the fastest rate with running at the greatest level of hypoxia. This probably reflects a greater utilization of oxygen under severe hypoxic conditions in the muscle. Furthermore, running during all

levels of hypoxia produced a greater rate of oxygen utilization than walking. Taken together, running at high altitude provides the greatest hypoxic stress to exercising leg muscle. 2015.

EFFECTS ON ARTERIAL OXYGEN PRESSURE FOLLOWING WHOLE-BODY VIBRATION AT ALTITUDE. Tor Hansen. Norwegian Armed Forces Medical Services Institute of Aviation Medicine. *Email: t.a.s.hansen@flymed.uio.no*. Introduction: Most helicopter operations in the Royal Norwegian Air Force (RNoAF) are carried out at altitudes below 10 000 ft. At these altitudes, there is low risk of the crew experiencing hypoxia. For that reason, supplementary oxygen is not standard equipment onboard RNoAF helicopters. Due to RoNAF's engagement in Afghanistan, high-altitude operations have become more frequent - as have the chances of the crew experiencing hypoxia. Altitudes up to 10000 ft are considered safe with respect to hypoxia. However, there are indications that air-crew do get significantly oxygen-desaturated at 10000 ft., particularly crew members that are physically active during flight. These findings indicate that relatively low physical activity can cause desaturation under conditions that are normally considered safe. Helicopter crew is subjected to a high load of whole-body vibration compared with crew of fixed-winged aircraft. Whole-body vibration increases muscle work, with increased oxygen consumption as a result. We hypothesize that whole-body vibration, experienced by helicopter crew will cause additional oxygen desaturation under hypoxic conditions. Methods: Data was collected from ten subjects. They were all exposed to six different pressure altitudes, ranging from 1000 ft. to 16000 ft. Arterial blood-gasses were drawn following 15 minutes of rest, followed by 15 minutes of whole-body vibration (17 Hz at 1.1 ms^-2), at each altitude. The subjects were breathing ambient air at all times. Results: There is no significant effect of whole-body vibration on arterial oxygen pressure, at altitudes up to 16000 ft. Conclusion: We contribute the lack of effect to the low vibration intensity used in this study. Since this vibration intensity is higher than is experienced by helicopter crew during flight, we conclude that whole-body vibration does not contribute to hypoxia during high altitude operations. Acknowledgements: Study funded by Norwegian Armed Forces. 2011.

ELECTROCARDIOGRAPHIC CHANGES IN HEALTHY INDIVIDUALS DURING A PROLONGED STAY ON MT EVEREST. George Rodway[1], Jeremy Windsor[2], Mike Grocott[2], Hugh Montgomery[2], Caudwell Xtreme Everest Research Group[2]. [1]Univ of Utah, SLC, UT; University College London, London, England, UK, [2]University College London, London, England, UK. *Email: gwrodway@hotmail.com*. Introduction: Whilst multiple changes in the human electrocardiogram (ECG) are known to occur on acute ascent to altitude, few studies have examined responses over longer periods of exposure, nor their resolution upon returning to low altitude. We sought to address these issues. Methods: Resting 12-lead ECGs were recorded in 16 men at 75m, on three occasions over a period of 60 days at 5300m, and again within 3 days of return to 1300m. Results: All of the participants remained in sinus rhythm throughout each recording. Resting heart rate

(HR) and QT interval corrected for heart rate (QTc) increased on ascent to altitude. HR increased from 60.3 +/- 15.2 beats per minute (bpm) to 72.8 +/- 13.0 ($P<0.05$), whilst QTc rose from 417.7 +/- 31.44 to 436.5 +/- 17.0 ($P<0.05$). On descending to low altitude these measurements returned to baseline values. Changes in QRS axis, P and T wave amplitudes were also found to occur at altitude. QRS Axis increased from 51.6 +/- 49.3 degrees to 74.1 +/- 65.4 degrees (NS), the sum of the P waves in II, III and aVF rose from 3.1 +/- 1.3 mV to 4.2 +/- 1.2 mV ($P<0.05$) and T wave amplitude in V1 decreased from 1.2mV +/- 1.6 mV to -1.3mV +/- 2.1mV ($P<0.05$). Although the QRS axis and the sum of the P wave amplitudes rapidly returned to normal following descent, the T wave amplitude in V1 remained below baseline values ($P<0.05$). No other morphological changes were observed. Conclusion: A prolonged stay at extreme altitude can result in marked ECG changes in healthy male adults. Although most of these changes resolve rapidly on descent, others can persist at lower altitudes. Acknowledgements: CXE research and logistics was funded from: John Caudwell; BOC Medical; Eli-Lilly Critical Care; Smiths Medical; Deltex Medical; The London Clinic; Rolex; and CXE volunteer trekkers. 2011.

ELECTRORETINOGRAPHIC ASSESSMENT OF RETINAL FUNCTION AT HIGH ALTITUDE. Gabriel Willmann[1], Andreas Schatz[1], Manuel Dominik Fischer[1], Kai Schommer[2], Andre Messias[3], Eberhart Zrenner[1], Karl-Ulrich Bartz-Schmidt[1], Florian Gekeler[1]. [1]Centre for Ophthalmology, Univ Tübingen, Tübingen, Germany, [2]Dept Sports Medicine, Medical Clinic, Univ Hospital Heidelberg, Heidelberg, Germany, [3]Dept Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, School of Medicine of Ribeirão Preto-Univ São Paulo, São Paulo, Brazil. *EMAIL: Gabriel.Willmann@googlemail.com* INTRODUCTION: Retinal hypoxia constitutes an important pathology in many potentially blinding retinal diseases. However, little is known about the effect of high altitude hypoxia on retinal function of healthy subjects. The current study investigates retinal function during high altitude hypoxia. This work is related to the Tübingen High Altitude Ophthalmology (THAO) project. METHODS: Electroretinography (ERG) was performed on 14 subjects in Tübingen (341 m) and at high altitude at the Capanna Margherita (4559 m) using a Ganzfeld ERG. Extended ERG protocols were used to estimate retinal function on a cellular level. Oxygen saturation (SpO_2), heart rate and scores of acute mountain sickness (AMS-c) were ascertained. Comparisons between baseline and high altitude measurements and correlations between ERG parameters and SpO_2, heart rate and AMS-c were performed. RESULTS: Significant differences of the maximum response for the rod sensitivity function (Naka and Rushton), the b-wave amplitude and implicit times of a- and b-wave amplitudes of mixed rod-cone responses were detected between high altitude and baseline. The a-wave slope was significantly lower at high altitude. The photopic ERG revealed an impairment of the photopic negative response and the i-wave. The strongest significant correlation was found for PhNR and SpO_2 ($r=0.68$; $p<0.05$). However, of all parameters photopic b-wave implicit time (10 cd.s/m2) correlated with AMS-c ($r=0.57$; $p<0.05$). CONCLUSION: High altitude hypoxia induced a functional impairment of inner, outer and ganglion

cell layers. Interestingly, most of the functional impairment was detected in a subset of ERG parameters related to rod–cone interaction. ACKNOWLEDGEMENTS: This study was supported by the Wilderness Medical Society (WMS), Novartis, Bausch & Lomb, Alcon, Allergan, Ursapharm, Optima Pharma, IMS Gear and Diagnosys LLC. 2015.

ELEVATED INTRACRANIAL PRESSURE IS ASSOCIATED WITH INCREASED RIGHT ATRIAL PRESSURE, DECREASED CEREBRAL PERFUSION PRESSURE AND DECREASED CAROTID ARTERY FLOW IN A SWINE MODEL OF ACUTE HYPOXIA. Todd Larabee[1], Jenny Campbell[1], Charles Little[1]. [1]University of Colorado Denver SOM, Denver, Colorado, USA. *Email: Todd.Larabee@ucdenver.edu*. Introduction: The physiologic mechanisms causing elevated intracranial pressure (ICP) associated with acute hypoxia are poorly defined. Elevated ICP is implicated in severe acute mountain sickness (AMS) and high altitude cerebral edema (HACE). Objective: To compare baseline physiologic parameters related to ICP, cerebral perfusion, and cerebral venous drainage to those measured after exposure to acute hypoxia in a swine model. Methods: Sixteen swine were monitored and instrumented with central aortic (AOP), right atrial (RAP) and saggital sinus (SSP) pressure-transducing catheters while under general anesthesia. An arterial flow probe was placed on a carotid artery (CAF). Continuous EKG, AOP, RAP, SSP and CAF readings were digitized and recorded to disk. Continuous cerebral perfusion pressures (CePP) and mean arterial pressures (MAP) were calculated and recorded. The animals were then ventilated with a hypoxic gas mix (10% O2/90% N2). Baseline MAP, mean CePP, mean SSP, mean CAF and mean RAP measured over one minute prior to hypoxia exposure were compared with values measured over one minute after 20 minutes of hypoxia. Comparisons were made using paired T-tests ($p<0.05$ considered significant). Results: Mean SSP (19.7 vs. 26.3 mmHg, $p<0.001$) and mean RAP (9.6 vs. 17.1 mmHg, $p<0.001$) were significantly elevated from baseline after acute hypoxia exposure. Mean CePP (44.9 vs. 16.1 mmHg, $p<0.001$), CAF (344.5 vs. 237.3 ml/min, $p<0.001$), and MAP (64.6 vs. 42.4 mmHg, $p<0.001$) were significantly decreased from baseline after acute hypoxia exposure. Conclusion: Elevated ICP is associated with elevated mean RAP and with decreased mean CePP, MAP and mean CAF in this swine model of acute hypoxia. These findings suggest that hemodynamic parameters related to cerebral venous drainage (RAP) are important mechanistically for developing elevated ICP after exposure to acute hypoxia. This model may provide insights into the pathophysiology of AMS and HACE. Acknowledgements: This work was supported by NIH/NHLBI-SBIR grant #2R44HL080826-03A1. 2011.

ELEVATED LEVELS OF UTEROGLOBIN AS A BIOMARKER FOR OZONE-INDUCED LUNG INJURY IN MOUNTAINEERS DIAGNOSED WITH HAPE IN THE HIMALAYA. John Semple[1], Andrea Conroy[1], Kent Moore[1], Molly Murphy[2], Prativa Pandey[2], Paolo Cristofanelli[3], Paolo Bonasoni[3], Kevin Kain[1]. [1]Univ Toronto, Toronto, Canada, [2]CIWEC Clinic, Kathmandu, Nepal, [3]National

Research Council, Bologna, Italy. *EMAIL: john.semple@wchospital.ca*
INTRODUCTION: Exposure to ozone (O3) impairs lung function, induces airway inflammation and alters epithelial permeability. We have previously shown that pre-monsoon O3 levels on Mount Everest are similar to those found in industrialized cities. The appearance of uteroglobin in plasma has been proposed as a sensitive marker of lung epithelial injury specific to O3 exposure. Here, we use uteroglobin as a biomarker of O3 exposure injury in mountaineers from the Himalaya diagnosed with AMS, HACE or HAPE. Healthy climbers were used as controls. Surface and satellite O3 levels from the Himalaya were established during same exposure periods. METHODS: Participant climbers evacuated from the Himalaya were treated at CIWEC Clinic in Kathmandu. Plasma samples from climbers with AMS (n=54), HACE (n=17), HAPE (n=71), or controls (n=50) were tested for uteroglobin by ELISA. Presentation history, Lake Louise Scoring, altitude and demographic details were also collected. O3 levels were established from TOMS satellite archival data and data from the PYRAMID Station (5,079m) in the Khumbu Valley during the period of observation. RESULTS: Median uteroglobin plasma concentrations were significantly increased in climbers diagnosed with HAPE (14.8ng/mL) compared to healthy climbers (11.1ng/mL) or climbers with AMS (11.1ng/mL) (p=0.04 and 0.05 respectively by Mann Whitney U test with Holm's correction for multiple comparisons). The difference in uteroglobin levels between HAPE vs. HACE was not significant (p=0.09). A positive association was observed between increased uteroglobin concentrations and O3 levels where 30 Dobson Units more total column ozone was observed (10% above mean) on enrollment dates of climbers diagnosed with HAPE. CONCLUSION: Uteroglobin may have clinical utility as a biomarker for ozone-induced lung epithelial damage in association with HAPE. Health-effects of air pollutants at high altitude are compounded by cold, hypoxia and hyperventilation. Further studies are required to elucidate these mechanisms further. ACKNOWLEDGEMENTS: K Kain, CIHR, Canadian Research Chair. 2015.

EMERGENCE OF ANESTHESIA ON 100% OXYGEN CAUSES POSTOPERATIVE HYPOXEMIA IN COPD GOLD 2 PATIENTS. Susanne Truebsbach[1], Kleinsasser Axel[1]. [1]Dept Anesthesiology & CCM, MUI Innsbruck. *EMAIL: axel.kleinsasser@i-med.ac.at*
INTRODUCTION: During emergence from anesthesia, breathing 100% oxygen is frequently used to provide a safety margin towards hypoxemia in case an airway problems occurs. Oxygen breathing has been shown to cause pulmonary gas exchange disorders in healthy individuals. This study investigates how oxygen breathing during emergence affects lung function in patients with chronic obstructive pulmonary disease (COPD). METHODS: Fifty-three patients with an FEV1/FVC < 0.70 and an FEV1 < 80% of the predicted value were randomly allocated to breathe either 100% or 30% oxygen balanced with nitrogen during emergence of anesthesia. Arterial blood gas measurements were taken before induction and at 5, 15 and 60 minutes after extubation. RESULTS: Patients treated with 100% oxygen had a higher alveolar-arterial oxygen pressure gradient ($AaDO_2$) compared to patients treated with 30% oxygen (25 vs. 20 mmHg) and compared to their baseline at the 60 minute measurement (25 vs. 17 mmHg). At

the 60 minute measurement, arterial partial pressure of oxygen was lower in the 100% group (62 vs. 67 mmHg). Arterial partial pressure of carbon dioxide and pH were not different between groups or measurements. CONCLUSION: In this experiment we examined oxygen breathing during emergence - a widely practiced maneuver known to generate pulmonary blood flow heterogeneity. In the observed cohort of patients already presenting with pulmonary blood flow disturbances, emergence on oxygen resulted in deterioration of oxygen-related blood gas parameters – or put differently in an intensification of the preoperative pathology. In the perioperative care of patients with COPD, oxygen breathing during emergence may need reconsideration. ACKNOWLEDGEMENTS: This study has been performed on institutional funding only. 2015.

END-TIDAL TO ARTERIAL PO_2 GRADIENT WITH GRADED HYPOXIA IN ACUTELY ACCLIMATIZED LOWLANDERS AND ANDEAN HIGHLANDERS AT 3600M. Alexandra Mardimae, Marat Slessarev, David Preiss, Dahlia Balaban, Alex Vesely, Jay S Han, James Duffin, Gustavo Zubieta-Calleja, Luis Zubieta-Calleja, Richard Greene, Joseph A Fisher. University of Toronto, University of British Columbia, New Mexico Highlands University. *Email: mardimae@gmail.com.* Approximation of PaO_2 from end-tidal PO_2 ($PETO_2$) would aid altitude research. We have previously shown that ventilating via a sequential rebreathing circuit eliminates the $PETCO_2$ - $PaCO_2$ difference, through reducing regional heterogeneity in pulmonary gas concentrations. The purpose of the current study was to quantify the $PETO_2$ - PaO_2 gradient (PO_2-Diff) while ventilating through a sequential rebreathing circuit throughout a range of hypoxic PO_2 levels applied at altitude. A system for prospectively targeting end-tidal PCO_2 ($PETCO_2$) and $PETO_2$ (RespirAct™ TRI, Toronto, Canada) was used to control end-tidal gas values in 5 healthy lowlanders (L) (2 females) following 2 weeks at 3600m, and 8 chronically acclimatized Andean natives (H) (3 females). $PETCO_2$ was maintained at subjects' resting levels while $PETO_2$ was targeted at 100, 70, 60, 50, and 40 mmHg in steps lasting 2 minutes. An arterial sample was drawn in the final 30 seconds of each step and analyzed for PO_2. We graphed $PETO_2$ vs. PO_2-Diff. The line of best fit through these points was PO_2-Diff = 0.21*($PETO_2$) - 4.7 for L and PO_2-Diff = 0.19*($PETO_2$) - 6.3 for H. At $PETO_2$ = 100, the calculated PO_2-Diff is 16.4 mmHg for L and 13.0 mmHg for H. At $PETO_2$ = 40, the calculated PO_2-Diff was 3.8 mmHg for L and 1.4 for H. We attribute this progressive decrease in PO_2-Diff with severity of hypoxia to the intensification of hypoxic pulmonary vasoconstriction and reduction in shunt. 2009.

ENDURANCE PERFORMANCE IS MORE DECREASED IN HYPOBARIC HYPOXIA THAN IN NORMOBARIC HYPOXIA. Jonas Saugy[1,2], Thomas Rupp[3,4], Raphael Faiss[1,2], Alexandre Lamon[1], Nicolas Bourdillon[1,2], Gregoire Millet[1,2]. [1]Institute of Sport Sciences, Faculty of Biology and Medicine, University of Lausanne, Switzerland, [2]Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Switzerland; [3]Joseph Fourier University & CHU Grenoble, HP2 Laboratory, F-38043, Grenoble, France; [4]National Institute for

Health and Medical Research, U1042, F-38043, Grenoble, France. *Email: jonas.saugy@unil.ch.* Introduction: Recently research has focused on the potential differences between normobaric hypoxia (NH) and hypobaric hypoxia (HH). Slight physiological differences between acute exposure in NH and HH have been reported. Taken together, these differences suggest different physiological responses to hypoxic exposure in simulated (NH) versus real altitude (HH). On purpose, in the present study we aimed to directly compare the time-trial performance after acute hypoxia exposure (26 h, 3450 m) with the same subjects in three different conditions: normobaric hypoxia, hypobaric hypoxia and normobaric normoxia. Regarding all precedent studies made on differences between these hypoxic conditions, we hypothesized greater performance impairment in HH compared to NH. Methods: The experimental design consisted of three sessions: NN (Sion, FiO_2 20.93), NH (Sion, hypoxic room, FiO_2 13.8, BP 716 mmHg), HH (Jungfraujoch, FiO_2 20.93, BP 478 mmHg). The performance was evaluated at the end of each session with a cycle time-trial of 250 kilojouls. Results: The mean of TT duration in NN was significantly lower than the two hypoxic conditions ($P<0.001$). In addition, the mean duration in NH was significantly lower than in HH ($P<0.01$). The mean of SpO_2 during the TT was significantly lower for HH than NH ($P<0.05$), and was significantly higher in NN than for the two other sessions ($P<0.001$). Conclusion: As previously suggested, HH seems to be a more stressful stimulus, and NH and HH should not be used interchangeability with endurance performance as main objective. The main factor of this performance difference between hypoxic conditions seems to be the lower peripheral oxygen saturation in HH at rest and further during the exercise. Further research is needed to better understand the physiological mechanisms responsible for these differences, as cerebral blood flow, muscle oxygenation or pacing strategies. 2015.

EPO PLAYS A KEY ROLE IN THE NEURAL CONTROL OF HYPERCAPNIC VENTILATORY RESPONSE. Jeton Florine[1], Pichon Aurélien[1], Marchant Dominique[2], Quidu Patricia[2], Soliz Jorge[3], Richalet Jean-paul[1], Voituron Nicolas[1]. [1]Paris 13 University, Sorbonne Paris Cité, UFR SMBH, Laboratory "Hypoxia and lung", EA 2363, 93017 Bobigny, France - Laboratory of Excellence (Labex) GR-Ex, PRES Sorbonne Paris Cité, [2]Paris 13 University, Sorbonne Paris Cité, UFR SMBH, Laboratory "Hypoxia and lung", EA 2363, 93017 Bobigny, France, [3]Department of Pediatrics, Centre de Recherche de I'Hôpital St-François d'Assise (CR-SFA), Centre Hospitalier Universitaire de Québec (CHUQ). *Email: florine.jeton@gmail.com.* Introduction: Erythropoietin (Epo) is involved in the hypoxic ventilatory response but its effect on the hypercapnic ventilatory response remains to be determined. The objective was therefore to assess the role of Epo on the ventilatory response to hypercapnia (HcVR) and establish if Epo has an effect on peripheral and/or central ventilatory pathways. Methods: HcVR was evaluated by whole body plethysmography on intact Epo-deficient (Epo-TAgh) mice and intact or chemodenervated wild type (WT) mice after intravenous (IV) or intracisternal (IC) injection of Rhu-Epo. Furthermore, histological approach was used to determine the respiratory areas involved in this process. Results: In normocapnia, female

Epo-TAgh had higher ventilation compared to other groups. In hypercapnia, WT and Epo-TAgh mice showed hyperventilation but we observed different ventilatory patterns between WT and Epo-TAgh: hypercapnia induced an increase of tidal volume in WT and an increase of tidal volume associated with an increase of breathing frequency in Epo-TAgh. Histological approach showed that several brainstem areas involved in CO_2 response were normally activated in WT mice but were understimulated in Epo-TAgh mice. Injection of Epo (IV and IC) decreased HcVR only in Epo-TAgh females. Conclusion: Epo deficiency alters the ventilatory response to CO_2 due to weak response of the ventral surface of the medulla and modification of the ventilatory pattern without any impairment in the quantitative HcVR. This suggests the implication of other respiratory areas in CO_2 response and a plasticity of the respiratory network. Moroever, Epo could interact with sexual hormones. Acknowledgements: This work was partially financed by the Red Cell Group of Excellence. 2015.

EV-DVT: THE MOUNT EVEREST DEEP VENOUS THROMBOSIS. Ken Zafren, Joanne Feldman, Robert J Becker. Stanford University School of Medicine and Himalayan Rescue Association. *Email: zafren@alaska.com.* Objective. This study was done to determine the incidence of deep venous thrombosis (DVT) in asymptomatic high altitude climbers. Methods. On-site personnel enrolled a convenience sample of climbers at Mt. Everest Base Camp, elevation 5340 m (17,500 ft), during the 2006 spring climbing season. The subjects completed a questionnaire to evaluate their risk factors for DVT, and then a D-dimer test was performed in asymptomatic individuals. If D-dimer test was negative, DVT was ruled out. Ultrasound was available to perform lower extremity compression ultrasounds to evaluate for DVT in case D-dimer was positive. Results. 76 high altitude climbers were enrolled. There were no positive D-dimer tests. Conclusion. The absence of positive D-dimer tests suggests that there is a low incidence of DVT in asymptomatic high altitude climbers. 2009.

EVALUATION OF THE BALANCE ERROR SCORING SYSTEM (BESS) TEST IN THE DIAGNOSIS OF ACUTE MOUNTAIN SICKNESS AT 4380 M. Martin MacInnis[1], Jim Rupert[1], Michael Koehle[1]. [1]University of British Columbia. *Email: mmacinni@interchange.ubc.ca.* Introduction: Ascent to altitude is associated with a decrease in balance; however, in previous studies, the effect of acute mountain sickness (AMS) status on balance is variable depending on the test used and the altitude at which the test is performed. We tested the utility of the balance error scoring system (BESS) test in the diagnosis of AMS at 4380 m (Gosainkunda, LangTang, Nepal). Methods: Subjects (n=37) were recruited through the Himalayan Rescue Association temporary medical clinic at the 2010 Janai Purnima festival. All of the subjects completed a shortened BESS test (mBESS) while a subset (n=27) completed the full BESS test. Physicians trained in high altitude medicine made the AMS diagnoses using the Lake Louise Score (LLS). Pulse oximetry was used to measure heart rate and oxygen saturation, and blood pressure was measured by sphygmomanometer.

Balance test scores and physiological measurements were compared between AMS+ and AMS- groups. Receiver-operator characteristic (ROC) curves were used to assess the potential of the BESS test as a diagnostic tool. Results: Subjects with AMS scored significantly higher on the mBESS (6.6 ± 3.5 vs. 2.7 ± 1.7, P = 0.018) and BESS tests (19.2 ± 8.8 vs. 10.4 ± 6.0, p = 0.001) indicating inferior balance. Both mBESS and BESS test scores were correlated with AMS status (R^2=0.561 and R^2=0.293, respectively), but the area under the ROC curve was significantly greater for the BESS test (0.895 vs. 0.690, p=0.02), implying that the full BESS test had a greater value as a diagnostic test. Conclusion: With this study, we have demonstrated the BESS test to be an easily administered and portable field test that could be useful in the diagnostic and prognostic assessment of AMS. Acknowledgements: We would like to thank the Himalayan Rescue Association for assisting with translation, AMS diagnosis, and subject recruitment. MM is the recipient of a UBC Four Year Fellowship and a UBC Affiliated Fellowship. 2011.

EVIDENCE OF RESPIRATORY SYSTEM REMODELING IN A COMPETITIVE FREEDIVER. Leigh Seccombe[1], Christine Jenkins[1], Peter Rogers[1], Mark Pearson[2], Matthew Peters[1]. [1]Thoracic Medicine, Concord Hospital, Sydney, Australia, [2]Nuclear Medicine, Concord Hospital, Sydney, Australia. *EMAIL: leigh.seccombe@sswahs.nsw.gov.au* INTRODUCTION: Glossopharyngeal insufflation (GI) is commonly employed by freedivers to increase oxygen stores for depth and duration events. Lung barotrauma has been associated with GI, which raises the possibility that use of this technique results in significant lung damage and long-term physiological impairment. We sought to characterise the nature of any changes in the respiratory system that may be associated with GI, using physiological measurement and imaging. METHODS: The research data from a healthy competitive freediver who practised regular GI training was reviewed. This included respiratory function, including volumes achieved with a maximal GI manoeuvre. Non-contrast computed tomography (CT) of the thorax taken at baseline and following GI on a single occasion were segmented for 3D analysis of lung tissue. RESULTS: Lung function was measured over a period of eight years (from age 25 to 33 years) on four occasions. There was a total >800mL increase in baseline vital capacity, functional residual volume and total lung capacity (TLC). There was no evidence of gas trapping as residual volume remained unchanged, and transfer factor was preserved. The subject was adept at GI, however there was an observed limit to maximal absolute volume achieved above traditional TLC despite ongoing GI training. 3D images of lung tissue demonstrated significant lung volume following GI, with intercostal bulging of lung tissue, mediastinal distortion and flattening of the diaphragm. CONCLUSION: We present a healthy freediving subject with increasing lung volumes associated with repeated performance of an intervention used to enhance athletic performance. However, the upper limit of TLC with GI was stable. The repeated use of GI over time in this case appears to have altered respiratory system mechanics without any functionally important macroscopic lung damage, at least as evidenced by CT scans and measures of gas exchange. 2015.

EVIDENCE THAT DRUGS USED TO TREAT ACUTE MOUNTAIN SICKNESS AND HAVE AN OFF-TARGET EFFECT ON NRF2 INDUCTION MAY BE MORE EFFICACIOUS THAN THOSE THAT DON'T. Christina Lisk[1], Joe McCord[1], Swapan Bose[1], Karyn Hamilton[2], Thies Schroeder[3], Ian Connor[1], Elena Forchielli[1], David Irwin[1]. [1]University of Colorado Denver, [2]Colorado State University, [3]Duke University. *Email: christina.lisk@ucdenver.edu*. Introduction: Acute mountain sickness (AMS) is a well-known syndrome that affects 60%< of individuals ascending to high altitude, yet the etiology is still unclear. Recently it has been proposed that reactive oxygen species (ROS) formed during acute high altitude exposure contribute to brain vascular leak and development of AMS. Current treatments for AMS are targeted at vasodilation, enhancing fluid clearance, and increasing ventilation rate. To date, up-regulation of the antioxidant genome has not been tested. Nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) is a transcription factor that controls transcription of greater than 90% of the body's antioxidant genes. We postulated that some drugs currently used to treat AMS symptoms also have an off target effect on Nrf2 activation. We further hypothesized that inducing Nrf2 activation and increasing antioxidant status, prior to an oxidative stress challenge, would decrease vascular damage from oxidative stress. Methods: A breast cancer MCF7-derived cell line, AREc32, stably transfected with a luciferase gene driven by an antioxidant response element (ARE, the known Nrf2 promoter) was utilized to screen drugs used to treat AMS against a known Nrf2 activator, Protandim. Drugs were also screened for synergism with Protandim and in combination with each other. All compounds were also tested for their ability to decrease brain leak (measured by Evans blue dye) in male Sprague-Dawley rats exposed to high altitude (48 h, 19,000 ft). Results: Protandim, Nifedipine, Methazolamide, and Sitaxentan all showed strong Nrf2 activation and an ability to synergize with Protandim. Yet, the combination of Nifedipine and Methazolamide had the greatest synergism to activate Nrf2. Dexamethasone, Sildenafil, Acetalozamide, Formoterol, and Theophylline, all showed no Nrf2 activation. With the exception of Dexamethasone, only the drugs that showed Nfr2 activation in vitro, decreased brain vascular leak in vivo. Conclusion: Nrf2 activators are effective at reducing brain vascular leak caused by acute high altitude exposures. Our results suggest that due to the off target effects of Nrf2 activation: (1) Methazolamide may offer better protection against AMS, compared to Acetazolamide and (2) In addition to its known vasodilatory activities in the lung, Nifedipine may provide protection against AMS symptoms by decreasing brain vascular leak as well. 2011.

EXERCISE IN NORMOBARIC HYPOXIA IMPROVES CARDIOVASCULAR REHABILITATION IN GERIATRIC PATIENTS. Stephan Pramsohler[1], Nikolaus Netzer[1]. [1]Hermann Buhl Institute for Hypoxia and Sleepmedicine Res. Univ. Innsbruck. *Email: Nikolaus.netzer@uibk.ac.at.* *Introduction:* In the last years training under hypoxic conditions has been under a lot of attention. The evidence suggests that while physical effort seems reduced, training in high altitude can be very effective. Therefore moderate endurance training in altitudes around 3000m could be useful to improve cardiovascular rehabilitation while reducing the physical effort

for geriatric patients. They could benefit especially during early stages of recovery when normal stress is not yet allowed due to physical Impairment after surgery. Methods: 20 patients (11 female, 9 male) from the rehabilitation hospital (Fachklinik Ghersburg für Geriatrische Rehabilitation) attached to the study center were included in the study. The subjects had to be older than 65 years without cognitive impairment assessed via MMSE. After assessment of anthropometric data sonography was performed to determine the ejection fraction (EF). Target pulse was calculated as 80% of VO2 peak. 5bpm were added due to the transfer from cycle to treadmill. The patients had to perform seven trainings during their three weeks stay in the hospital. Each training included a stay of at least 30 minutes inside the altitude chamber. According to the assigned group (experimental / control) the altitude chamber was either containing hypoxic air equivalent to an altitude of 3000m or sham hypoxia. Results: Evaluating the training we've seen a tendency in effectiveness towards the experimental group. Calculating the mean pulse for each group during the training sessions we've seen that the experimental group could reach the target pulse in almost every session. The control group failed to train on 80% of their Vo2 peak value and stayed about 10% lower even though their physical effort was higher. Results showed a higher EF in the experimental group during posttest. In the control group EF was enhanced by 2.4% while in the experimental group by 8.2%. The maximum heart rate was enhanced in the control group by 3.8 bpm and reduced in the experimental group by 6 bpm. The maximum power in the posttest increased in the experimental group (4.3 Watt). Conclusion: Training for geriatric rehabilitation patients in hypoxia is more effective than in normoxia. 2015.

EXERCISE-INDUCED ARTERIAL HYPOXEMIA AND EXERCISE-INDUCED BRONCHOCONSTRICTION IN COMPETITIVE SWIMMERS. Jane Labreche[1], Eric A. Carter[1], Walter Karlen[2], Michael S. Koehle[1,3], A. William Sheel[1], Donald C. McKenzie[1,3]. [1]The School of Kinesiology, [2]Electrical and Computer Engineering in Medicine Group, [3]Allan McGavin Sports Medicine Centre, The Univ British Columbia, Vancouver, British Columbia, Canada. *EMAIL: labreche9@gmail.com*
INTRODUCTION: Exercise-induced arterial hypoxemia (EIAH) has been shown to occur in approximately 50% of well-trained athletes in a variety of sports, but has not been fully explored in the swimming athlete. Sex differences have been documented with a higher prevalence in females. Pool swimmers, exposed to chlorine on a regular basis, commonly exhibit respiratory dysfunction. This irregular airway response to exercise could be associated with the development of EIAH. Therefore, the purpose of this research was to examine the existence of EIAH and its relationship to exercise-induced bronchoconstriction (EIB) in competitive swimmers. METHODS: Twenty-one (10 male, 11 female) swimmers completed a eucapnic voluntary hyperpnea (EVH) test and an incremental swim test to exhaustion with pre/post exercise spirometry. Metabolic data (VO_2, VCO_2, ventilation and heart rate) along with oxyhemoglobin saturation (SpO_2) were collected throughout exercise. RESULTS: Eleven of the twenty-one subjects (52%) tested positive for EIB. No subjects fulfilled our criteria for EIAH ($SpO_2 \leq 95\%$ or $\geq 3\%$ drop from resting values) despite a small mean drop in SpO_2 from rest to maximal exercise in

both males (99.6%±0.5, 97.1±1.1) and females (99.7%±0.4, 97.5±1.1). CONCLUSION: These findings suggest that the unique nature of swimming exercise provides protection against the development of EIAH in this population. ACKNOWLEDGEMENTS: This project was funded by the BC Sports Medicine and Research Foundation. 2015.

EXHALED CARBON MONOXIDE IS NOT RELATED TO ACUTE MOUNTAIN SICKNESS IN NEPALI PILGRIMS TO 4380 METERS. Eric Carter[1], Martin MacInnis[1], Michael Freeman[2], Bidur Pandit[3], Ashmita Siwakoti[3], Ankita Subedi[3], Utsav Timalsina[3], Nadia Widmer[4], Kamal Thapa[3], Jim Rupert[1], Michael Koehle[4]. [1]School of Kinesiology, Univ British Columbia, Canada, [2]Univ Glasgow, Scotland, [3]Maharajgunj Medical Campus, Institute Medicine, Tribhuvan Univ, Nepal, [4]Faculty of Medicine, Univ British Columbia, Canada. *EMAIL: eric.a.carter@me.com* INTRODUCTION: Humans traveling above 2500 meters must acclimatize to the hypoxia. When acclimatization occurs too slowly for a given rate of ascent, acute mountain sickness (AMS) develops. Our understanding of the pathophysiology of AMS is incomplete. A single physiological variable has yet to show a strong enough relationship to the development of AMS to allow for accurate predictions. Several recent studies have shown an association between smoking and AMS. Fraction of exhaled carbon monoxide (FECO) has been associated with AMS but hemoglobin concentration ([Hb]) was not assessed. The purpose of this study was to determine if the fraction of exhaled nitric oxide (FENO) and/or FECO could predict AMS susceptibility. METHODS: Subjects were recruited in Dhunche (1950m) and assessed for AMS at Lake Gosainkunda (4380m). Subjects were interviewed to determine demographic information and AMS score (Lake Louise Score). FENO, FECO, and [Hb], heart rate (HR), and oxygen saturation (SPO_2) were measured at rest in Dhunche prior to ascent. Data are presented as mean (standard deviation). RESULTS: Subjects suffering from AMS were significantly older (38.41 (15.62) years vs. 30.97 (11.39) years; $n = 85$; $p = 0.03$), had a significantly higher resting HR (93.44 (15.95) bpm vs. 83.87 (13.35) bpm; $n = 87$; $p < 0.01$), and significantly lower [Hb] (14.03 (2.60) g dl-1 vs. 15.26 (2.52) g dl-1; $n = 73$; $p = 0.05$). Smoking status, FENO, and FECO were not significantly associated with AMS. CONCLUSION: It is possible that differences in [Hb] are responsible for the relationship that previous studies have found between FECO and AMS. Our study took [Hb] into account when evaluating FECO in subjects at altitude. Further research is necessary to examine the usefulness of [Hb] as a predictor of AMS. Future predictive indices of risk may benefit from including age, moderate altitude HR and [Hb] as factors. ACKNOWLEDGEMENTS: Funding provided by the B.C. Sports Medicine Research Foundation. 2015.

EXHALED NITRIC OXIDE AND SYSTOLIC PULMONARY ARTERY PRESSURES DURING GRADED ASCENT TO HIGH ALTITUDE. Joseph J Donnelly, Douglas C Cowan, David J Yeoman, Philip N Ainslie, Keith R Burgess, Robin Taylor. Department of Physiology Otago New Zealand, University of Sydney. *Email: donjo492@student.otago.ac.nz.* Ascent to high altitude is associ-

ated with increases in pulmonary artery pressure; however, the mechanisms of this pressure increase are unclear. To further elucidate the importance of nitric oxide (NO) in regulating pulmonary vascular tone during ascent to high altitude, we measured exhaled NO (a summary measure of pulmonary NO production, consumption, and transfer to airways) and systolic pulmonary artery pressure in 11 healthy sea level residents during graded ascent to an altitude of 5050m. Exhaled NO was measured with a handheld nitric oxide analyser (NIOX MINO) and corrected (1). Systolic pulmonary artery pressure was assessed with Doppler echocardiography. Exhaled partial pressure of NO progressively decreased from 16.5 ± 11.4 nm Hg (mean, standard deviation) at sea level to 9.1 ± 5.5 nm Hg at 5050m (P = 0.012), while systolic pulmonary artery pressure increased in a curvilinear fashion from 22.7 ± 3.2 mm Hg at sea level to 38.4 ± 3.4 mm Hg at 5050m (P<0.001). There was a strong negative (r = -0.94) correlation between the mean exhaled NO for each altitude and the associated systolic pulmonary artery pressure (P < 0.001). However, when all individual measurements of NO and pulmonary artery pressure at all altitudes were pooled together, the correlation was weaker (r = -0.228, P=0.06). These findings further support the concept that NO plays a role in the regulation of pulmonary vascular tone and that at high altitude, a decrease in NO availability may lead to pulmonary hypertension. This study was supported by the Otago Medical Research Foundation and the Italian National Research Council who kindly provided use of the EV-K2-CNR research laboratory. 1. Hemmingsson T, Horn A, and Linnarsson D. FENO with NIOX MINO at extreme altitude. Paper presented at American Thoracic Society International Conference 2008. 2009.

EXPOSURE TO INDOOR AIR POLLUTION INDUCES ENDOTHELIAL DYSFUNCTION IN NEPALESE HIGH-ALTITUDE DWELLERS. Lorenza Pratali[1], Rosa Maria Bruno[1], Enrico Duo[2], Kamal Thapa[3], Manuela Bartesaghi[4], Ramesh Sharma[3], Luca Polidori[2], Buddha Basnyat[3], Annaluisa Cogo[2]. [1]Institute Clinical Physiology, Pisa, Italy, [2]Biomedical Sport Studies Center, Univ Ferrara, Italy, [3]Nepal International Clinic, Kathmandu, Nepal., [4]Dept Experimental Medicine, Laboratory of Clinical Physiology and Sport Medicine, Univ Milano-Bicocca, Italy. *EMAIL: lorenza@ifc.cnr.it* INTRODUCTION: The aim of this study was to evaluate endothelial function, in high-altitude dwellers in the Kumbu Valley (Nepal), where outdoor pollution is virtually absent, in relationship to indoor air pollution (IAP). METHODS: 82 subjects without cardiovascular disease (age range 15-65 years, mean age 35.5 ± 14 years, men 38%, hypertention 13%, smokers 5%, obese 6%), living in the Kumbu Valley (Nepal) between 2500-3850m, were enrolled. Endothelial function in the brachial artery was studies by ultrasound technique and automated image analysis (flow-mediated dilation, (FMD), response to glyceryl trinitrate, (GTN). Exposure to IAP was evaluated by interview; kitchen ventilation was also evaluated. RESULTS: In the study population, 88% used biomass fuels (dung /wood), 63% worked in the kitchen; 43% had no chimney, 21% had reduced ventilation coefficient. FMD was related with age (r=-0.19, p=0.04) and hyperemic shear rate (r=0.35, p=0.003), but was not influenced by smoking,

blood pressure, BMI, O_2 saturation (p=ns for all). FMD was not different between subjects with and without traditional risk factors (4.2±0.8 vs 5.2±0.4%, p=ns). Conversely, FMD was significantly reduced in people using biomass fuels (5.0±0.4 vs 6.4±1.1, p<0.05) and in those with no chimney in the kitchen (4.3±0.5 vs 5.7±0.5, p<0.05). In logistic regression analysis, age- and sex-adjusted, performed among biomass fuels users, absence of chimney (OR 3.4 , CL95% 1.1-9.9) remained an independent predictor of reduced FMD (below the median value, 4.84%). Main determinants of brachial artery diameter and response to GTN were age and hypertension, but not IAP-related characteristics. CONCLUSION: In rural Nepalese high- altitude dwellers using biomass fuels, insufficient ventilation system is associated with selective impairment of conduit-artery endothelial function, the first step of atherosclerosis. This study suggests that simple interventions can reduce the cardiovascular burden of exposure to IAP. ACKNOWLEDGEMENTS: Funded by Ev-K2-CNR, Italy. 2015.

FACTOR ANALYSIS AND RELIABILITY ANALYSIS OF THE LAKE LOUISE SCORE QUESTIONNAIRE IN NEPALESE PILGRIMS AT 4380 M. Martin MacInnis[1], Jim Rupert[1], Michael Koehle[1]. [1]School of Kinesiology, Univ British Columbia. *EMAIL: martin@alumni.ubc.ca* INTRODUCTION: The structure and reliability of the Lake Louise Score Questionnaire (LLSQ) have not been determined in a large population at high-altitude; however, these analyses are critical for the application of the LLSQ. METHODS: A large group of Nepalese pilgrims (n = 491) was assessed for AMS with the LLSQ after rapidly ascending from 1950 m to 4380 m. Based on polychoric correlations, a confirmatory factor analysis (CFA) was performed and an ordinal alpha coefficient was calculated to determine the structure and the internal consistency reliability of the LLSQ, respectively. RESULTS: A one-factor model with all five items of the LLSQ was statistically acceptable. Four items (headache, gastrointestinal upset, fatigue/weakness, and dizziness/lightheadedness) loaded strongly on this factor (>0.7), but sleep quality had a much lower factor loading (0.327). The ordinal alpha coefficient for the LLSQ was 0.79, but removing the sleep quality item improved this value to 0.84. Similar results were observed in a smaller (n = 135) dataset collected the morning after arrival to 4380 m. CONCLUSION: The sleep quality item was weakly related to the other items of the LLSQ. For research purposes, it may be useful to consider impaired sleep quality as a separate condition from AMS. ACKNOWLEDGEMENTS: Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC), and MM was funded by an NSERC Canada Graduate Scholarship. Data were collected by members of the 2012 Janai Purnima Research Expedition with assistance from the Mountain Medicine Society of Nepal and the Himalayan Rescue Association. 2015.

FACTORS INFLUENCING CLINICAL BREAST EXAMINATION AND MAMMOGRAPHY SCREENING. Parisa Parsa. Child and Mother Care Research Center and Department of Mother and Child Health, Hamedan University of Medicine and Health Sciences, Iran. *Email: pparsa2003@yahoo.com*. Introduction: Breast cancer is the most common cause of cancer incidence and cancer mortality

among women worldwide. Methods: A cross-sectional study was conducted to determine the rates and factors related to clinical breast examination (CBE) and mammography among 425 female teachers in Malaysia. Results: Only 25% and 13.6% of the eligible women ever had a CBE and a mammography, respectively. Results showed higher susceptibility to breast cancer, higher benefits of doing CBE and regular visit with a physician were significant predictors for undergoing CBE ($p<0.05$). Higher perceived susceptibility to breast cancer and regular CBE were significant predictors for having a mammography. Conclusion: Findings suggest a need for improving women awareness on breast cancer screening, its importance and recommended guidelines. 2011.

FEASIBILITY OF MEASURING RESPIRATORY CHEMOREFLEXES IN SCHOOL AGED CHILDREN. Joanna MacLean[1], Des Fuhr[2], Kristie DeHaan[1], Michael Stickland[2], James Duffin[3]. [1]Dept Pediatrics, Univ Alberta, [2]Dept Medicine, Univ Alberta, [3]Departments of Anaesthesia and Physiology, Univ Toronto. *EMAIL: joanna.maclean@ualberta.ca* INTRODUCTION: Studies of respiratory chemoreflex in childhood have focused on infants with little data beyond this period. The aim of this study was to determine whether protocols established for adult subjects could be successfully used in school aged children. METHODS: Children aged 8-12 years of age with a history of extreme premature birth (<28 weeks gestational age) were recruited to participate along with aged matched control children. Testing included a 5 minute baseline period breathing room air, 3 minute periods of SpO_2 targeted at 90% and 85% respectively, and 5 minutes of hyperventilation followed by hypoxic isoxic rebreathe (Duffin protocol; reservoir containing O_2 4%, CO_2 6%, nitrogen balanced; isoxic PO_2 40mmHg, maximum end tidal CO_2, $ETCO_2$, 50mmHg). Children were coached during hyperventilation for a target decrease in $ETCO_2$ of at least 10mmHg. Testing was completed with subjects lying in a supine position and breathing through a standard mouth piece with nose clips in place. RESULTS: Of the first 12 children recruited, 9 were able to complete the protocol. The children (2 controls, 7 ex-preterm) were aged 11.5±0.7 years. All children reported the hyperventilation as the most difficult part of testing. Change in $ETCO_2$ during hypoxia varied widely; 1.9±3.8mmHg and 2.7±4.9mmHg at target SpO_2 of 90% and 85% respectively. Hyperventilation resulted in a decrease in $ETCO_2$ of 8.9±3.8mmHg from baseline to an absolute level of 25.9±2.3mmHg. Maximum $ETCO_2$ before rebreathe termination was 45.3±7.5mmHg with an increase from start of rebreathe of 19.4±7.3mmHg. Data was sufficient to calculate ventilatory response threshold (VRT; 39.7±2.4mmHg) and CO_2 sensitivity (S1 slope; 2.8±1.4 L/min/mmHg) for 6 children. CONCLUSION: This preliminary data supports the feasibility of measuring respiratory chemoreflex in school aged children. Minor modification to the Duffin protocol may be necessary to increase successful data collection. 2015.

FIRST OBSERVATIONS OF SURFACE OZONE ON MOUNT EVEREST. John L Semple, GWK Moore. University of Toronto. *Email: john.semple@wchospital.ca.* Objective: The presence of ozone at extreme altitudes represents a health risk

for climbers and remote Sherpa communities. Ozone is highly toxic and can produce a variety of acute pulmonary effects, including decreased lung function, an inflammatory reaction, increase of epithelial permeability and increased airway resistance. Here we report the first surface ozone measurements from the summit of Mount Everest. Methods: In May 2005 we recorded ozone concentrations at different elevations on the Tibetan (North) side from Base Camp (5,676m) to the summit (8,848m) using a portable ozone meter (Model: OMC-1108: Ozone Solutions Inc). Back-trajectory analysis of prevailing winds and Total Column Ozone (TCO) satellite analysis were performed on the days with the highest ozone recordings. Results: Ozone concentrations ranged as high as 70 ppb. Simultaneous measurements recorded at different altitudes indicated an ascending gradient showing higher ozone concentrations at higher altitudes. Two distinct sources of ozone were identified. The first being stratospheric in origin, the result of deep convection associated with the arrival of the Asian monsoon and the second source was identified as long distance transport of polluted air masses from Nepal, the Indian subcontinent and South East Asia. Conclusions: Ozone is a recognized global risk to human health. Areas such as the Himalaya have previously been thought to have the world's cleanest air. We present, here the first ever surface ozone measurements on Mount Everest showing contributions from both the stratosphere and the long distance transport of pollution via the Indian sub continent and south East Asia. These ozone values are similar to those recorded in nearby Kathmandu. Ozone exposure at extreme altitude may represent a new health risk for mountaineers and remote populations in the Himalaya. 2009.

FLOWING STRIDES: HIGH ALTITUDE EXERTION INDUCED MICROPARTICLE ANALYSIS: THE MARSTSE MIK LA EXPERIMENT. Col Prosenjit Ganguli[1], Lt Col Surinderpal Singh[2], Rehan Ahmed[1], Amit Prabhakar[3], Zahid Ashraf[3], Sanjaya Mallick[4], Maj Y Uday[1], Maj Gen Velu Nair AVSM,VSM**[5].[1]Army Hospital (Research& Referral) Delhi Cantt, [2]Armed Forces Medical College Pune, [3]Defense Institute Of Physiology & Allied Sciences Timarpur Delhi, [4]BD Biosciences Kolkata, [5]Senior Consultant (Medicine), O/o DGAFMS, Ministry Of Defense, Govt Of India. *Email:* prosenjitganguli@gmail.com Introduction: Microparticles (MPs) represent physiology and/or pathophysiology of the originating cell; be it endothelial, platelet, or leukocyte. Venous thrombosis has been reported in lowlanders within hours to weeks of ascent to HA. Systemic Endothelial Dysfunction (ED) has been reported in cases of HAPE. An increase in circulating MP's on exposure to hypoxia may suggest ED as a common mechanism for initiation of thrombosis and occurrence of HAI all of which involves vascular leak Method: We hypothesized that circulating MPs increase with increased hypoxia and maybe enhanced by exertion at altitude.15 ALL were evaluated after 2 years baseline stay at 11-14000 feet (4300 m) with a total break of 90 days at sea level per year. ALL evaluated at 14,000 feet ascended to17,000 feet (5100 m) by motor transport and within an hour were re-evaluated by Flowcytometry, Complete Blood Count (CBC), Arterial Blood Gas (ABG) and dry chemistry by I-stat (ABBOT). Following an ascent of 1000 feet and back to 17000

feet over 3 hours by foot they were re-evaluated. ANOVA & Paired T Test analysis (SPSS 20) was done. Results: The experiment demonstrated an increase in MPs with increase in altitude. The increase in Anx-V, CD14 & Anx-V co-positive MP's was more marked on induction at 17000 feet from the baseline whereas CD41 labelled MP's increased post exercise. Hemoglobin, MCV, MCH, MCHC, Lymphocytes and Mixed Population done on 3 part Cell counts reduced post exercise whereas Polymorphs and White cell counts increased significantly. Conclusions: The MP's response on ascent is more marked than that following moderate exertion at target altitude. Post exercise increase in platelet MP's was seen. This supports the hypothesis that sub-acute hypoxia induces MP's which might be a feature of ED in asymptomatic healthy subjects. Acknowledgements: Special thanks to Mr Manoj Jagota, Mr. Diwakar Sharma & Dr Paresh Jain of BD-BioSciences for facilitating the Experiments. 2015.

FLUORESCEIN ANGIOGRAPHY DURING ACUTE EXPOSURE TO HIGH ALTITUDE REVEALS LEAKAGE. Gabriel Willmann[1], Manuel-Dominik Fischer[1], Andreas Schatz[1], Kai Schommer[2], Eberhart Zrenner[1], Karl-Ulrich Bartz-Schmidt[1], Florian Gekeler[1]. [1]University Eye Hospital and the Institute for Ophthalmic Research, University of Tübingen, Tübingen, Germany, [2]Medical Clinic and Policlinic, Department of Sports Medicine, University of Heidelberg, Heidelberg, Germany. *Email: Gabriel.Willmann@googlemail.com*. Introduction: High altitude exposure (HAE) is -due to constantly rising numbers of mountaineers and trekkers- a clinically relevant cause of complex pathophysiological events leading to alterations in all parts of the visual system. The purpose of our study was to evaluate the effects of HAE on retinal blood flow using fluorescein angiography (FA) and assess a correlation to acute mountain sickness (AMS). An increased capillary permeability due to a HIF-1α mediated upregulation of vascular endothelial growth factor (VEGF) has been discussed to be involved in the pathophysiology of AMS. Methods: 14 healthy lowlanders ascended from Tübingen (341m, Germany) to the Capanna Margherita (CM; 4554m, Italy) during the Tübingen High Altitude Ophthalmology (THAO) research expedition within 24 hours including one overnight stay at 3611m. AMS scores (Lake Louise and AMS-c scores) were assessed twice daily, oxygen saturation (SpO2) and heart rate were monitored daily. FA was performed one day after arrival at the CM using Spectralis HRA+OCT (Heidelberg Engineering, Germany). Baseline recordings were taken before and at least 2 weeks after HAE at the University Eye Hospital in Tübingen. Results: One day after arrival at CM, FA revealed considerable peripheral dye concentrated in the temporal quadrant in 7 out of 14 subjects. Incidence of AMS according to AMS scores was 50% on day 2. Only 2 subjects with leakage showed clinically relevant AMS scores. Subjects with AMS had lower SpO_2 (71.7±6.54%) and higher heart rate (85.7±9.0/min) compared to those without AMS (74.3±5.48% and 79.8±9.9/min respectively). At baseline FA showed no leakage and all AMS scores were negative. Conclusion: FA revealed leakage during HAE. While incidence for leakage and AMS was found to be 50%, leakage did not correlate with symptoms of AMS. Our data support a hypoxia-induced increased vascular permeability, but do not support

its potential role in the pathogenesis of AMS. Acknowledgements: Wilderness Medical Society (WMS) and Heidelberg Engineering. For more supporters please visit www.thao-project.com 2011.

FREEZING AND FROSTBITE ON MOUNT EVEREST: NEW INSIGHTS INTO WIND CHILL AND FREEZING TIMES AT EXTREME ALTITUDE. Kent Moore[1], John Semple[1]. [1]University of Toronto. *Email: gwk.moore@utoronto.ca.* Introduction: Cold injury is an acknowledged risk factor for those who, for occupational or recreational purposes, venture into high altitude regions. There is however little quantitative information on this risk that can be used to implement mitigation strategies. Here we provide the first characterization of the risk of cold injury near the summit of Mount Everest. Methods: This is accomplished through the application of a meteorological dataset that has been demonstrated to characterize conditions in the region as inputs to new parameterizations of wind chill equivalent temperature (WCT) and facial frostbite time (FFT). Results: Throughout the year, the WCT near the summit of Everest is always less -30°C and the FFT is always less than 20 minutes. During the spring climbing season, WCTs of -50°C and FFTs of 5 minutes are typical; while during severe storms they approach values found during the winter. Furthermore, we show that the summit barometric pressure is an excellent predictor of summit WCT and FFT. Conclusion: Our results provide the first quantitative characterization of the risk of cold injury on Mount Everest and also allow for the possibility of using barometric pressure, an easily observed parameter, to be used in real-time to characterize this risk and to implement mitigation strategies. They also provide additional confirmation as to the extreme environment experienced by those attempting to summit Mount Everest and other high mountains. Acknowledgements: KM was supported in this research by the Natural Sciences and Engineering Research Council of Canada. 2011.

FRONTAL AND MOTOR CORTEX OXYGENATION DURING MAXIMAL EXERCISE IN NORMOXIA AND HYPOXIA. Andrew W Subudhi, Brittany R Miramon, Matthew E Granger, Robert C Roach. University of Colorado, Colorado Springs and Denver. *Email: asubudhi@uccs.edu.* Reductions in prefrontal oxygenation near maximal exertion may limit exercise performance by impairing executive functions that influence the decision to stop exercising; however, it is unknown if deoxygenation also occurs in motor regions that more directly affect central motor drive. METHODS: Multichannel near infrared spectroscopy (NIRS) was used to compare changes in prefrontal, premotor and motor cortices during exhaustive exercise. Twenty-three subjects performed two sequential, incremental cycle tests (25 W/min ramp) during acute hypoxia (PIO_2 = 79 mmHg) and normoxia (PIO_2 = 117 mmHg) in an environmental chamber. Test order was balanced and subjects were blinded to chamber pressure. RESULTS: In normoxia, bilateral prefrontal oxygenation was maintained during low- and moderate- intensity exercise, but dropped 9.0 ± 10.7 % (mean ± SD; $P < 0.05$) prior to exhaustion (maximal power = 305 ± 52 W). The pattern and magnitude of deoxygenation was similar in prefrontal, premotor and motor regions ($R2 > 0.94$). In hypoxia, prefrontal oxygenation was reduced

11.1 ± 14.3% at rest ($P < 0.01$) and fell another 26.5 ± 19.5% ($P < 0.01$) at exhaustion (maximal power = 256 ± 38 W, $P < 0.01$). Correlations between regions were high ($R2 > 0.61$), but deoxygenation was greater in prefrontal than in premotor and motor regions ($P < 0.05$). CONCLUSIONS: Prefrontal, premotor and motor cortex deoxygenation during high-intensity exercise may contribute to an integrative decision to stop exercise. The accelerated rate of cortical deoxygenation in hypoxia may hasten this effect. 2009.

GENDER INTERACTS WITH THE CONSTITUTIVE ERYTHROPOIETIN DEFICIENCY ON THE HYPERCAPNIC VENTILATORY RESPONSE IN MICE. Nicolas VOITURON[1], Florine JETON[1], Maxime BRUMM[1], Dominique MARCHANT[1], Patricia QUIDU[1], Jorge SOLIZ[2], Jean-Paul RICHALET[1], Aurelien PICHON[1]. [1]Université Paris 13, Sorbonne Paris Cité, UFR SMBH, Laboratoire "Réponses cellulaires et fonctionnelles à l'hypoxie", EA 2363, 93017 Bobigny, France., [2]Dept Pediatrics, Centre de Recherche de I'Hôpital St- François d'Assise (CR-SFA), Centre Hospitalier Universitaire de Québec (CHUQ), Faculty of medicine. *EMAIL: nicolas.voituron@univ-paris13.fr* INTRODUCTION: Erythropoietin (Epo) synthesized by neurons and astrocytes modulates the ventilatory response in a gender-dependent manner to reduced oxygen supply. This effect has been attributed to the presence of Epo receptors in brainstem respiratory centers, central and peripheral chemosensors. However, the impact of Epo on the ventilatory response to hypercapnia remains unknown. Therefore, we aimed to assess the interaction effects between Epo and gender on the ventilatory response to hypercapnia. METHODS: We used male and female wild type and transgenic mice constitutively deficient for erythropoietin (Epo-TAgh). The ventilatory parameters were assessed by whole body plethysmography. Furthermore, anatomical approaches were used to determine the neuronal network in the brainstem that is involved in this process. RESULTS: Wild type mice showed the expected hyperventilation upon hypercapnic stimulus, while Epo-TAgh mice displayed a drastic reduction in the ventilatory response to hypercapnia. We found a clear gender dimorphic response to hypercapnic stimulus with a relative respiratory frequency decline associated with a significant increase in tidal volume in Epo-TAgh females that was not observed in Epo-TAgh males. CONCLUSION: These results imply that Epo plays a key-regulating role in the neural control of hypercapnic response and specifically in females. This novel finding suggests for the first time that Epo regulates the ventilatory responses to hypercapnia in a gender-dependent-manner. ACKNOWLEDGEMENTS: Univ Paris 13 and Laboratory of Excellence GR-Ex (FJ fellowship). 2015.

GENE EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS DURING EXPOSURE TO HYPOBARIC HYPOXIA. Giacomo Strapazzon[1], Giulio Ceolotto[2], Georg Hofer[3], Karla Balkenhol[1], Alessia Martellini[2], Marika Falla[4], Hermann Brugger[1]. [1]EURAC Institute Mountain Emergency Medicine, Bolzano, Italy, [2]Dept Medicine, Univ Padova, Padova, Italy, [3]Dept Anaesthesiology and Critical Care Medicine, General Hospital of Silandro, Silandro, Italy, [4]Dept

Neurology and Psychiatry, Sapienza Univ Roma, Roma, Italy. *EMAIL: giacomo.strapazzon@eurac.edu* INTRODUCTION: Gene expression data during acute and subacute exposure to hypobaric hypoxia are often lacking. Circulating peripheral blood mononuclear cells (PBMCs) have been advocated both as a time-persistent and readily accessible model capable of reflecting the whole-organism conditions. The aim of the study was to investigate gene expression during pure hypobaric hypoxia exposure in a group of 15 healthy lowlanders. METHODS: Blood was drawn into PAXgene Blood RNA Tubes before and during hypobaric hypoxia exposure (24 and 72 hours and 1 week) at an altitude of 3,830 m (12,566 ft). Total RNA was purified manually with the PAXgene Blood RNA Kit (Qiagen). Reverse trascription was performed using iScript complementary DNA synthesis kit (Bio-Rad). Subunit 1alpha of Hypoxia-Inducible Factor 1 (HIF-1alpha) gene expression was determined by quantitative real-time PCR using the Bio-Rad iQ5 system according to manufacturer instructions with SsoFast EvaGreen PCR Supermix, using beta-Actin as housekeeping gene. RESULTS: A median RNA amount of 50 ng/ul (range 20-60 ng/ul) in the samples collected at high altitude was measured determining concentration at 260 nm and purity using the 260/280 ratio by a Nano-Drop spectrophotometer. Preliminary expression analysis of HIF-1alfa showed a variable response at 24h (range -60-24%), followed by a relative up-regulation of 72% (range 43-101%) and 125% (range 108-155%) at 72 hours and 1 week, respectively. CONCLUSION: The preliminary data describe the pure expression response of human HIF-1alpha in PBMCs to hypobaric hypoxia without the effects of physical activity and altitude variations. The expression of selected genes with HIF-1-dependent transcription will be quantified and correlated with clinical and expression parameters. Moreover, the results validate the method for detection and quantification of intracellular RNA transcripts suitable for studies at high altitude and in remote areas. 2015.

GENETIC ASSOCIATION STUDY OF ACUTE MOUNTAIN SICKNESS SUSCEPTIBILITY. Pei Wang, Michael S Koehle, Jim L Rupert. University of British Columbia. *Email: wang76@interchange.ubc.ca.* Purpose: Acute mountain sickness (AMS) frequently develops in individuals who ascend to altitude above 2500 m rapidly. The recurrence rates of AMS in individuals suggest some people may be more susceptible than others, possibly due to a genetic predilection. Bradykinin is a circulating vasoactive peptide that stimulates vasodilatation, primarily via activating endothelial B2 receptors. This study investigated the impact of variants in the genes encoding B2 receptor gene (BDKRB2) on the susceptibility to AMS. Methods: Nepalese lowlanders (80 male, 23 female) were recruited at the 2005 Janai Purnima Festival at Lake Gosain Kunda at 4380 m in Lang Tang, Nepal. Two polymorphisms (9/-9 in exon1 and -58 C/T in promoter region) in the BDKRB2 gene that previously have been associated with the expression of BDKRB2 gene and hypertension were investigated. AMS was assigned to individuals who were clinically diagnosed and had a Lake Louise score of 3 or greater. Buccal cells were collected from individuals (AMS (LLS›3 & clinical AMS) vs non-AMS (LLS<3 and non-clincial AMS) (32: 52)). DNA prepared, genotyped, and allele frequencies

compared between the two groups using Chi-Square analysis. Results: No association was found between AMS status and the alleles at the 9/-9 polymorphism in the BDKRB2 gene. Conclusions: Our data of the 9-9 polymorphism suggest that variants in the BDKRB2 genes do not contribute to AMS susceptibility in the Nepalese. Acknowledgments: Pei Wang is a recipient of a U.B.C. University Graduate Fellowship. 2009.

GENETIC EVIDENCE OF HIGH ALTITUDE ADAPTATION IN TIBETAN HIGHLANDERS. Wuren Tana[1], Ri-Li Ge[1]. [1]Research Center for High-Altitude Medicine, Qinghai Univ Medical School, Xining, Qinghai China. *EMAIL: geriligao@hotmail.com* INTRODUCTION: The genetic architecture underlying adaptive physiological characteristics for high altitude is unfolding. We wished to distinguish Tibetan highland population from Chinese (CHB) and Japanese (JPT) lowland populations. METHODS: To detect positive selection on particular genetic variants, we used two different statistical tests: cross-population extended haplotype homozygosity (XP-EHH), and high integrated haplotype score (iHS), RESULTS: We found 10 genes in Tibetans among 240 genes related to hypoxia pathway in GO categories that were differently expressed from Chinese and Japanese. These candidate genes include EPAS1, PHD2 (EGLN1), and PPARA. Individuals with more copies of haplotype PHD2 and PPARA have significantly lower hemoglobin concentration, suggesting that they are protected against polycythemia. One study of both Andean and Tibetan populations also revealed that both populations have been through positive selection on HIF pathway genes, include PHD2 (EGLN1). We also found a novel missense mutation of the PHD2 gene which together with another previously reported but invalidated PHD2 SNP that results in missense mutation, correlates with hemoglobin levels in Tibetan highlanders but not lowlanders. Considering the lack of differentiation of codons in Han Chinese and Tibetans, it is also possible that many genetic targets of selection are in non-coding, regulatory regions of the genome. CONCLUSION: Our analyses highlight a miRNA near the PPARA gene and a non-coding highly conserved region in Tibetan population that may contribute to altitude adaptation. ACKNOWLEDGEMENTS: This work supported by National Basic Research Program of China(No.2012CB518200), Program of International S&T Cooperation of China (No.0S2012GR0195), National Natural Science Foundation of China (No.30393133). 2015.

GENETIC SIGNATURES OF ACUTE MOUNTAIN SICKNESS. Megan Wilson[1], Tzu Phang[2], Bifeng Gao[3], Andrew Subudhi[4], Colleen Julian[1], Vaughn Browne[1], Mark Geraci[2], Robert Roach[1]. [1]Altitude Research Center, Department of Emergency Medicine, School of Medicine, University of Colorado Denver, Aurora, CO, [2]Pulmonary Sciences/Critical Care Medicine, School of Medicine, University of Colorado Denver, Aurora, CO, [3]Cancer Center, Microarray Core, School of Medicine, University of Colorado Denver, Aurora, CO, [4]Altitude Research Center, Department of Emergency Medicine, School of Medicine University of Colorado Denver, and Department of Biology, University of Colorado at Colorado Springs. *Email: megan.wilson@ucdenver.edu*. Introduction: Acute mountain sickness

(AMS) is the most common illness for sojourners to high altitude. Among people who travel from sea level to stay at moderate altitudes (1920-2960m), 27% exhibit AMS (Honigman 1993). Currently, there is no accurate way to predict who will get AMS among people never before exposed to high altitude. Additionally, the molecular mechanisms leading to AMS are relatively unexplored. This study seeks to identify AMS gene expression hallmarks, both before ascent to simulated high altitude to discover candidate biomarkers for the prediction of AMS, and during the illness. Methods: In this study, 24 subjects were exposed to simulated high altitude (PB=425Torr, 4875m) for ten hours using a hypobaric chamber. Peripheral blood mononuclear cells were collected from the subjects at baseline, one day prior to hypoxic exposure in ambient conditions (1600m; Aurora, CO), and after nine hours of hypoxic exposure. Genome-wide microarrays measuring greater than 47,000 transcripts were used to profile gene expression changes with altitude exposure as well as gene expression in subjects who developed AMS compared with those who remained healthy. Results: Exposure to hypoxia triggered the expression of genes in hypoxia-sensing pathways, as well as angiogenic and inflammatory pathways. Subjects with AMS compared with those who remained healthy showed increased expression of genes involved in hypoxia-sensing pathways and processes that particularly affect the brain. Using class prediction with seven different methods, a six-gene signature was established to predict AMS before exposure to hypoxia with 98% accuracy. Conclusion: A parsimonious gene signature to predict AMS before ascent as presented could help prevent AMS in millions of people worldwide. Acknowledgements: This work was funded by the National Heart, Lung, and Blood Institute (HL-070302) and by Intramural funds from the Altitude Research Center, Department of Emergency Medicine, University of Colorado Denver. 2011.

GESTATIONAL STRESS DELAYS MATURATION OF THE HYPOXIC VENTILATORY RESPONSE: AN IN VIVO AND IN VITRO STUDY. Richard Kinkead, Stèphanie Fournier, Sèbastien Fournier. Laval University. *Email: Richard.Kinkead@crsfa.ulaval.ca.* Exposure to stress during gestation disrupts CNS development; however, its effects on the respiratory control system are unknown. We tested the hypothesis that exposing dams to a predator odor during gestation disrupts the maturation of the hypoxic ventilatory response (HVR) of newborn rats. From the 9th to the 19th day of pregnancy, females subjected to gestational stress (GS) were placed in a cage containing 35 ·l of TMT, a component of fox odor for 20 min. For sham treatment, gestating females were exposed to a strong odor (butyric acid, 105 ·l). Controls were undisturbed. Ventilatory activity was measured at post natal days 0, 2 and 4 using plethysmography (in vivo) and "en bloc" brainstem spinal cord preparations (in vitro). Measurements were performed under baseline conditions and hypoxia. In vivo: unlike controls, P4 male pups born to GS dams could not sustain a significant increase in breathing frequency through out the hypoxic period (12% O_2, 20 min). This effect of GS was not observed in females. In vitro: at the end of hypoxia (7% O_2, 20 min), preparations from GS pups showed a smaller phrenic burst frequency decrease than controls (-58% vs -76%). No sexual dimorphism was observed in vitro. GS delays maturation of the hypoxic ventilatory

response. Comparison of in vivo vs in vitro data suggests that GS affects peripheral chemoreceptor development and/or integration of afferent signal in the CNS. Supported by the CIHR and the Canada Research Chair in Respiratory Neurobiology. 2009.

HAEMATOLOGICAL ACCLIMATION AND RE-ACCLIMATION TO NORMOBARIC HYPOXIA IN MICE. Meaghan J MacNutt, James L Rupert, A. William Sheel. University of British Columbia. *Email: mjmacnutt@hotmail.com.* We recently presented evidence that the human haematological response to hypoxia is enhanced on re-exposure versus initial exposure to high altitude. To further investigate, we exposed and re-exposed adult female C57Bl6-J mice to normobaric hypoxia (PIO_2=80mmHg). Groups of 5 mice were terminally sampled after 1, 3, 7, 14 and 28d of initial acclimation (IA) and re-acclimation (RA). RA animals experienced 2wk IA followed by 2wk normoxic de-acclimation (DA) before hypoxic re-exposure. Blood was collected via cardiac puncture and complete blood counts and reticulocyte counts were determined using standard automated methods. As anticipated, hypoxic exposure elicited significant increases in red blood cell count (ΔIA vs. ΔRA; 20 vs. 22%), haematocrit (Hct; Δ36 vs. 26%), [haemoglobin] (Δ22 vs. 21%) and reticulocyte count (Δ65 vs. 54%). These changes were completely reversed after 14d DA. None of the above variables showed an improvement in the rate or magnitude of change in RA vs. IA, and surprisingly, the Hct response was lower during RA than IA ($p<0.01$). Mean corpuscular volume increased throughout IA ($p<0.01$) but did not change from baseline during RA. Also, cell distribution width was higher during RA than IA ($p<0.01$), suggesting an increased presence of small, immature erythrocytes during RA. We provide no evidence that haematological acclimation to hypoxia is improved by recent hypoxic exposure such that the O_2 carrying capacity of blood is enhanced during RA vs. IA. However, despite identical baseline values prior to each exposure, both Hct and erythrocyte size clearly respond to hypoxia differently between IA and RA. This suggests a persistent effect of the previous hypoxic stimulus (or perhaps its removal) on haematological acclimation during re-exposure to hypoxia in mice. Acknowledgements: NSERC, Heart and Stroke Foundation of Canada. 2009.

HAEMOGLOBIN MASS IS GREATER IN NATIVE HIMALAYAN HIGHLANDERS THAN NATIVE LOWLANDERS. Edward Gilbert-Kawai[1], Ronan Astin[1], Adam Sheperdigian[1], Michael Grocott[1], Walter Schmidt[2], Hugh Montgomery[1], Daniel Martin[1]. [1]UCL Centre for Altitude, Space and Extreme Environment Medicine, London, UK, [2]Sports Physiology, University of Bayreuth, Germany. *Email: e.gilbert@ucl.ac.uk.* Introduction: The objective of this study was to accurately measure the total haemoglobin mass (tHb-mass) of Himalayan high altitude natives of Nepal (Sherpas). Sherpas display exceptional physical performance while hypoxic, the physiological basis for which is unknown. Reports have consistently shown their haemoglobin concentrations ([Hb]) are similar to lowland natives who have ascended to a similar altitude. However, [Hb] depends upon both tHb-mass and plasma volume, and is thus a crude index of oxygen carrying capac-

ity. Methods: 59 lowlanders (32 male) and 40 Sherpas (20 male) had tHb-mass measured in London (lowlanders, 35m above sea level) or Kathmandu (Sherpas, 1300m above sea level). tHb-mass was measured using the carbon monoxide (CO) re-breathing technique (1). Venous [Hb] was measured by Seimens Rapidpoint500® blood gas analyser which also provided HbCO% measurements for tHb-mass calculation. Results: In whole cohort comparison, [Hb] was not significantly different between groups (lowlanders mean ± SD 151 gL-1 ± 1.3 vs. Sherpas 146 gL-1 ± 1.7, p= 0.08). Haematocrit also did not differ (lowlanders 43.1L/L ± 3.9 vs. Sherpas 43.5 L/L ± 4.1, p=0.6). However, tHb-mass was significantly higher in Sherpas (lowlanders 9.3 g/kg ± 1.8 vs. Sherpas 10.3 g/kg ± 2.3, p=0.02) - findings consistent with concurrently expanded plasma volumes (35.1 mL/kg ± 5.6 vs. 40 mL/kg ± 9.2) and thus blood volume (61.7 mL/kg ± 9.2 vs. 70.9 mL/kg ± 14.6, lowlanders vs Sherpas respectively p<0.001). Comparing males only, tHb-mass remained significantly greater in Sherpas (data p=0.01) whereas the difference did not reach significance in females (data p=0.07). Conclusion: Whilst [Hb] is similar in lowlanders and Sherpas, this hides the fact that tHb-mass and blood volume are elevated in the latter. Oxygen carrying capacity is thus greater. Using [Hb] to calculate oxygen carrying capacity can be misleading. 2015.

HAF PROMOTES HIF-2 TRANSACTIVATION AND PROMOTES POOR PROGNOSIS IN KIDNEY CANCER. Mei Yee Koh[1], Galina Kiriakova[1], Robert Lemos[1], Eric Jonasch[1], Garth Powis[1]. [1]U.T. M.D. Anderson Cancer Center Houston TX 77030 USA. *EMAIL: mykoh@mdanderson.org* INTRODUCTION: The hypoxia inducible factors, HIF-1 and HIF-2, are critical mediators of the hypoxic response, and play non-redundant roles. HIF-1 regulates acute metabolic changes required for hypoxic survival, whereas HIF-2 is important for adaptation to chronic hypoxia. Clear cell renal carcinoma (RCC) is the most common form of kidney cancer, and is frequently initiated by pVHL loss. HIF-2 is an important driver of RCC, whereas HIF-1 plays a tumor suppressor role and can predict for good patient prognosis. However, the mechanism determining HIF-1 versus HIF-2 activation is unclear. The hypoxia associated factor (HAF), ubiquitinates HIF-1α independently of oxygen and pVHL, thus targeting HIF-1α for proteasomal degradation, without affecting HIF-2α levels. Instead, HAF promotes HIF-2 transactivation, thus mediating the switch from HIF-1 to HIF-2 or the 'HIF switch'. Our aim was to delineate molecular mechanisms of the HAF-mediated HIF switch and its impact on RCC progression. METHODS: The impact of HAF on HIF-2 specific transcriptional activity was assessed using chromatin immunoprecipitation (ChIP) and TaqMan gene expression assays. The effect of HAF on RCC growth was assessed in vitro and in mice. The clinical relevance of HAF was evaluated using immunohistochemistry in patient samples. RESULTS: HAF promotes the binding of HIF-2α to the promoters of target genes thereby increasing its transcriptional activity. HAF co-localizes with HIF-2α in cells and patient tissue. HAF overexpression promotes RCC tumor growth in mice, and RCC patients with high HAF levels show decreased progression-free survival. CONCLUSION: HAF, by degrading HIF-1α and promoting HIF-2α transactivation, provides a mechanism by which cells can switch from

HIF-1α to HIF-2α dependent transcription. HAF may be novel target for the treatment of RCC and other HIF-2 driven tumor types. ACKNOWLEDGEMENTS: CA098920. 2015.

HAVING A SWELL TIME AT HIGH ALTITUDE: INVESTIGATION OF WHOLE BRAIN WHITE MATTER IDENTIFIES ALTERED WATER MOBILITY IN THE PATHOGENESIS OF HIGH-ALTITUDE HEADACHE. Justin Lawley[1], Samuel Oliver[1], Paul Mullins[2], Jamie Macdonald[1]. [1]Extremes Research Group, Bangor Univ, Bangor, Gwynedd, LL57 2AS, United Kingdom, [2]Bangor Imaging Center, Bangor Univ, Bangor, Gwynedd, LL57 2AS, United Kingdom. *EMAIL: JustinLawley@texashealth.org* INTRODUCTION: Objective. Utilize a multi-imaging approach to examine the impact of high-altitude on cerebral white matter water mobility and high-altitude headache. METHODS: Thirteen male volunteers were recruited. A diffusion-weighted, spin-echo, single-shot, echo-planar imaging sequence and a mono exponential T2 relaxation sequence were obtained for the whole brain after 2 and 10 hrs of exposure to normoxia and high-altitude (12%O_2) on two separate days. Headache intensity was measured after 10 hrs at high-altitude using a visual analogue scale. Using the FMRIB's Diffusion Toolbox, fractional anisotropy (FA) and mean diffusivity (MD) were calculated for the whole brain. Whole-brain voxelwise analysis of FA, MD and T2 relaxation (T2) was first analyzed using tract-based spatial statistics (TBSS) and then using the more conservative summation of pre-defined regions of interests (mROI) technique and ANOVA. RESULTS: TBSS revealed a reduction in MD throughout the left posterior hemisphere after 2 hrs at high-altitude, which was exacerbated throughout the white matter after 10 hrs. No changes in T2 or FA were observed using this technique. Using the mROI we observed similar results, although FA was also slightly increased by 0.01 after both 2 and 10 hrs ($P<0.001$). TBSS identified an association between changes in MD, FA and T2 both supra and subtentorially after 2 and 10 hrs with headache intensity. Using the mROI identified a positive relationship between the change in T2 ($r=0.69$, $P=0.012$) and a trend towards a positive relationship with the change in MD ($r=0.51$, $P=0.09$) after 10 hrs and headache intensity. CONCLUSION: Acute high-altitude results in a substantial increase in intracellular water but not interstitial (vasogenic) edema. Changes in brain water were related to the intensity of high-altitude headache. ACKNOWLEDGEMENTS: This study was funded by an unrestricted Bangor Univ 125 Anniversary Scholarship. 2015.

HEART RATE AND METABOLIC RATE OF BAR-HEADED GEESE FLYING IN HYPOXIA. Jessica Meir[1], Julia York[2], Wilhelmina Jardine[2], Beverly Chua[2], William Milsom[2]. [1]Harvard Medical School / Massachusetts General Hospital, Boston, MA, U.S.A., [2]Univ British Columbia, Vancouver, B.C., Canada. *EMAIL: jmeir@partners.org* INTRODUCTION: Bar-headed geese accomplish the extraordinary feat of migrating over the Himalayas, where oxygen (O_2) levels are only ½ - 1/3 those at sea-level. Although physiological responses and adaptations relevant to this species' success at high altitude have been previously documented, only one study has measured physiological variables in this bird during flight, and

only under conditions of normoxia. METHODS: In order to assess the roles of the cardiovascular and respiratory systems in maintaining oxygen delivery during flight in hypoxia, we trained bar-headed geese to fly in a wind tunnel while wearing our physiological data-logger and a mask system. We were successful in measuring heart rate and metabolic rate in flying geese under conditions of normoxia and hypoxia (10.5%O_2 and 7%O_2, equivalent to altitudes of ~5,500 and ~8,500 meters respectively). RESULTS: Surprisingly, bar-headed geese exhibited a remarkably wide range of heart rates and metabolic rates while flying at their preferred flight speed, even on an individual bird basis. Based on preliminary data, mean heart rate during flight changed very little with increasing levels of hypoxia, though mean metabolic rate was reduced. CONCLUSION: This suggests that the birds settled into more efficient flight patterns in the more challenging hypoxic environment. Bar-headed geese sustained the same flight durations at equivalent flight speeds under conditions of normoxia and severe hypoxia. In addition, we will present in-flight examples of arterial and venous PO_2, the first continuous measurements of PO_2 in any flying bird. ACKNOWLEDGEMENTS: National Science Foundation International Research Post-doc Fellowship; NSERC. 2015.

HEART RATE AND OXYGEN SATURATION IN CHILDREN WITH CHRONIC (AYMARAS AND NON-AYMARAS) AND ACUTE (NON-AYMARAS) EXPOSURE AT 3500 M (PUTRE, CHILE). Fernando Moraga[1], Vasthi Lopez[1]. [1]Universidad Católica del Norte, Coquimbo, Chile. *Email: fmoraga@ucn.cl.* Introduction: Our objective was to determine if there is a difference between children with chronic high altitude exposure (Aymaras and Non-Aymaras) in comparison with children acutely exposed to 3500 m (Putre, Chile). Methods: Heart rate (bpm) and pulse oxymetry (%) were evaluated in the children (8100M, Nonin) from Putre, and acute mountain sickness (AMS) symptoms were evaluated using the Children's Lake Louise Score (CLLS) in children acutely exposed. Results: No differences were observed in oxygen saturation between chronic children (Aymaras and non-Aymaras). However, a significant difference in oxygen saturation was found when comparing chronic children (Aymaras and non Aymaras) with children acutely exposed ($p<0.0001$). The heart rate (HR) of chronic Aymaras children was less than that observed in non-Aymaras ($p<0.05$), whereas acute children had a significantly increased HR as compared to chronic children ($p<0.001$). Inverse and higher correlations were observed between heart rate and oxygen saturation in all groups, and a higher AMS score was observed in acute children. Conclusion: Our results suggest that children chronically exposed to high altitude exhibit a lower heart rate and higher oxygen saturation than children acutely exposed. Correlation between oxygen saturation vs heart rate suggest that chronic Aymaras have greater sympathetic tone, similar to that observed in acute children. In contrast, chronic non-Aymaras children have a lower sympathetic tone suggesting an early blunting to hypoxia in these children. Further studies are needed to understand the physiological mechanisms in this population group. Acknowledgements: We are grateful to the Director of Posta Rural General de Putre and the Government of Province of Putre-Parinacota for their support and Grant INNOVA-CORFO 07CN13ISM-152. 2015.

HEAVY EXERCISE DOES NOT ALTER SPECIFIC VENTILATION HETEROGENEITY. Vincent Tedjasaputra[1], Rui Carlos Pereira Sa[2], Tatsuya J. Arai[2], Sebastiaan Holverda[2], William T. Chen[2], Peter D. Wagner[2], Chris K. Davis[3], Rebecca J. Theilmann[2], Gordon Kim Prisk[2], Susan R. Hopkins[2]. [1]San Diego State University – School of Exercise and Nutritional Sciences, and University of California, San Diego – Pulmonary Imaging Laboratory, [2]University of California, San Diego – Pulmonary Imaging Laboratory, [3]Rady Children's Hospital, San Diego, CA. *Email: vtedjasaputra@ucsd.edu*. Introduction: Ventilation-perfusion (VA/Q) inequality increases with exercise, and is suggested to result from interstitial edema compressing small airways and blood vessels. Forty-five min of heavy exercise increases the spatial heterogeneity of pulmonary perfusion in athletes. We hypothesized that specific ventilation (SV, ratio of volume fresh air entering lung region to regional end-expiratory volume) heterogeneity would similarly increase, consistent with airway compression secondary to the development of exercise-induced interstitial pulmonary edema. Methods: Trained subjects (4 men, 2 women VO_2max=58±3mL/kg/min) cycled 45min at ventilatory threshold (270±48W). Arterial blood gases were measured at rest (R), exercise (E), and recovery (REC) to calculate alveolar-arterial O_2 Difference (A-aD O_2). O_2 enhanced MRI was used to quantify SV before and after exercise (40 and 90min) in a sagittal slice of the supine right lung (resolution: 1.6x1.6x15mm). SV heterogeneity was assessed by relative dispersion (RD=SD/mean). Results: Partial pressure of O_2 decreased from 107±3torr R, to 89±8torr, $p<0.001$ during E, returning to 100±7torr at REC. A-aDO_2 increased from 6.0±3.6torr to 23.6±5.9torr during E , $p<0.001$ and was 7.3±4.3torr at REC. No significant changes in mean SV were seen with exercise: before (0.343±0.07), post 40 min (0.325±0.03), post 90 min (0.308±0.05),p=0.108. Similarly there was no change in RD: before (0.80±0.17), post 40 (0.85±0.15), post 90 min (0.80±0.09) p=0.404. Conclusion: Combined with the previously documented increase in perfusion heterogeneity the unchanged mean SV or RD of SV post-exercise suggests that increased VA/Q inequality with exercise is due to changes in the distribution of perfusion not matched by ventilation. Perhaps airways are more resistant than blood vessels to the development of changes, or resolve them more quickly. Alternately exercise-induced bronchodilation may mitigate the effect of peribronchial cuffing and compression caused by fluid efflux. Acknowledgements: Funding provided by NIH HL081171, PI Hopkins. 2011.

HEMATOPOIETIC RESPONSE SUBJECTED TO LONG-TERM HYPOXIC CONDITION IN MICE. Tomonori Harada[1], Isao Tsuboi[1], Yukio Hirabayashi[1], Hideki Oshima[1], Hiroyuki Hara[1], Tohru Inoue[1], Shin Aizawa[1]. [1]Department of Functional Morphology, Nihon University School of Medicine, Tokyo, Japan. *Email: harada.tomonori@nihon-u.ac.jp*. Introduction: The responses of erythroid, myeloid, lymphoid and mast cell lineages in hematopoietic tissues in mice subjected to long-term hypoxic condition were investigated. Methods: C57BL/6J mice (7-13 week-old) were subjected to 10% O_2 for 35 days in a isolated normobaric chamber. Control mice were maintained in room air. Peripheral blood, femoral bone marrow (BM) and spleen were obtained on day 2, 7, 14, 21 and 35. Peripheral blood

parameters and the numbers of hematopoietic progenitor cells in BM and spleen were measured. Results: Body weights in mice after hypoxic exposure were temporally decreased to 90% of pre-treatment levels on day 2 and were returned to pre-treatment levels on day 7. Hematocrit values in mice after hypoxic exposure began at 55%, then were continuously increased to 70% until day 21 and remained unchanged thereafter. White blood cell (WBC) number in mice after treatment were temporally increased by 150% of control mouse levels and returned to control mouse levels. The oscillation of platelets number in treated mice was comparable with that in WBC. The number of mature erythroid progenitor cells such as CFU-E in spleen were rapidly increased by 600% of control value then returned near to control mice levels at day 14, whereas CFU-E numbers in BM were gradually increased to 250%. Increased number of both immature erythroid progenitor cells (BFU-E) and myeloid progenitor cells (CFU-GM) in spleen and BM was observed at day 21 and day 14, respectively. Increased number of femoral B cell progenitors (CFU-preB) was observed at day 7. The number of mast cell progenitors (CFU-mast) in spleen was increased by 250% of control value at day 21. Conclusion: Long-term hypoxic condition affected the growth and maturation not only in erythroid lineage but also in other lineages including myeloid, lymphoid and mast cell. 2011.

HIF-1-INDEPENDENT ADENOSINE A2A RECEPTOR-MEDIATED INDUCTION OF TYROSINE HYDROXYLASE IN HYPOXIC PC12 CELLS. Gaetano Cairo, Elena Gammella, Lorenza Tacchini. University of Milan. *Email: gaetano.cairo@unimi.it.* Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of catecholamines, which are released by carotid bodies in response to hypoxic conditions. Increased HIF-1-dependent TH gene expression during hypoxia has been demonstrated in O_2-sensitive PC12 cells (Schnell et al J Neurochem 2003). Adenosine is released in response to hypoxia in the central nervous system and CGS21680, an adenosine A2A receptor agonist, enhances AP-1 and CREB-dependent TH transcription (Chae and Kim Mol Brain Res 1997). As we have shown A2A receptor-mediated induction of HIF-1 in macrophages (De Ponti et al J Leukoc Biol, 2007), we investigated the involvement of HIF-1 in the adenosine-mediated induction of TH expression. Exposure to adenosine or CGS21680 increased TH mRNA and protein levels in PC12 cells. The transcription of a reporter gene under the control of the wild type rat TH promoter was induced 3.5-fold in CGS21680-treated PC12 cells, but both the mutation of the hypoxia responsive element in the TH promoter and the co-transfection of a dominant negative of the HIF-1 b subunit did not prevent the increase in transcription. Furthermore, CGS21680 increased CREB binding activity but did not induce HIF-1 DNA binding activity and protein levels. To investigate whether this HIF-1 independent transcriptional activation was involved in hypoxia-mediated TH induction, PC12 cells were exposed to hypoxia in the presence of the A2A receptor antagonist ZM241385. In spite of concomitant HIF-1 activation, ZM241385 completely prevented hypoxia-dependent TH induction. In line with this finding, pharmacological inhibition of HIF-1 by Chetomin did not abolish TH induction in hypoxic PC12 cells.

These results indicate that a typical hypoxia-regulated gene like TH, which is key in the systemic adaptation to reduced oxygen availability, is not regulated by HIF-1, the primary modulator of the response to hypoxia, but by the adenosine A2A receptor-mediated signalling pathway. 2009.

HIF-1α OVEREXPRESSION IN DUCTAL CARCINOMA IN SITU OF THE BREAST IN HEREDITARY PREDISPOSED WOMEN. Petra van der Groep[1], Paul J van Diest[1], Fred H Menko[2], Joost Bart[3], Elisabeth GE de Vries[3], Elsken van der Wall[1]. [1]University Medical Center Utrecht, Utrecht, Netherlands, [2]Free University Medical Center, Amsterdam, Netherlands, [3]University Medical Center Groningen, Groningen, Netherlands. *Email: p.vandergroep@umcutrecht.nl*. Introduction: Recent studies have revealed that BRCA1 and BRCA2 germline mutation related invasive breast cancers show frequent overexpression of HIF-1α, often next to necrosis, suggesting a role of hypoxia in carcinogenesis and progression. Little is known about the role of hypoxia in precursors of these hereditary cancers like ductal carcinoma in situ (DCIS). The aim of this study was to evaluate HIF-1α expression in DCIS as a putative precursor lesion of BRCA1 and BRCA2 related breast cancer. Methods: DCIS lesions of 16 BRCA1 and 7 BRCA2 germline mutation carriers and their accompanying invasive lesions were stained by immunohistochemistry for HIF-1α, its downstream proteins Glut-1 and CAIX, and for ER, PR, HER-2/neu, Ck5/6, Ck14, EGFR and Ki67. Expression in DCIS and the invasive counterparts was compared. Results: 11/16 (69%) of the BRCA1 DCIS cases and 5/7(86%) of the BRCA2 DCIS cases showed HIF-1α overexpression. Glut-1 and CAIX were expressed in 11/16(69%) and 9/16(56%) of BRCA1 related DCIS and in 6/7(86%) and 5/7(71%) of BRCA2 related DCIS. Glut-1 and CAIX expression was seen in 11/16 (69%) of BRCA1 and in 4/7 (57%) of the BRCA2 invasive counterparts, especially in cases of HIF-1α overexpression. HIF-1α expression in DCIS matched that in invasive counterparts in 16/23 cases (70%), for Glut-1 in 15/23(65%) and for in CAIX 18/23(78%) cases. Conclusion: BRCA1 and BRCA2 germline mutation related DCIS shows frequent HIF-1α overexpression, usually similar to that of invasive counterparts. This suggests that hypoxia may already play a role in the DCIS stage of BRCA1 and BRCA2 germline mutation related carcinogenesis. Acknowledgements: Supported by unrestricted educational grants from Aegon Inc and Pink Ribbon The Netherlands. 2011.

HIF-2α IS AN OXYGEN-REGULATED TRANSLATION FACTOR. James Uniacke[1], Chet Holterman[1], Aleksandra Franovic[1], Josianne Payette[1], Stephen Lee[1]. [1]University of Ottawa, Ottawa, Ontario, Canada. *Email: juniacke@uottawa.ca*. Introduction: Metazoan organisms adapt to fluctuations in environmental oxygen tension by inducing a systemic reorganization of biochemical processes. Hypoxia-inducible factor is responsible for orchestrating the transcriptional response to hypoxia by activating an array of genes involved in cellular oxygen homeostasis. Results: Here, we demonstrate that HIF is an oxygen-regulated translation factor that recruits mRNA for protein synthesis during periods of low oxygen tension. HIF assembles with the RNA-binding protein RBM4 at the 3' UTR of specific mRNAs,

including the epidermal growth factor receptor. HIF/RBM4 complex targets mRNA to polysomes by capturing their 5' cap thereby evading repression of cap-dependent translation by mTOR. Conclusion: These results demonstrate a novel role for HIF as a translation factor involved in coordinating the protein synthesis response to hypoxia. Acknowledgements: This work was funded by the National Cancer Institute of Canada (NCIC) and the Canadian Institutes of Health Research (S.L.). S.L. is a recipient of the NCIC Harold E. Johns Award. J.U. holds a Terry Fox Foundation Fellowship through the NCIC. 2011.

HIGH ALTITUDE HEADACHE (HAH) AND ACUTE MOUNTAIN SICKNESS (AMS) ARE COMMON IN PILGRIMS AFTER RAPID ASCENT TO 4380 M. Ken Zafren[1], Matiram Pun[2], Nirajan Regmi[2], Gobinda Bashyal[3], Bhuwan Acharya[3], Subarna Gautam[2], Sujan Jamarkattel[2], Shankar Raj Lamichhane[2], Suman Acharya[2], Buddha Basnyat[4]. [1]Stanford University Medical Center, Stanford, CA USA, [2]Mountain Medicine Society of Nepal, Kathmandu NEPAL, [3]Himalayan Rescue Association, Kathmandu NEPAL, [4]Oxford University Clinical Research Unit - Nepal, Kathmandu NEPAL. *Email: kenzafren@gmail.com.* Introduction: The goal of the study was to characterize high altitude illness in pilgrims who ascended rapidly to 4380 m. Methods: We kept standardized records for patients seen at the Himalayan Rescue Association (HRA) Temporary Health Camp at Gosainkund Lake (4380 m) in the Nepal Himalaya during the annual Janai Purnima Festival in 2014. Records included rate of ascent and Lake Louise Score (LLS). HAH was headache alone or LLS≤2). AMS was LLS≥3. High Altitude Cerebral Edema (HACE) was AMS with ataxia or altered mental status. The number of pilgrims was based on the official estimate of the Gosainkunda Area Development Committee. We report descriptive statistics. Results: An estimated 10,000 pilgrims ascended rapidly, most in 1-2 days, from Dunche (1960 m) to Gosainkund Lake (4380 m). We saw 769 patients, of whom 86 had HAH. There were 226 patients with AMS, including 11 patients who had HACE. We treated patients with HACE using dexamethasone and supplemental oxygen prior to rapid descent. Each patient with HACE descended carried by a porter. There were no fatalities due to HACE. There were no cases of High Altitude Pulmonary Edema (HAPE). Conclusion: HAH and AMS were common in pilgrims ascending rapidly to 4380 m. There were 11 cases of HACE, treated with dexamethasone, supplemental oxygen and descent. There were no fatalities. There were no cases of HAPE. Acknowledgements: The study was funded by the Himalayan Rescue Association, a non-profit organization. 2015.

HIGH ALTITUDE ILLNESS IN SOUTH POLE WORKERS: ANTARCTIC STUDY OF ALTITUDE PHYSIOLOGY (ASAP). Paul J Anderson, Andrew D Miller, Kathy A O'Malley, Maile L Ceridon, Ken C Beck, Bruce D Johnson. CDC/NIOSH, Mayo Clinic. *Email: PJAnderson@cdc.gov.* Background: The US Antarctic Program rapidly transports scientists and support personnel from sea level (SL) to the South Pole station (SP-9300ft). The combination of rapid transport (3hr), low barometric pressure, cold arid environment, and physical exertion increases altitude

illness risk. This unique natural laboratory allowed quantification of AMS incidence, and the frequency, severity and timing of symptoms in polar workers. Methods: Medically pre-screened adults (N=246, age=37±11yr, 30% female, BMI=26±4kg/m^2) were recruited. All underwent SL and SP physiologic evaluation and completed 9 symptom questionnaires during the first week at HA. Acetazolamide was voluntarily used by 40% of participants. Results: Heart rate and O_2 saturation were 71±12bpm, 97±1% at SL and 83±13, 89±3% after the second night at SP ($p<0.05$). Overall, 52% developed AMS with incidence decreasing until day 7 when only 5% met criteria. Acetazolamide use did not affect AMS incidence ($p>0.05$). Common symptoms reported were exertional dyspnea (87%), sleeping difficulty (74%), headache (66%), fatigue (65%), dizzy/lightheaded (46%). Symptoms peaked on days 1-2 at SP, yet in 20% exertional dyspnea, fatigue and sleep problems persisted through day 7. Residence<3,000ft, female gender, previous altitude symptoms, and mild symptoms at SL all predicted AMS. Conclusion: AMS is common in medically pre-screened polar workers rapidly transported to high altitude. Many symptoms linger beyond the initial 2-3 days. The effectiveness of acetazolamide during unsupervised field use did not appear to match expected efficacy observed in placebo controlled clinical trials. NSF Grant ANT-0540710. 2009.

HIGH ALTITUDE PULMONARY HYPERTENSION IS ASSOCIATED WITH SLEEP APNEA IN KYRGYZ HIGHLANDERS. Tsogyal D. Latshang[1], Michael Furian[1], Sayaka Aeschbacher[1], Silvia Ulrich[1], Aizat K. Myrzaakmatova[2], Talant Sooronbaev[2], Almaz Aldashev[3], Konrad E. Bloch[1]. [1]Pulmonary Division and Sleep Disorders Center, Univ Hospital of Zurich, Switzerland, [2]National Center for Cardiology and Internal Medicine, Bishkek, Kyrgyzstan, [3]Research Institute for Molecular Biology and Medicine, Bishkek, Kyrgyzstan. *EMAIL: konrad.bloch@usz.ch* INTRODUCTION: We evaluated the hypothesis that sleep apnea is more common in highlanders with high altitude pulmonary hypertension (HAPH) compared to healthy highlanders without HAPH. METHODS: The study was performed in Aksay, Kyrgyzstan, at 3250 m. Life-long residents of the area were invited to undergo clinical examinations, echocardiography, polysomnography and arterial blood gas analysis. According to a case control design, participants with HAPH (defined as mean pulmonary artery pressure by echocardiography mPAP >30 mmHg, in the absence of excessive erythrocytosis, heart or lung disease) were compared to healthy highlanders without HAPH. RESULTS: Of 91 consecutive participants, 36 (16 women) had HAPH (mPAP range 31-42 mmHg); 54 (21 women) were healthy controls without HAPH (mPAP range 13-28 mmHg, $P<0.01$ vs. HAPH). Highlanders with HAPH were older (53±10y) had a lower nocturnal oxygen saturation (87±4%), a higher number of cyclic oxygen desaturations (>3%) due to sleep apnea (40±26 events/h), and a higher body mass index (28.7±4.7 kg/m2) compared to highlanders without HAPH (age 39±10y, nocturnal oxygen saturation 90±3%, oxygen desaturation events 19±14/h, body mass index 24.0±3.9 kg/m2, $P<0.001$ all instances). Daytime arterial PCO_2 was similar in highlanders with HAPH (4.43±0.52 kPa) and without HAPH (4.31±0.49 kPa, P=.245). In 48 participants, 24 with HAPH and 24 controls matched for age, gender and body mass index, conditional logistic

regression indicated that the number of cyclic oxygen desaturations was an independent predictor of HAPH (odds ratio 1.16, 95% CI 1.004 to 1.346, P=0.044). CONCLUSION: HAPH is associated with sleep apnea and older age. Randomized trials are warranted to evaluate whether treatment of sleep apnea improves hemodynamics in highlanders with HAPH. ACKNOWLEDGEMENTS: Grant support: OPO Foundation and Zurich Lung League. 2015.

HIGH RESOLUTION EXPRESSION ANALYSIS OF THE WHOLE BLOOD TRANSCRIPTOME OF MICE ACCLIMATED TO HYPOXIA. Jim L Rupert, Gallo E Maria, Thomson J Cynthia , Matthew N Fedoruk. University of British Columbia. *Email: rupertj@interchange.ubc.ca.* PURPOSE: As part of our work on the application of transcriptional analysis to detect EPO doping, we used Serial Analysis of Gene Expression to compare the whole blood transcriptome of control mice to that of mice who had their hematocrits raised by hypoxia exposure. METHODS: Mice were exposed to 13.7% O_2 for 28 days, resulting in a ~10% increase in hematocrit. Whole blood RNA was extracted, pooled and used to prepare longSAGE (21 bp) libraries. The libraries were sequenced using Solexa 1G Sequencing. DiscoverySpace 4.0 software was used to compare tag frequency (each tag represents a single mRNA transcript) and to map the tags to genes. RESULTS: The total number of tags sequenced in the control and the hypoxia library was 8141875 and 7770746 respectively. The hypoxia library was more complex (993,221 distinct tag-types) than the control library (681662 distinct tag-types). In the control library, 6.5% of the tags were singletons (78.0% of tag types) while in the hypoxia library 10.07% of the tags were singletons (78.9% of tag types). There were 17514 tag-types over-represented in the hypoxia mice (13774 common, 3770 unique) compared to control while 3,217 tag-types were more common in the controls (2496 common, 721 unique). Many of the tags did not map to known genes. CONCLUSIONS: Whole blood is a transcriptionally complex tissue with patterns of gene expression that respond to changes in the external environment. Acclimatization to hypoxia results in a substantial in crease in the complexity of the blood transcriptome, although the significance of this, and the functions of the up-regulated transcripts, has yet to be elucidated. As the number of tag-types exceeds the number of protein-coding genes in the mouse by several fold, much of this transcriptional complexity must be due to either alternate splicing or as yet uncharacterized functional RNAs. ACKNOWLEDGMENTS: This work is funded by the World Anti-doping Agency. 2009.

HIGH-ALTITUDE ILLNESS AND HYPOXIC CHEMORESPONSIVNESS: A UNIFYING VIEW OF INDIVIDUAL SUSCEPTIBILITY. Hugo Nespoulet[1], Bernard Wuyam[1], Renaud Tamisier[1], Carole Saunier[2], Denis Monneret[1], Judith Rémy[1], Olivier Chabre[3], Jean-Louis Pépin[1], Patrick Lévy[1]. [1]HP2 Laboratory INSERM U 1048, School of Medicine, Grenoble, France, [2]CHU de Grenoble, Grenoble, France, [3]CHU de Grenoble, France. *Email: hnespoulet@chu-grenoble.fr.* Introduction: High-altitude illness remains a major cause of mortality at high-altitude. Ventilatory response to hypoxia during wakefulness, periodic breathing

during sleep, functional changes in pulmonary circulation in response to hypoxia may be informative to predict altitude intolerance. Methods: We compared twelve altitude-illness susceptible individuals (i.e. with recurrent and severe acute mountain sickness, pulmonary and/or cerebral edemas) to matched controls by evaluating hypoxic ventilatory sensitivity, ventilator patterns when sleeping in a hypoxic tent and pulmonary circulation adaptive response to exercise during a hypoxic challenge. Results: A lower mean blood oxygen saturation (SpO_2) (81·6±2·6 vs. 86·0±2·4%, $p<0·01$) during nighttime was observed in altitude intolerant subjects compared to controls. Intolerant subjects also exhibited impaired pulmonary circulation recruitment with inappropriate elevation in pulmonary arterial pressures in response to hypoxia. iHVR5, an index of peripheral hypoxic chemoresponsiveness, represented the best single predictive factor for altitude intolerance. An 80% risk to develop high-altitude illness was present when iHVR5 was lower than a threshold value of 0·58 l.min-1.%SpO_2-1. Conclusion: Owing to their lower hypoxic chemoresponsiveness, case subjects were exposed to more severe hypoxic conditions during night. They exhibited less periodic breathing and more severe oxygen desaturation than controls. They also presented with higher pulmonary pressure changes in response to hypoxia. Although both findings may play a role in High-Altitude Illness development, the reduction in chemosensitivity and related aggravated hypoxic exposure seem to be critical. Our study provides insights regarding both mechanisms and clinical prediction of altitude intolerance. Acknowledgements: The authors are grateful to the DRCI CHU de Grenoble, AGIRadom, Région Rhône-Alpes for financial support. 2011.

HIGH-INTENSITY FOREARM EXERCISE INFLUENCES ARTERIOVENOUS DIFFERENCES OF NITRATE. Norbert Maassen[1,3], Karina Sutmöller[1], Henning Starke[1], Mirja Maassen,[3] Alexandra Schwarz[2], Anke Böhmer[2] and Dimitrios Tsikas[2]. [1]Institute of Sport Medicine, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany, [2]Institute of Clinical Pharmacology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany, [3]Institute of Sport Science, Leibniz University Hannover, Am Moritzwinkel 6, 30167 Hannover, Germany. *Email: Maassen.Norbert@mh-hannover.de.* *Introduction:* In recent years the role of nitrate in physical exercise has been investigated in several studies, but reports on arteriovenous (A/V) differences in humans are scarce. The aim of the present study was to investigate A/V differences of nitrate and nitrite concentrations in blood draining working muscles during and after exhaustive forearm exercise. Methods: Nine male healthy subjects performed two maximal hand grip exercise bouts lasting 30 s. Arterialized blood of a heated dorsal vein of the non-dominant arm and cubital venous blood draining the working muscles were drawn before, during and after exercise, and heparinized plasma was generated. Plasma concentrations of nitrate and nitrite were determined by stable-isotope dilution gas chromatography-mass spectrometry (GC-MS). Forearm blood flow was determined by venous occlusion plethysmography. Results: Lactate concentration in cubital venous plasma increased to 13.5 ± 3.2 mM at the end of exercise (EE). At that time, PCO_2 was 102.3 ± 9.5 mmHg, HbO_2 was 26.4 ± 6% and the pH value was 7.12 ± 0.03 in

venous blood samples. Blood flow increased from 2.0 ± 0.65 mL/100 mL tissue×min at rest to 30.3 ± 17.2 mL/100 mL tissue×min at EE. There was a significant positive A/V difference in nitrate concentration at EE ($P < 0.001$), due to a significant decrease in venous plasma nitrate concentration ($P < 0.001$) indicating a nitrate loss from plasma. Maximal nitrate loss was noted at EE (0.65 ± 0.75 μmol/L tissue×min, $P < 0.001$). Such changes have not been seen for nitrite. Conclusions: These findings strongly suggest that circulating nitrate may serve as a readily accessible store for NO production, presumably via intermediate reduction of nitrate to nitrite, during high-intensity exercise of the forearm. The nitrate→nitrite→NO pathway is likely to have been facilitated by falls in SO_2 and pH in venous blood. Enzymes involved in the nitrate→nitrite→NO conversion may be xanthine oxidoreductase and deoxyhaemoglobin and carbonic anhydrases. 2015.

HIGHLAND ANCESTRY ALTERS THE HYPOXIA ACCLIMATION RESPONSE IN DEER MICE. Graham Scott[1], Mikaela Lui[1], Todd Elogio[1], Grant McClelland[1], Zachary Cheviron[2], Jay Storz[3]. [1]McMaster Univ, Hamilton, Canada, [2]Univ Illinois at Urbana-Champaign, Urbana, USA, [3]Univ Nebraska-Lincoln, Lincoln, USA. *EMAIL: scottg2@mcmaster.ca* INTRODUCTION: The objective of this study was to compare the hypoxia acclimation responses of deer mice (Peromyscus maniculatus) from highland and lowland ancestry. METHODS: Mice were caught on the summit of Mount Evans (4350m elevation) in Colorado and in eastern Nebraska (430m), and were then used to establish captive breeding populations. First generation adult mice that were born and raised in captivity at sea level were compared before and after acclimation to hypobaric hypoxia (60 kPa, equivalent to 4300m) for 6-8 weeks. RESULTS: Acclimation to hypobaric hypoxia improved aerobic capacity (VO2max) during treadmill exercise in hypoxia in both populations, but hypoxic VO2max was consistently higher in the highland deer mice. In contrast, highland ancestry had no effect on VO2max measured in normoxia. Associated with the heightened capacity for hypoxic exercise in highlanders was a higher density of oxidative fibres in the locomotory muscle (gastrocnemius). However, there were no population-specific differences in lung mass or heart ventricle mass. Blood haemoglobin content increased after hypoxia acclimation, but did not differ between populations. CONCLUSION: Highland ancestry has therefore improved the capacity of deer mice to exercise in hypoxia, and has altered at least some of the underlying determinants of oxygen transport and utilization. ACKNOWLEDGEMENTS: Supported by NSERC of Canada. **2015.**

HUMAN SKELETAL MUSCLE MITOCHONDRIA AT HIGH-ALTITUDE: ADAPTATION AND ACCLIMATISATION. Daniel Martin[1], James Horscroft[2], Aleksandra Kotwica[2], Verna Laner[3], Edward Gilbert-Kawai[1], David Howard[1], Hugh Montgomery[1], Michael Grocott[4](presenting author), Denny Levett[1], Erich Gnaiger[5], Andrew Murray[2]. [1]UCL Centre for Altitude, Space and Xtreme Environment Medicine, London, UK, [2]Dept of Physiology, Development and Neuroscience, University of Cambridge, UK, [3]Oroboros Instruments, Innsbruck, Austria, [4]University Hospital Southampton / University of Southampton, NIHR Respiratory

Biomedical Research Unit, [5]Daniel Swarovski Laboratory, Medical University of Innsbruck, Austria. *Email: daniel.martin@ucl.ac.uk.* Introduction: Exposure of lowland natives to high-altitude is associated with metabolic reprogramming in skeletal muscle and loss of mitochondrial density, though this might depend upon duration/severity. Meanwhile, little is known about metabolic adaptations in the muscle of high-altitude natives and whether alterations occur upon exposure. We therefore set out to investigate mitochondrial function in unacclimatised lowlanders and Sherpas before and after an identical ascent from Kathmandu (1300 m) to Everest Base Camp (EBC; 5300 m). Methods: Vastus lateralis biopsies were taken and saponin-permeabilised fibre bundles prepared for high-resolution respirometry. Sherpa biopsies were taken in Kathmandu (baseline) and at EBC after short-term exposure to altitude (12-14 d). Lowlander biopsies were taken in London (50 m, baseline), and at EBC after short-term (16-21 d) and long-term exposure (55-60 d). Results: At baseline, Sherpa muscle had a lower respiratory capacity than lowlander muscle, and most notably a 34% lower capacity during fatty acid oxidation (with octanoyl-carnitine). Moreover, the Sherpas' LEAK/OXPHOS ratio, a measure of mitochondrial uncoupling, was 21-23% lower, suggesting greater metabolic efficiency. Following short-term exposure, LEAK respiration during fatty acid oxidation fell by 28% in lowlanders, whilst in Sherpas there was no change in mitochondrial respiratory capacity. Following long-term exposure, mitochondrial complex II-supported respiration fell by 10% in lowlanders. Conclusions: Our findings demonstrate metabolic adaptation to high-altitude in Sherpa muscle, characterised by greater efficiency and lower fatty acid oxidation, which might underlie more economical oxygen use. The acclimatisation of lowlanders' mitochondria to altitude, decreasing uncoupled respiration and electron transport chain capacity, might achieve a similar effect. Acknowledgements: On behalf of the the Xtreme Everest 2 Research Group. 2015.

HYPERVENTILATION, ACCLIMATIZATION AND CEREBRAL OXYGENATION AT ALTITUDE. Matthew Sanborn[1], Heng Yow[2], Meeri Kim[3], Daniel Martin[2], Kay Mitchell[2], Mark Edsell[4]. [1]Hospital of the University of Pennsylvania, Philadelphia, PA, USA, [2]Centre or Altitude, Space and Extreme Environment Medicine, University College London, London, England, [3]University of Pennsylvania, Philadlephia, PA, USA, [4]St. George's Hospital, London, England. *Email: Matthew.Sanborn@uphs.upenn.edu*. Introduction: There is a paucity of human-subjects data on cerebral oxygenation during acclimatization particularly in response to a provocative challenge such as hyperventilation. Methods: 11 subjects were studied at 75m above sea level and then 2 and 7 days after ascent to 4,559m. Cerebral oxygenation ($SctO_2$) was assessed using near-infrared spectroscopy. Peripheral oxygen saturation (SpO_2) and end-tidal carbon dioxide ($PETCO_2$) were also monitored. After obtaining baseline measurements in all modalities subjects were asked to hyperventilate to reduce their baseline $PETCO_2$ by 50%. After 3 minutes of maintaining this $PETCO_2$ a further set of measurements were obtained. Results: At sea-level, $SctO_2$ was reduced by hyperventilation from 67.4 +/-3.1% to 61+/-1.6% ($p < .01$). Following ascent to 4,559m, hyperventilation caused an increase $SctO_2$ from 65+/-6.5% to

70.8+/-7.4% (p <.001). By the 7th day at altitude, there was no significant difference between $SctO_2$ before (63 +/-5%) or after (64.3 +/-5.6%) hyperventilation. In contrast, SpO_2 was unaffected by hyperventilation at sea-level (98.28+/-1.80% to 98.85+/-1.07%) but improved following hyperventilation on day to at altitude [80.86+/-4.41% to 95.85+/-5.43% p<0.01)]. After 7 days, baseline SpO_2 continued to improve with acclimatization and similarly increased with hyperventilation from 87.00+/-1.90% to 98.50+/-0.84% (p< 0.01). $PETCO_2$ was 4.51+/-0.77 kPa at sea level and decreased to 3.37+/-.39 kPa after 2 days at 4,559m and 3.19+/-.42 kPa after 7 days. There was a positive correlation between $PETCO_2$ and $SctO_2$ (r=0.4001, N=34, p = 0.019) and between SpO_2 and $SctO_2$ (r=0.3867, n=34, p=0.0239). Conclusion: $SctO_2$ and $PETCO_2$ decreased after 2 days at altitude and decreased further at 7 days despite a rebound in SpO_2. Since $SctO_2$ is determined by a combination of cerebral blood flow and tissue oxygenation, this implies an increase in cerebrovascular resistance between days 2 and 7. Although hyperventilation at altitude initially increases $SctO_2$, after acclimatization it has no effect. Interestingly, there was a slightly more robust correlation between $PETCO_2$ and $SctO_2$ than between SpO_2 and $SctO_2$. Acknowledgements: Xtreme Alps Investigators, American Alpine Club Research Grant. 2011.

HYPOXIA AND BLOOD-BRAIN BARRIER DYSFUNCTION: IS HIF-1 THE CULPRIT? Omolara Ogunshola[1], Sabrina Engelhardt[1], Abraham Al-Ahmad[1]. [1]Institute Veterinary Physiology, Univ Zurich. *EMAIL: larao@access.uzh.ch* INTRODUCTION: Hypoxia, a characteristic state of many brain diseases, leads to disturbance of blood-brain barrier (BBB) function, increased vascular permeability and injury progression. Activation of hypoxia inducible factor-1 (HIF-1) mediated signalling is instrumental for cells and tissues to adapt to oxygen deprivation, but can also be a double-edged sword and induce cell death. At the BBB it is unclear to what extent this pathway modulates cell-specific responses and/or barrier integrity. Indeed such knowledge could directly impact the future use of HIF stabilizers as protective drugs. METHODS: We are investigating the consequences of HIF-1 induction on endothelial barrier function using novel in vitro (using endothelial and astrocyte mono- and co-cultures subjected to 1% O_2 for up to 48h). HIF-1 modulation was performed using CoCl2, DMOG, DFO, 2ME and YC1 as well as knockdown and over expression studies. Barrier function is measured using permeability assays. RESULTS: Our studies clearly demonstrate that pharmacological stabilization of HIF-1alpha under normoxic conditions in endothelial monolayers increases barrier permeability and inhibition of HIF-1 during oxygen deprivation improves barrier function. In correlation with these findings localization and levels of tight junction proteins was also altered by HIF-1 modulation. These changes are at least partially regulated by MAPK signalling as inhibitors of this pathway also preserved barrier integrity. Our current data also shows that astrocyte and pericyte interactions with vascular endothelial cells reduce the degree of injury experienced by the monolayer in terms of oxidative stress and HIF-1 induction. CONCLUSION: Overall, our work suggests that HIF-1 stabilization may have detrimental consequences for barrier function. We have now generated new animal models with inducible cell-specific knockdown of HIFs at the BBB to further investigate the impact of

cell-specific HIF-1 stabilization on vascular function. ACKNOWLEDGEMENTS: Supported by SNF grant project number 31003A-133016 to O.O. 2015.

HYPOXIA AND CARBON MONOXIDE EXPOSURE BOTH CAUSE SYMPATHETIC ACTIVATION, BUT ONLY HYPOXIA INCREASES HEART RATE AND VENTILATION. Mikael Sander, Carsten Lundby. National Hospital Copenhagen Denmark. *Email: sanders@dadlnet.dk.* Hypoxia engages chemoreflexes to cause increases in sympathetic activity, heart rate and ventilation. Carbon monoxide (CO)-induced changes have been sparingly studied, but in one human study short-term CO-exposure has been shown to induce an immediate increase in sympathetic nervous activity. By tight binding to hemoglobin, CO causes decreases in saturation and hence decreases in oxygen content (CaO_2) without changing arterial oxygen tension (PaO_2). We asked whether steady-state CO-exposure in humans was accompanied by sympathetic activation. In 8 healthy volunteers, we measured arterial blood gasses, blood pressure (MAP), heart rate (HR), ventilation (VE), and arterial norepinephrine levels (NE) during 8 hours exposure to CO, hypoxia or normoxia. The normoxia time-control caused no changes in hemodynamic parameters. Hypoxia caused saturation to decrease to 83±0.4%, and NE, HR, and VE to increase. CO-exposure caused HbCO to increase to 17,5±0,6%, i.e. HbO_2 similar to the hypoxia-exposure. NE-increases were similar during hypoxia and CO, and the NE-increase was delayed by about 1 hour during both trials. During CO, there were no changes in HR or VE. Interestingly, MAP did not change during either hypoxia or CO. These data suggests 1) during CO-exposure, the decrease in HbO_2 is responsible for sympathetic activation, perhaps due to activation of central nervous chemosensitive neurons, which would be responsive to decreases in tissue oxygenation; 2) such central nervous chemoreceptors are not involved in the control of HR and VE; 3) The mechanism causing delayed increases in NE-levels during hypoxia is dependent on decreased HbO_2; and 4) The local vascular mechanism off-setting hypoxia-induced sympathetic activation is also dependent on decreases in HbO_2. Funding: Novo Nordisk Foundation. 2009.

HYPOXIA AND INFLAMMATORY PHENOTYPE IN PROSTATE CANCER CELLS. Linda Ester Ravenna[1], Lorenzo Principessa[1], Matteo Antonio Russo[2], Luisa Salvatori[1], Giuseppe Coroniti[2], Elisa Petrangeli[2]. [1]National Council of Research, [2]University of Rome Sapienza. *Email: linda.ravenna@ifo.it*. Introduction: Inflammation and hypoxia have an interconnected role in the pathogenesis and progression of many cancers such as prostate, mesothelioma and breast. This study investigates the role of hypoxia in the modulation of the pro-inflammatory phenotype in cancer prostate LNCaP, PC3 and DU145 cells. Methods: We performed real-time and western blot analysis to evaluate gene expression. After oxygen withdrawal, we compared the activation kinetics of HIF-1A, 2A and 3A and of NF-kB (p65 e p50) and we evaluated the expression levels of a number of genes (VEGF, RAGE, P2X7R, COX2, HMOX1, CXCR4) involved in inflammation and metastasis. Results: Nuclear translocation of HIF-1A followed comparable kinetics in all prostate cells: early start, top after four hours and decline by 24. Expression of HIF1A mRNA was stable for 4 hours, then abruptly decreased and stabilized under

the base level. HIF-2A did not seem to respond to reduced oxygen. HIF3A was expressed only in DU145 and both mRNA and protein were up-regulated concomitantly with the decrease of HIF1A mRNA expression. Hypoxia dependent increase in NF-kB nuclear translocation was observed in PC3 and DU145 cell lines but not in the more differentiated LNCaP. When expressed, a clear up-regulation of almost all the studied pro-inflammatory and metastasizing genes was observed, peaking after 24 h (DU145, LNCaP) or between 48 and 72h (PC3). Conclusion: Our data show that prostate tumor cells express molecules of the inflammatory response and that hypoxic microenvironment strongly modulates this phenotype. By silencing HIF isoforms and p65 we intend to clarify the individual contribution of each transcription factor to the pro-inflammatory phenotype in prostate and identify potential therapeutic targets. 2011.

HYPOXIA CHANGES THE SENESCENCE OF ENDOTHELIAL PROGENITOR CELLS. Markus de Marees, Dennis Nebe, Tobias Weber, Julia Kolling, Birgit Bolck, Klara Brixius, Jochen Mester, Wilhelm Bloch. German Sport University Cologne. *Email: demarees@dshs-koeln.de.* Physical activity as well as hypoxia is the major stimuli for processes which support angiogenisis as well as vascular reparation. Increasing evidence suggests that endothelial progenitor cells (EPC) play an important role for both of these processes. To examine the effects of hypoxia on the senescence of EPC we set up two different studies. In study one, we investigated the acute effect of hypoxia. Athletes performed an incremental cycling ergometer test to individual exhaustion under normoxia and normobaric hypoxic conditions (12.5% pO_2). Before, 0, 10 and 240 min after the test blood samples were taken. The second study investigates the influence of chronic hypoxia. Members of the national track and field team underwent a hypoxic (15.6% pO_2) training camp (28 d) according to the live high-train high (LH-TH) design, while the control group underwent a training camp under sea level conditions. Before and after the training camp incremental running tests under normoxic conditions were carried out and additional blood samples were taken. To characterize the senescence of EPC, the fl-galactosidase assay was used. The preconditioned serum in study one induced a significant decrease in senescence of EPC for time point 0,10,240 min. The results of the second study showed an increase of the senescence of EPC after the LH-TH training camp compared to the values before. The control group did not show such a huge increase in the senescence of EPC compared to the altitude group although it was significant increased to the values before the camp. The decrease in EPC-senescence caused by acute hypoxia indicates an increasing capacity of the regeneration potential of EPC. While the increased senescence of EPC induced by the chronic hypoxia may lead to a more inactive state of EPC. 2009.

HYPOXIA IN PATIENTS STARTING HOME MECHANICAL VENTILATION. Michael Laub, Bengt Midgren. Copenhagen University Hospital, Lund University. *Email: laub@rh.dk.* Methods: From The Swedish Home Mechanical Ventilation Register we identified 352 patients with neuromuscular disease and we looked at circumstances (acute vs. elective) and clinical motives for

starting ventilatory support. Results: The patients were moderately hypercapnic ($PaCO_2$: 7.0 kPa, SD: 1.3). Mean vital capacity was close to 40% of predicted, but significantly lower in the Duchenne patients (26% of predicted). None of the clinical motives were related to the $PaCO_2$ level. Average PaO_2 was above 8 kPa in all groups, but lowest in the Post-polio and Dystrophia myotonica patients. Conclusions: As to the hypoxia per se, we have especially in the Dystrophia myotonica group no explanation for this, which cannot be explained by age or known cardio-pulmonary disorders. Our finding is in accordance with one previous study where the baseline PaO_2 level in DM patients was even lower (7.1 kPa) than in our study. In the literature, there seems to be no explanation for this phenomenon. 2009.

HYPOXIA INDUCED CHANGES IN LUNG FLUID BALANCE IN HUMANS IS ASSOCIATED WITH BETA-2 ADRENERGIC RECEPTOR DENSITY ON LYMPHOCYTES. Bruce D Johnson, Eric M Snyder. Mayo Clinic, University of Arizona. *Email: johnson.bruce@mayo.edu.* Background. Previous studies have demonstrated an important role for β2 adrenergic receptors (β2AR) in lung fluid clearance. The purpose of this investigation was to examine the relationship between β2AR density on lymphocytes and indices of lung water in healthy humans exposed to ~18hr of hypoxia (FIO_2 12.5% in a hypoxia tent). Methods. Thirteen adults (age=31±3yr, BMI=24±1 kg/m2, VO_2Peak=40±2 ml/kg/min) participated. Key methods included CT imaging for measures of lung tissue volume (Vtisñtissue, blood & water), pulmonary function as well as measures of lung diffusing capacity for carbon monoxide (DLCO) & nitric oxide (DLNO) to obtain estimates of alveolar-capillary conductance (DM), pulmonary capillary blood volume (Vc) and lung water (CT VtisVc), and β2AR density on lymphocytes using radioligand binding. Arterial oxygen saturation (SaO_2), cardiac output (Q), right ventricular systolic pressure (RVSP) and blood pressure (BP) were also monitored. Results. After ~18hr hypoxia, SaO_2 dropped from 97±1 (rm air) to 82±4% and RVSP increased from 15±9 (rm air) to 28±4 mmHg ($p<0.05$) with little change in Q or BP ($p>0.05$). Vc and DM both increased with hypoxia with small changes in the DM/Vc ratio ($p>0.05$). Tissue volume decreased and lung water was estimated to decline 7±13%. β2AR density averaged 1511±720 receptors/lymphocyte and increased 21±34% with hypoxia (range 31 to +86%). The change in β2AR density with hypoxia was associated with the change in lung water ($r=0.64$), with the subjects with the greatest increase in density demonstrating the largest decline in lung water. Conclusions. Lung water change with 18hr normobaric hypoxia is associated with changes in beta adrenergic receptor density on lymphocytes in healthy adults. NIH Grant HL71478. 2009.

HYPOXIA PREVENTS THE INCREASE IN MITOCHONDRIAL OXYGEN CONSUMPTION INDUCED BY STIMULATION OF AMPK IN CARDIOMYOCYTES. Heimo Mairbäurl[1], Emel Baloglu[2], Ying Zhang[3]. [1]Medical Clinic VII, Sports Medicine, University of Heidelberg, and Translational Lung Research Center (TLRC-H; part of the German Center for Lung Research, DZL), Germany, [2]Medical Clinic VII, Sports Medicine, University of Heidelberg,

[3]Department of Biomedical Sciences, Beijing Sport University, China. *Email: heimo.mairbaeurl@med.uni-heidelberg.de.* Introduction: The AMP-activated protein kinase (AMPK) balances cellular energy demand and ATP production by increasing PGC-1α-dependent expression of mitochondrial proteins. Hypoxia decreases mitochondrial activity in order to decrease mitochondrial production of ROS. Therefore, we tested the hypothesis that stimulation of AMPK to simulate increased metabolic demand blunts the impaired mitochondrial function in hypoxia to prevent functional impairment of cardiomyocytes. Methods: H-10 cardiomyocytes were treated with AICAR to stimulate AMPK in normoxia and hypoxia (1.5 % O_2). Results: AICAR, hypoxia, and their combination increased phosphorylated AMPK indicating stimulation. AICAR induced a 3x increase in mitochondrial oxygen consumption (JO_2) that depended on ERRα, whereas hypoxia decreased basal JO_2 (-20%) and prevented the AICAR-induced increase. Hypoxia also prevented the AICAR-induced increase in mitochondrial proteins. Both, hypoxia and AICAR decreased the mitochondrial membrane potential measured with TMRE. Interestingly, hypoxia did not prevent the AICAR-induced increase in pyruvate dehydrogenase (PDH) activity despite increased PDK expression. The HIF-dependent genes GAPD (1.5x) and BNIP3 (4x) were increased in hypoxia, also in the presence of AICAR. Hypoxia decreased H_2O_2 production. Caspase-3 activity, an indicator of BNIP3-dependent apoptosis was not affected by the treatments. Conclusion: These results indicate that hypoxia prevents the AMPK-dependent stimulation of mitochondrial metabolism thus disproving our hypothesis. The inhibition of mitochondrial respiration by hypoxia seems to be caused by decreasing the expression of enzymes of the electron transfer chain by blocking signaling downstream of AMPK rather than by decreasing PDH-mediated substrate flux into mitochondria. Together this indicates that hypoxia decreases cardiomyocyte oxidative metabolism despite increased metabolic demand. 2015.

HYPOXIA RELATED CHANGES IN PHOTORECEPTOR FUNCTIONS IN YOUNG SUBJECTS ACUTELY EXPOSED TO HIGH ALTITUDE. Sarper Karakucuk[1], Burcu Polat[1], Ertugrul Mirza[1], Hatice Arda[1], Koray Gumus[1], Ayse Oner[1]. [1]Erciyes Univ Faculty of Medicine Dept. of Ophthalmology, Kayseri, Turkey. *EMAIL: sarperkarakucuk@gmail.com* INTRODUCTION: Hypoxia related changes in human retina can occur during prolonged unacclimatized ascent to high altitudes (over 8000ft/2438m) however it is not known whether these can occur after relatively short exposures. Here we present the evaluation of retinal changes after a short exposure to high altitude. METHODS: Contrast sensitivity testing (CST), electroretinography (ERG) and optical coherence tomography (OCT) were performed on 23 healthy subjects aged between 17-29 years (mean age ±S.E.M = 20.88±0.64 years) at the Eye clinic of Erciyes Univ Faculty of Medicine located at an altitude of 1080m/3543ft. On the following week, the subjects were transported from 1080m/3543ft to a station at 2630m/8628ft on Mt. Erciyes by buses and cable-cars within one hour. After resting for one hour at 2630m/8628ft, the group started to climb to 2650m/8694ft altitude, completed a route within 2 hr. and returned back to 2630m/8628ft and then were transported back to the city. The tests were repeated

within the 3 days following the altitude exposure. The test results before and after the exposure were compared with each other. RESULTS: Average pre-exposure CST on the 20/25 scale was 0.12 logMar units on the right and 0.13 logMar units on the left eye. After the altitude exposure, these values decreased to 0.14 logMar units (p=0.02) and 0.14 log mar units (p=0.01), on the right and left eye, respectively. Average pre-exposure ERG flicker amplitude on the left eye was 58.64mV and decreased to 52.05mV after the exposure (p=0.05). No significant differences were observed in the OCT values. CONCLUSION: Hypoxia during an acute and unacclimatized short exposure to high altitude can negatively affect some of the photopic photoreceptor functions in young subjects and this effect can last a few days following the exposure. ACKNOWLEDGEMENTS: Participation of Residents, Medical Students and Technicians of Erciyes Univ Medical Faculty and technical help of mountaineers and skiing instructors from Mt. Erciyes region during the related activity is greatly acknowledged. 2015.

HYPOXIA SIGNALING PATHWAY GENE EXPRESSION PROFILE IN HAPE PATIENTS. Wuren Tana[1], Bai Zhenzhong Bai[2], uhong Li[3], Yingzhong Yang[3], Ri-Li Ge[3]. [1]Research Center for High-Altitude Medicine, Qinghai Univ Medical School, Xining, Qinghai, China, [2]Research Center for High-Altitude Medicine, Qinghai Univ Medical School, Xining, Qinghai China, [3]Research Center for High Altitude Medicine, Qinghai Univ, Xining Qinghai, China. *EMAIL: geriligao@hotmail.com* INTRODUCTION: High altitude pulmonary edema (HAPE) is a life-threatening illness that for high altitude (>2500 m) newcomers is primarily caused by hypoxic stress, however the underlying changes in the hypoxia gene expression network remain elusive. We have designed this study, based on the clinically accepted use of the peripheral blood as a surrogate for tissue expression, to profile expression in the hypoxia signaling pathway. METHODS: This study was conducted using peripheral blood obtained from 12 Han Chinese HAPE patients and 6 healthy Han Chinese subjects at 3700m. All subjects were measured under clinical parameters, and RNA was extracted from 2.5ml whole blood. The RT2 profiler PCR array for the hypoxia signaling pathway (SABiosciences) was used to perform gene expression profiling and the data were analyzed using the provided web based portal (SABiosciences) with 2-Δ Ct methods to determine relative quantification. RESULTS: Of 89 genes tested using the specific PCR array, 31 were significantly up-regulated (>2 fold differences) with 1 gene significantly down regulated (-2 fold differences) in AMS patients. The validated genes are categorized into HIF-1 related transcription factors, in addition to genes which participate in angiogenesis, coagulation, DNA damage signaling and repair, metabolism, apoptosis regulators, cell proliferation regulators, transporters, channels & receptors. CONCLUSION: Importantly, to allow translation of our work, this is the one of the first time that profiled the hypoxia signaling pathway gene expression in HAPE patients in high altitude, it suggest that the manifestation of HAPE is a multi-gene process. Furthermore, these novel up-regulated genes need to be assessed in functional studies. Their confirmation could lead to novel diagnostic approaches for acute mountain sickness. ACKNOWLEDGEMENTS: This work supported by Supported by National Basic

Research Program of China(No.2012CB518200), Program of International S&T Cooperation of China (No.0S2012GR0195), National Natural Science Foundation of China (No.30393133). 2015.

HYPOXIA TOLERANCE GENES IN DROSOPHILA: DEVELOPMENT, TISSUE SPECIFICITY, AND ADULT SURVIVAL IN RESPONSE TO ACUTE, LOW OXYGEN EXPOSURE. Rachel Zarndt[1], Priti Azad[1], Gabriel Haddad[2]. [1]University of California San Diego, La Jolla, California, [2]Rady Children's Hospital San Diego; University of California San Diego, La Jolla, California,. *Email: rzarndt@ucsd.edu*. Introduction: In a screen of over 2300 Drosophila lines with individual gene disruptions, called p-element insertions, we identified a subset of novel genes that increased survival in hypoxia. Of this subset, we performed detailed assays of eclosion rates and adult survival in p-element lines whose known roles in gene regulation and cell growth suggest they are promising candidates for co-regulating hypoxic tolerance: tna and Alh. These genes are transcriptional modifiers in the Polycomb group (PcG), whose composition and functional roles are conserved in humans. Methods: To assay hypoxia tolerance at specific life stages, we screened development (egg to pupae) and eclosion rates (pupae to adult) at an $FIO_2 = 0.05$ and acute survival of adults at an $FIO_2 = 0.02$. To characterize Alh and tna gene expression for conferred hypoxia tolerance, we used the F1 progeny of a cross of either a UAS- (over-expression) or RNAi-line (knockdown) with GAL4 drivers for ubiquitous expression, or for specific heart, muscle, hemocyte, neuronal, or glial tissue expression. Results: We found decreased eclosion rates at $FIO_2 = 0.05$ with knockdown of tna or Alh, and increased eclosion with over-expression of Alh. Ubiquitous knockdown of Alh and tna appeared non-advantageous to adult survival at $FIO_2 = 0.02$, while ubiquitous or selective over-expression of Alh in brain and heart tissue increased adult survival. Further, knockdown of either gene in hemocytes greatly increased adult survival at $FIO_2 = 0.02$. Conclusion: Selective regulation by single genes, such as for Alh and tna may be advantageous for flies in hypoxia to sustain growth or control cell proliferation, given that tissue-specific expression improves hypoxic tolerance. Further, as PcG transcriptional modifiers, these genes may act on a number of targets to modulate response to hypoxia. Acknowledgements: Biomedical Sciences Graduate Program, UCSD. 2011.

HYPOXIA-INDUCED INTRAPULMONARY ARTERIOVENOUS SHUNTING INCREASES OVER TIME IN HUMANS AT REST. Steven S Laurie, Ximeng Yang, Jonathan E Elliot, Kara M Beasley, Andrew T Lovering. University of Oregon. *Email: slaurie@uoregon.edu.* We have recently identified a hypoxic threshold necessary to induce IPAV shunting in all subjects at rest (see accompanying poster). However it is still unknown if shunting will qualitatively increase during sustained exposures (>5-10 min) to hypoxia. Therefore, the purpose of this study was to determine if the duration of hypoxic exposure altered intrapulmonary shunting in humans at rest in a semi-recumbent position. Thirteen healthy subjects (6 female) breathed four different levels of hypoxia (FIO_2=0.16, 0.14, 0.12, 0.10) in either ascending or descending order for 30 min with each FIO_2 separated by a

15 min normoxic break. Saline contrast echocardiography was used to detect the passage of bubbles through large diameter IPAV shunts. The number of bubbles in the left heart was scored on a 0-5 scale: 0= 0 bubbles; 1=1-3 bubbles; 2=4-12 bubbles; 3= >12 bubbles; 4= heterogeneously filled left ventricle; 5= homogeneously filled left ventricle. A bubble score ≥2 was defined as an IPAV shunt. Bubble scores at 30 min of exposure were greater than scores at 5 min exposure for an FIO_2=0.16, 0.14, 0.12, and 0.10 in 2/13, 5/13, 6/13, and 9/13 subjects, respectively. These data suggest that there is a temporal effect on the degree of hypoxia-induced IPAV shunting. Support: OHSU MRF Grant. 2009.

HYPOXIA-INDUCED MUSCLE DYSFUNCTION IN NEONATAL RATS: NO ROLE OF OXIDATIVE STRESS. Jayne Carberry, Prof. Aidan Bradford, Ken O'Halloran. University College Dublin, Royal College of Surgeons in Ireland. *Email: jayne.carberry@yahoo.com.* We have previously shown that acute and chronic hypoxia impairs contractile and endurance properties of rat respiratory muscles during development with evidence for age-related effects. In adult animal studies, antioxidants have been shown to improve respiratory muscle performance under hypoxic conditions. The aim of this study was to determine the putative role of oxidative stress in hypoxia-induced muscle dysfunction in neonates. Wistar rats aged postnatal day (P)19 were killed humanely and the paired sternohyoid (SH, a representative pharyngeal dilator) muscles were removed. Isometric contractile properties of isolated muscle strips were measured in tissue baths containing physiological salt solution (PSS) at 30°C under hyperoxic ($95\%O_2/5\%CO_2$) or hypoxic ($95\%N2/5\%CO_2$) conditions. In separate studies, the muscles were incubated in PSS containing the antioxidants: N-acetyl-L-cysteine (NAC 10mM) or Tempol (10mM). Force-frequency relationship and fatigue index (ie ratio of force at 5min of fatigue to initial force) were examined. Hypoxia-decreased specific force in the SH (peak force was 9.7±1.5 vs 3.3± 0.6*; mean±SEM N/cm2, hyperoxia (n=15) vs. hypoxia (n=9), P<0.05 ANOVA) and significantly reduced muscle endurance (fatigue index was 60±3% vs 22±2%*; mean±SEM N/cm2, hyperoxia vs. hypoxia, P<0.05 ANOVA). Tempol had a positive inotropic effect on SH force during hypoxia but this was only significant for peak tetanic force (1.8±0.4 vs 3.2±0.6*N/cm2, hypoxia (n=7) vs. tempol-incubated hypoxia (n=9) muscles, P<0.05 ANOVA). Tempol had no effect on muscle endurance. NAC had no effect on SH force or endurance under hyperoxic or hypoxic conditions. These studies suggest that oxidative stress has no role in respiratory muscle impairment during acute hypoxia in neonates. Future studies will examine the efficacy of antioxidant treatment in preventing SH dysfunction following chronic hypoxia in neonatal rats. Supported by the Health Research Board of Ireland and UCD. 2009.

HYPOXIA-INDUCED RESPIRATORY MUSCLE PLASTICITY IS UNAFFECTED BY NITRIC OXIDE INHIBITION IN THE RAT. Clodagh McMorrow, Aidan Bradford, Ken D O' Halloran. University College Dublin, Royal College of Surgeons in Ireland. *Email: clodaghmc1@hotmail.com.* Chronic hypoxia (CH) is encountered in pulmonary diseases as well as in healthy individuals exposed

to altitude and it is known to affect skeletal muscle structure and function. Nitric oxide (NO) has been shown to be an important modulator of respiratory muscle function especially under in vitro hypoxic conditions and hypoxic tolerance in cardiac muscle is NO-dependent. The aim of this study was to 1) test the hypothesis that CH improves respiratory muscle performance under in vitro hypoxic conditions and 2) determine if CH-induced plasticity is NO-dependent. Adult male Wistar rats (n=23) were exposed to normobaric normoxia or hypobaric hypoxia (380mmHg) for 6 weeks. Diaphragm strips were mounted isometrically in an organ bath containing aerated physiological salt solution maintained at 30oC. Force-frequency relationship and fatigue was assessed under hypoxic conditions (95%N2, 5% CO_2). Muscles from normoxic and CH rats were assessed in the absence and presence of the nitric oxide synthase (NOS) inhibitor L-NNA (1mM). CH exposure for 6 weeks significantly improved diaphragm endurance, whereas the force-frequency relationship was unaffected. The fatigue index (ie. ratio of force at 2 min of fatigue to initial force) was significantly altered by CH (68±3 vs. 97±7*; % of initial force, mean+/-SEM, control vs. hypoxia, P<0.05, ANOVA). L-NNA had no effect on the contractile properties of the normoxic or CH diaphragm. In conclusion, CH elicits functional plasticity in the diaphragm muscle. The reduced diaphragm fatigue following CH is suggestive of an adaptive response and is consistent with studies of the human COPD diaphragm. Acute NOS inhibition following CH does not influence the improved endurance in CH diaphragm. Future studies manipulating NO in vivo during CH will determine if CH-induced plasticity is NO-dependent. Supported by the Health Research Board (Ireland). 2009.

HYPOXIA, HYPERCAPNIA AND HYPOTHERMIA IN AVALANCHE BURIAL: A RANDOMISED PROSPECTIVE PORCINE PILOT STUDY. Giacomo Strapazzon[1], Peter Paal[2], Patrick Braun[2], Guenther Sumann[3], Andreas Werner[4], Volker Wenzel[2], Markus Falk[5], Hermann Brugger[1]. [1]EURAC Institute Mountain Emergency Medicine, Bolzano, Italy, [2]Dept Anesthesiology and Critical Care Medicine, Innsbruck Medical Univ, Innsbruck, Austria, [3]Dept Anesthesiology and Critical Care Medicine, General Hospital Voecklabruck, Voecklabruck, Austria, [4]Institute Physiology, Charité Berlin, Campus Benjamin Franklin, Berlin, Germany, [5]Province College for Health-Care Professions Claudiana, Bolzano, Italy. *EMAIL: giacomo.strapazzon@eurac.edu* INTRODUCTION: Asphyxia is the leading cause of death in avalanche victims who sustain complete burial within 35min. After 35min, survival is possible only in the presence of a patent airway; an accompanying air pocket around the face may improve survival. At this stage hypothermia is assumed to be an important factor for survival because rapid cooling decreases oxygen consumption. The aim of this study was to investigate the combined effects of hypoxia, hypercapnia and hypothermia in a porcine model of avalanche burial. METHODS: Eight piglets were anaesthetized, intubated and buried under snow, randomly assigned to an air pocket (AP, n=5) or ambient air (AA, n=3) group. The mean snow density around the artificial air pockets was 431.8(SD 25.6) kg/m3. Blood gases, temperature and haemodynamic parameters were recorded. The endpoint was asystole. RESULTS: In AP group arterial oxygen partial pressure

(p=.001), arterial pH (p=.002), cardiac output (p=.002) and time to asystole (p=.025) were lower, while arterial carbon dioxide partial pressure (p=.007) and serum potassium (p=.042) were higher compared to AA group. Asystole occurred in the AP group between 22 and 53 min with a core body temperature between 31.1 and 34 degrees Celsius and in the AA group between 83 and 178 min with a core body temperature between 20.8 and 27.6 degrees Celsius. Mean cooling rates in the first 10 min of burial were -19.7(SD 4.7) degreeCelsius/h in the AP group and -13.0(SD 4.4) degreeCelsius/h in the AA group (p=.095); between 10 and 30 min, cooling rate flattened in the AP group. CONCLUSION: The results demonstrate that severe asphyxia may occur even in the presence of an air pocket, being influenced by AP volume and snow density. Moreover, serum potassium increased in the AP group only, indicating severe acidosis and very likely lysis of hypoxia-sensitive cells in the case of death from asphyxia in avalanche victims. Finally, hypothermia may develop in the early phase of avalanche burial but a continued decrease in temperature is related to an efficient cardiac output. 2015.

HYPOXIC EXERCISE TEST IS USEFUL FOR A BETTER IDENTIFICATION OF PERSONS INTOLERANT TO HIGH ALTITUDE. Jean-Paul Richalet[1], François Lhuissier[1], Florence Canouï-Poitrine[2]. [1]Université Paris 13, Sorbonne Paris Cité, Laboratoire "Réponses cellulaires et fonctionnelles à l'hypoxie", [2]AP-HP, Groupe Henri Mondor-Albert Chenevier, Service de Santé Publique. *EMAIL: richalet@smbh.univ-paris13.fr* INTRODUCTION: Factors predisposing to Severe High Altitude Illnesses (SHAI) are clinical, physiological or environmental. Clinical factors (previous history of severe AMS, HAPE or HACE, history of migraine) are very few and well identified. Environmental factors were, up to now, limited to the speed of altitude gain. Previous studies had not clearly identified physiological factors leading to a decreased tolerance to hypoxia, although many works showed that a reduced ventilatory response to hypoxia could induce a greater oxygen desaturation and therefore a higher risk for SHAI. METHODS: In our medicine outpatient consultation we were able to evaluate cardiac and ventilatory response to hypoxia at rest and exercise in around 5000 persons before their stay at high altitude. The clinical tolerance to high altitude was estimated through a questionnaire filled during the stay at high altitude. A prospective epidemiological study was completed through a multivariate analysis. RESULTS: Independent risk factors of SHAI were: history of SHAI (OR=12.82), migraine (OR=2.28), speed of ascent >400m/day (OR=5.89), low ventilatory response to hypoxia (OR=6.68), high desaturation at exercise in hypoxia (OR=2.5). A score, calculated from these variables and others obtained from the univariate analysis (geography, sex), allowed us to predict with a good precision the potential risk of SHAI (area under ROC curve= 0.91). Acetazolamide reduced the risk of SHAI about 50%. When adding physiological variables to clinical variables in the logistic regression model, the efficiency of prediction (C-statistic) increased from 0.806 to 0.883 (p<0.001). We evidenced a decrease in cardiac response to hypoxia and an increase in ventilatory response to hypoxia with ageing. These adaptations were blunted after menopause but could be maintained if women had a regular physical activity. Endurance training increased the exercise-induced

desaturation but preserved cardiac and ventilatory responses to hypoxia. CONCLUSION: The "Hypoxic Exercise Test" is a useful tool to ameliorate the information given to people planning to go to high altitude regions and for the prevention of severe illnesses that can be life threatening or, at least, provoke the interruption of trekking or climbing activities. 2015.

HYPOXIC VENTILATORY RESPONSE AND INSPIRATORY TIME IN PLATEAU PIKA ARE REGULATED BY SEROTONIN AND NITRIC OXIDE. Aurélien Pichon[1], Zhenzhong Bai[2], Nicolas Voituron[1], Tana Wuren[2], Florine Jeton[1], Dominique Marchant[1], Guoen Jin[2], Quanyu Yang[2], Qin Ga[2], Jean-Paul Richalet[1], Ri-Li Ge[2]. [1]Université Paris 13, Sorbonne Paris Cité, Laboratoire «Réponses cellulaires et fonctionnelles à l'hypoxie» EA2363, Bobigny, France, [2]Qinghai Univ Medical College, Research Centre for High Altitude Medicine, Xining, Qinghai, R. P. China. *EMAIL: aurelien.pichon@orange.fr* INTRODUCTION: We used pharmacological approach to test the influence of NMDA (memantine) and non-NMDA receptors (DNQX), serotonin (fluoxetine) and nitric oxide (NO, L-NAME) on ventilatory parameters and hypoxic ventilatory response in the adapted high altitude mammal 'Plateau Pika'. METHODS: Ventilatory parameters were measured before and after drug injections using a whole body plethysmographic method in conscious pikas at their natural living altitude (PIO_2 86 mmHg - PIO_2-86) and after a hypoxic challenge (PIO_2 57 mmHg - PIO_2-57). RESULTS: Tidal volume (Vt), respiratory frequency (fR) and minute ventilation (V_E) were increased and total time (Ttot) and inspiratory time (Ti) were decreased after hypoxic challenge (vehicle) whereas the Ti/Ttot ratio remained unchanged. Memantine or DNQX had no effect on HVR in pikas. The increase in fR observed after vehicle at PIO_2-57 (140±10 vs 177±36 c/min) was blunted under L-NAME (148±23 vs 159±27 c/min, $p<0.05$). At PIO_2-57, L-NAME also induced an increase in the Ti/Ttot ratio (0.59±0.03 vs 0.56±0.03, $p<0.05$) as compared to vehicle. The increase in V_E and Vt observed with vehicle at PIO_2-57 as compared to PIO_2-86 were inhibited after fluoxetine injection. CONCLUSION: We showed that serotonin and NO were involved in the ventilatory response to hypoxia. Moreover, we showed that the normally insensitive Ti/Ttot ratio was influenced by L-NAME injection, suggesting that this special ventilatory pattern that improves alveolar ventilation in the adapted Plateau Pika is regulated by NO. ACKNOWLEDGEMENTS: Univ Paris 13, Laboratory of Excellence Gr-Ex, National Basic Research Program (No.2012CB518200), Program of International S&T Cooperation (No.0S2012GR0195) and National Natural Science Foundation (No.30393133) of China. 2015.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PATHWAYS IN HYPOXIA AND HYPOTHERMIA. Hendrik Luuk[1], Christian Ansgar Hundahl[1]. [1]Dept Physiology, Univ Tartu, Estonia. *EMAIL: hendrik.luuk@gmail.com* INTRODUCTION: In clinical practice, hypothermia is used as a treatment alleviating hypoxic damage. However, the therapeutic mechanisms of hypothermia are not well understood. In the present study, a factorial experiment was conducted to determine differential gene expression responses after hypoxia and hypothermia in a

primary cell culture of mouse embryonic fibroblasts. METHODS: Large-scale gene expression profiling was performed using Mouse Exon 1.0 ST array (Affymetrix) according to manufacturer's protocols. Differential gene expression and pathway analysis was performed in R (www.r-project.org) using DEMI (Differential Expression from Multiple Indicators) - the software package implementing a novel probe-level framework for the different differential expression analysis on gene, transcript, exon, genome and pathway levels. Differential expression was established in relation to the normal cell culture conditions (atmospheric oxygen, 37 °C). RESULTS: The predominant effect of hypoxia was the down-regulation of numerous pathways whereas an up-regulation of glycolytic and hypoxia response pathways was detected. Hypothermia caused even more widespread changes in gene expression than hypoxia. Surprisingly, all of the differentially expressed pathways were up-regulated by hypothermia. In the group exposed to both hypoxia and hypothermia, the differential gene expression response exhibited the individual characteristics of both treatments. CONCLUSION: Both hypoxia and hypothermia resulted in the differential expression of numerous relevant pathways. To our knowledge this is the first study to systematically evaluate the effects of hypothermia and hypoxia on gene expression at a genome-wide level. ACKNOWLEDGEMENTS: HL would like to acknowledge financial support from Estonian Science Foundation (9099) and the Ministry of Science and Education (SF0180125s08). CAH would like to acknowledge financial support from the NOVO-Nordisk Foundation, Brødrene Hartmanns Family Foundation and The Foundation for Providing Medical Research. 2015.

IDENTIFICATION OF THE THRESHOLD FOR HYPOXIA-INDUCED INTRAPULMONARY ARTERIOVENOUS SHUNTING IN HUMANS AT REST. Steven S Laurie, Ximeng Yang, Jonathan E Elliot, Kara M Beasley, Andrew T Lovering. University of Oregon. *Email: slaurie@uoregon.edu.* Acute, brief (5-10 min) exposure to hypoxia (FIO_2=0.12) has been shown to open intrapulmonary arteriovenous (IPAV) shunt pathways in some but not all subjects during rest (Lovering et al., JAP, 2008). The purpose of this study was to identify an FIO_2 threshold that induced IPAV shunting in all subjects at rest in a semi-recumbent position. Thirteen healthy subjects (6 female) breathed four different levels of hypoxia (FIO_2=0.16, 0.14, 0.12, 0.10) in ascending (n=6) or descending (n=7) order for 30 min, with each FIO_2 separated by a 15 min normoxic break. Saline contrast echocardiography was used to detect the passage of bubbles through large diameter IPAV shunts. The number of bubbles in the left heart was scored on a 0-5 scale: 0= 0 bubbles; 1=1-3 bubbles; 2=4-12 bubbles; 3= >12 bubbles; 4= bubbles heterogeneously fill left ventricle; 5= bubbles homogeneously fill left ventricle. A bubble score $\geq$2 was defined as an IPAV shunt. IPAV shunt occurred within 30 min of breathing an FIO_2=0.16, 0.14, 0.12, and 0.10 in 3/13, 7/13, 10/13, and 13/13 subjects, respectively. These data indicate that breathing an $FIO_2 \leq 0.14$ (equivalent altitude ~3300m) induces IPAV shunting in the majority of male and female subjects at rest by 30 min. Previous work indicates that hypoxia will induce shunting at lower workloads compared to normoxia. Accordingly, this data indicate that low to moderate levels of physical

activity would induce IPAV shunting to occur in all subjects at moderate altitudes. Support: OHSU MRF Grant. 2009.

IMPACT OF ACETAZOLAMIDE ON VENTILATION AND CBF SENSITIVITY TO CO_2 DURING SUSTAINED HYPOXIA. Zachary Smith[1], David Dubowitz[1]. [1]Univ California San Diego. *EMAIL: zmsmith@ucsd.edu* INTRODUCTION: Acetazolamide provides symptomatic relief and accelerates acclimatization to high attitude hypoxia. We investigated how oral acetazolamide therapy impacts the hypercapnic ventilatory / cerebrovascular response to sustained hypoxia, and whether this differs between subjects who are susceptible vs. resistant to acute mountain sickness (AMS). METHODS: 14 healthy subjects participated (9 M, 5 F, age 31+/-11 years, 8 developed AMS). We measured resting ventilation, cerebral blood flow (CBF) and CO_2 reactivity using 3T MRI. Measurements were made at baseline and following 2-days sustained high altitude hypoxia with and without oral acetazolamide prophylaxis. RESULTS: Ventilation: The resting $ETCO_2$ set point for ventilation was reduced by 2 days acclimatization with an effective increase in resting ventilation (increased intercept of ventilation-$ETCO_2$ plot) (P =0.0005). Acetazolamide further reduced $ETCO_2$ and further increased resting ventilation (P=0.002). There was no acetazolamide * AMS interaction. CBF: Resting CBF was increased by 2 days acclimatization (P=0.01) with significant acetazolamide * AMS interaction (no CBF change in the no-AMS group with acclimatization, but increased resting CBF in the AMS group - P=0.049). Acetazolamide further increased CBF (p=0.047) with acetazolamide * AMS interaction (but reversed, no CBF change in the AMS group with acclimatization+Acetazolamide, but increased resting CBF in the no-AMS group - P=0.05). CONCLUSION: Acclimatization to hypoxia with or without acetazolamide increases CO_2 sensitivity for ventilation. Thus both AMS and no-AMS subjects have hyperventilation and reduced $ETCO_2$ during acclimatization or acetazolamide. The impact of this reduced $ETCO_2$ on CBF differs for the 2 groups; During altitude acclimatization alone, subjects with AMS have a higher resting CBF despite the ventilatory hypocapnia. Cerebral symptoms of AMS may relate to this higher resting CBF in this group. With addition of acetazolamide, subjects with AMS see reduced CBF (which may account for their reduced symptoms). ACKNOWLEDGEMENTS: Supported by NIH R01-NS053934(DJD) R21-NS075812(DJD). 2015.

IMPACT OF AMD-1 ON VASCULAR REMODELING IN HYPOXIA-IDUCED PULMONARY HYPERTENSION. Friederike Christine Weisel[1], Christina Kloepping[1], Alexandra Pichl[1], Jochen Wilhelm[1], Markus Roth[1], Karen Ridge[2], Hossein Ardeschir Ghofrani[1], Ralph Theo Schermuly[1], Friedrich Grimminger[1], Werner Seeger[1], Grazyna Kwapiszewska[3], Norbert Weissmann[1]. [1]Universities of Giessen and Marburg Lung Center (UGMLC), member of the German Center for Lung Research (DZL), Excellence Cluster Cardio-Pulmonary System (ECCPS), Giessen, Germany, [2]Division of Pulmonary and Critical Care Medicine Northwestern Univ, Chicago, Illinois, USA, [3]Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria. *EMAIL: Friederike.Weisel@innere.med.uni-giessen.de*

INTRODUCTION: Pulmonary Hypertension (PH) can be induced by chronic alveolar hypoxia which results in a vascular remodeling process. In this study, we investigated whether pulmonary vascular remodeling observed in the mouse model of hypoxia-induced PH could be reversed by re-oxygenation (reverse remodeling). Furthermore we sought to identify new genes that may trigger this process. RESULTS: Mice exposed to chronic hypoxia (21 days, 10%O_2) were re-exposed to normoxia (for up to 42 days). Reversal of PH during re-oxygenation was evident by decreased right heart hypertrophy, right ventricular pressure and muscularization of small pulmonary vessels compared to hypoxic controls. Microarray analysis from these mice revealed S-Adenosylmethionine decarboxylase 1 (AMD-1) as one of the most down regulated candidates. As AMD-1 was already shown to be important for polyamine synthesis and cell proliferation, we focused on the impact of AMD-1 in the development and the reversal of pulmonary hypertension. In-situ hybridization revealed AMD-1 localization in pulmonary vessels in mice and in humans, and quantitative PCR after laser-microdissection demonstrated elevated AMD-1 expression under pathological conditions. AMD-1 silencing by siRNA and in vivo resulted in decreased proliferation and diminished phosphorylation of PLC-γ1. Furthermore, AMD-1+/- mice exhibited attenuated PH when exposed to chronic hypoxia compared to wild-type controls. Promoter analysis revealed that AMD-1 could be regulated by Egr1 as a consequence of EGF stimulation. CONCLUSION: These findings indicate that in the animal model of hypoxia-induced PH, vascular remodeling can be reversed by re-oxygenation. AMD-1 may be involved in both, the remodeling of pulmonary arteries during chronic hypoxia and the process of reverse remodeling. ACKNOWLEDGEMENTS: EU pulmotension, DFG, ECCPS. 2015.

IMPACT OF HIGH ALTITUDE EXPOSURE ON ANTERIOR CHAMBER GEOMETRY OF THE EYE. Gabriel Willmann[1], Manuel Dominik Fischer[1], Andreas Schatz[1], Kai Schommer[2], Ahmad Zhour[1], Eberhart Zrenner[1], Karl-Ulrich Bartz-Schmidt[1], Florian Gekeler[1]. [1]Centre for Ophthalmology, Univ Tübingen, Tübingen, Germany, [2]Dept Sports Medicine, Medical Clinic, Univ Hospital Heidelberg, Heidelberg, Germany. *EMAIL: Gabriel.Willmann@googlemail.com*
INTRODUCTION: This study aimed to quantify the impact of acute exposure to high altitude on central corneal thickness and the geometry of the anterior chamber angle. This work is related to the Tübingen High Altitude Ophthalmology (THAO) study. METHODS: Anterior segment spectral domain optical coherence tomography was used to quantify changes of central corneal thickness, anterior chamber angle (ACA) and Angle Opening Distance (AOD in 14 healthy subjects between baseline recordings (341 m) and during acute exposure to high altitude (4559 m). RESULTS: Detailed longitudinal analysis revealed highly significant ($p < 0.0001$) increased central corneal thickness (CCT) in healthy subjects during acute altitude exposure (CCTbaseline = 517.53±28.28 µm vs. CCTaltitude = 539.87±31.28 µm; mean±sd). This change was completely reversible upon descent and no subject demonstrated persisting structural or functional sequels. Geometric measures of the anterior chamber angle remained consistent with no significant changes in angle opening distance (AOD) at 500 µm (AODbaseline = 695.96±190.00 µm vs.

AODaltitude = 673.71±179.59 μm; p = 0.52) and stable measurements of anterior chamber angle (ACA) in degree (ACAbaseline = 37.85±6.53 vs. ACAaltitude = 36.29±5.81 μm; p = 0.34). CONCLUSION: Significant changes of CCT occur in response to acute exposure to high altitude in healthy control subjects. This may be due to decreased atmospheric pressure and consequently decreased blood oxygen saturation (SpO_2) in non-acclimatized subjects and constitute a mild corneal edema formation. Interestingly, AOD at 500 μm and ACA remained stable during the acute challenge to hypoxic conditions at high altitude. This is the first time a quantitative approach has been used to assess changes of the anterior segment during acute, non-acclimatized high altitude exposure. As such, it might provide a basis for the debate on changes of intraocular pressure during exposure to high altitude. ACKNOWLEDGEMENTS: This study was supported by the Wilderness Medical Society (WMS), Novartis, Bausch & Lomb, Alcon, Allergan, Ursapharm, Optima Pharma, IMS Gear and Heidelberg Engineering. 2015.

IMPAIRED LUNG FUNCTION DUE TO BIOMASS SMOKE EXPOSURE IN NON SMOKERS HIGH ALTITUDE DWELLERS. Luca Pomidori[1], Manuela Bartesaghi[2], Enrico Duo[1], Buddha Basnyat[3], Rosa Maria Bruno[4], Lorenza Pratali[5], Ramesh Sharma[3], Manirai Neupane[3,4], Kamal Tapa[3], Giuseppe Miserocchi[2], Annalisa Cogo[1]. [1]Biomedical Sport Studies Center, Univ Ferrara, Italy, [2]Dept Experimental Medicine, Univ Milano-Bicocca, Milano, Italy, [3]Travel and Mountain Medicine Centre, Nepal International Clinic, Kathmandu, Nepal, [4]Dept Internal Medicine, Univ Pisa, Italy, [5]Institute Clinical Physiology, CNR-IFC, Pisa, Italy. *EMAIL: annalisa.cogo@unife.it* INTRODUCTION: Exposure to biomass smoke is an additive risk factor for chronic obstructive pulmonary disease and early lung function impairment. We aimed to investigate the effect of biomass smoke exposure on lung function in a peculiar population, not exposed to other noxious particles such as cigarette smoke, traffic and industries pollution. METHODS: Respiratory function was assessed in 4 villages of Khumbu Valley (Nepal) where people use biomass fuels for heating and cooking, often without a chimney. We also measured the ventilation inside the houses (Ventilation Index [VI]= window + door surface/ kitchen cubic meters), the environmental and exhaled carbon monoxide (ex CO) as a surrogate marker of indoor pollution. A total of 342 subjects performed acceptable and reproducible spirometry. We calculated the % of subjects with non-reversible bronchial obstruction (FEV1/FVC <0.70) and the % of subjects with an early impairment of lung function (FEF25-75 <65% of predicted). Note that in the last ten years indoor ventilation has been improved in the buildings of Pengboche, the village most frequented by trekkers. RESULTS: The % of subjects with impaired lung function is reported for the 4 villages: Thame 3900m 154 subjects (76M); Age 41.2 (14-84); FEV1/FVC<0.7 11.8%*; FEF25-75<65% 25.3%*; ex-CO na; VI 0.06±.02. Phakding 2500m 58 subjects (24M); Age 34.8 (16-73); FEV1/FVC<0.7 11.7%*; FEF25-75%<65% 15%*; ex-CO 9.1±5.3 ppm; VI 0.08±.07* Pengboche, 3900m 92 subjects (43M); Age 32.9(14-70);FEV1/FVC<0.7 2.2%;FEF25-75<65% 8.6%; ex-CO 9.6±7.7 ppm; VI 0.12±.07 Thamo 3700m 38 subjects (20M); Age 36.4(14-72); FEV1/FVC<0.7 15.7%*;FEF25-75<65% 42%*; ex-CO 13±8.1 ppm; VI

0.08±.08* * = p<0.05 compared to Pengboche A significant inverse correlation was found between CO and VI (p <0.01). In 3 villages the % of subjects with non-reversible bronchial obstruction and early impairment of lung function was higher than expected in a general population. CONCLUSION: The only exposure to biomass smoke is a risk factor comparable to exposure to many harmful particles. Improvement in inside ventilation and in cooking stoves seems to reduce the lung function impairment. ACKNOWLEDGEMENTS: Funded by EVK2CNR 2015.

IMPAIRED VENTILATORY RESPONSE TO HYPOXIA IN A MOUSE MODEL OF SICKLE CELL DISEASE. Joanna MacLean[1], Jun Ren[1], Xiuqing Ding[1], John Greer[1]. [1]University of Alberta, Edmonton, Canada. *Email: joanna.maclean@ualberta.ca.* Introduction: Hypoxia is critical to the pathophysiology of Sickle Cell Disease (SCD). Failure to augment ventilation in response to hypoxia would be deleterious. The objective of this study was to assess ventilatory response to hypoxia in SCD. Methods: The SAD transgenic mouse model of SCD was used for this study. Measurements on unrestrained SAD and wild type (WT) mice were performed in whole-body plethysomgraphs at P6, 2 months and 8-9 months of age. Following a 40 minute period of room air (RA), the delivered gas was switched to hypoxia (10%, 8% O2) for 5 minutes. During RA and after a 1 minute gas equilibration, respiratory parameters and behavioral observations were recorded. Results: At P6, abnormal breathing patterns in RA were seen in 45% of SAD mice (SAD+) and 0% of WT mice. In RA, SAD+ had more apneas (1.3±0.1/min) than WT (0.7±0.1/min, p<0.001) and SAD- (0.8±0.1/min, p<0.001) mice. In response to hypoxia, WT mice increased minute ventilation (VE). In response to 10% O2, SAD mice increased VE for 2 minutes followed by a period of marked apnea (5/5 SAD+, 3/6 SAD-) and behavioral seizures (SAD+ 5/5, SAD-4/6, p<0.05). At 2 months, there was no difference in RA breathing patterns or response to hypoxia between SAD and WT mice. At 8-9 months, in RA 52% of SAD mice had tachypnea and increased VE relative (SAD+) to WT mice. SAD mice without tachypnea (SAD-) had similar respiratory parameters to WT mice. There were no differences between SAD and WT responses to 10% O2. In response to 8% hypoxia, WT mice showed an increase in VE, decrease in apnea, and no behavioral change. SAD+ mice had a lesser increase in VE (139±7%) compared to WT (233±12%, p<0.001) and SAD- mice (203±13%, p<0.05). Apnea frequency and behavioral seizures followed a similar pattern. Conclusion: Ventilatory response to hypoxia is blunted in early life and adulthood in a SCD mouse model. Acknowledgements: Women & Children's Health Institute. 2015.

IMPLICATIONS OF ACUTE EXERCISE AND INSPIRATORY HYPOXIA FOR SYSTEMIC NO BIOAVAILABILITY. John Woodside, Philip E James, Damian M Bailey. English Institute of Sport, School of Medicine, Cardiff University, University of Glamorgan. *Email: John.Woodside@eis2win.co.uk.* PURPOSE: The present study examined the effects of physical exercise and hypoxia (H) on nitric oxide (NO) metabolites in the systemic circulation. Specifically, we hypothesized that exercise would result in a net loss of NO metabolites which would be compounded

in hypoxia due to a) PO_2 mediated re-apportionment and/or b) oxidative inactivation. METHODS: Fourteen healthy males aged 24 (mean) ± 5 (SD) years performed two incremental tests to volitional exhaustion in normoxia (N) (21%O_2) and H (~12%O_2). Maximal oxygen consumption (VO_2max) was measured off line using the Douglas bag method and arterial haemoglobin oxygen saturation (SaO_2) via pulse oximetry. Blood samples were taken from an antecubital vein before and immediately after exercise for determination of PO_2 and plasma concentrations of nitrite (NO•), nitrate (NO•) and S-nitrosothiols (RSNO) via ozone-based chemiluminescence using modified tri-iodide/vanadium reagents. Data were analyzed with a two-way repeated measures ANOVA and post-hoc Bonferroni-corrected paired samples t-tests and relationships between variables by a Pearson Product Moment correlation coefficient. RESULTS: H decreased SaO_2 (N: 96 ± 1 vs H: 77± 4%, $P < 0.05$) and subsequently VO_2max (N: 3.92 ± 0.30 vs H: 3.00 ± 0.17 L/min, $P < 0.05$). Total NO did not change as a result of exercise and hypoxia (N: -0.4 ± 6.3 vs H: -1.2 ± 7.7μmol). The exercise-induced reduction in NO (N; -137 ± 93 norm vs. H: -78 ± 99 nmol) was mirrored by an exercise-induced increase in RSNO (N: + 11 ± 31 vs. H: +21 ± 16 nmol ($P < 0.05$). A more marked reduction in NO was observed during N exercise ($P = 0.07$). CONCLUSION: These findings demonstrate an exercise induced re-apportionment of NO across the metabolite pool (from nitrite to RSNO) that may help conserve vascular O_2 delivery which is especially important in H. Alternatively the reduction in nitrite and tendency towards a decrease total NO may reflect oxidative inactivation. 2009.

IMPROVED ANOXIC TOLERANCE IN CHRONIC HYPOXIC RAT DIAPHRAGM IS NITRIC OXIDE-DEPENDENT. Clodagh McMorrow[1], James F.X. Jones[1], Aidan Bradford[2], Ken D. O'Halloran[1]. [1]University College Dublin, Ireland, [2]Royal College of Surgeons in Ireland. *Email: ken.ohalloran@ucd.ie*. Introduction: Hypoxic adaptation in skeletal muscle is well described but there remains a general paucity of information concerning the effects of chronic hypoxia on respiratory muscle function despite the clinical significance. The respiratory muscles face unique challenges during prolonged hypoxia, given that they must increase their workload to support hyperventilation. We recently reported that chronic hypoxia increases rat diaphragm muscle endurance and Na/K ATPase pump content [1]. In the present study, we sought to test the hypothesis that chronic hypoxia improves anoxic tolerance of rat diaphragm. Methods: Adult male Wistar rats were exposed to 6 weeks of normobaric normoxia (controls, N=6) or 6 weeks of hypobaric hypoxia (380mmHg, N=6). Following treatment, diaphragm muscle force and endurance was assessed in vitro, using isolated bundles of diaphragm mounted isometrically in tissue baths gassed with 95% N2/5% CO_2 in the absence and presence of the nitric oxide synthase (NOS) inhibitor L-NNA (1mM). Twitch force and contractile kinetics, force-frequency relationship and fatigue characteristics were determined. Tissue was harvested for MHC fibre type determination and assessment of oxidative enzyme (SDH) activity. Results: Peak force-generating capacity of the diaphragm was unaffected by chronic hypoxia, but diaphragm endurance was increased compared to normoxic diaphragms. NOS inhibition significantly

decreased force in normoxic and hypoxic diaphragms with no interaction, and reversed improved anoxic tolerance in chronic hypoxic diaphragm. Five-min fatigue index values were: 10±2%, 28±2%, 10±3%, and 11±2%; control, chronic hypoxia, control+L-NNA, and hypoxia+L-NNA; mean±SEM; % of initial force; p=0.008 for hypoxic treatment, p=0.027 for drug treatment and p=0.013 for interaction, two-way ANOVA. Diaphragm SDH activity was unaffected by chronic hypoxia. Conclusion: The results of this study indicate that improved anoxic tolerance in chronic hypoxic rat respiratory muscle is NO-dependent. Our results may have implications for respiratory disorders that are characterized by chronic hypoxia such as COPD. Acknowledgements: Supported by the Health Research Board (Ireland). [1]: McMorrow C, Fredsted A, Carberry J, O'Connell RA, Bradford A, Jones JFX, O'Halloran KD (2010). Eur Respir J, Epub ahead of print; PMID 21148231. 2011.

IMPROVED ANOXIC TOLERANCE IN CHRONIC HYPOXIC RAT DIAPHRAGM IS NITRIC OXIDE-DEPENDENT. Clodagh McMorrow[1], James F.X. Jones[1], Aidan Bradford[2], Ken D. O'Halloran[1]. [1]University College Dublin, Ireland, [2]Royal College of Surgeons in Ireland. *Email: ken.ohalloran@ucd.ie*. Introduction: Hypoxic adaptation in skeletal muscle is well described but there remains a general paucity of information concerning the effects of chronic hypoxia on respiratory muscle function despite the clinical significance. The respiratory muscles face unique challenges during prolonged hypoxia, given that they must increase their workload to support hyperventilation. We recently reported that chronic hypoxia increases rat diaphragm muscle endurance and Na/K ATPase pump content [1]. In the present study, we sought to test the hypothesis that chronic hypoxia improves anoxic tolerance of rat diaphragm. Methods: Adult, male, Wistar rats were exposed to 6 weeks of normobaric normoxia (controls, N=6) or 6 weeks of hypobaric hypoxia (380mmHg, N=6). Following treatment, diaphragm muscle force and endurance was assessed in vitro, using isolated bundles of diaphragm mounted isometrically in tissue baths gassed with 95% N_2/5% CO_2 in the absence and presence of the nitric oxide synthase (NOS) inhibitor L-NNA (1mM). Twitch force and contractile kinetics, force-frequency relationship and fatigue characteristics were determined. Tissue was harvested for MHC fibre type determination and assessment of oxidative enzyme (SDH) activity. Results: Peak force-generating capacity of the diaphragm was unaffected by chronic hypoxia, but diaphragm endurance was increased compared to normoxic diaphragms. NOS inhibition significantly decreased force in normoxic and hypoxic diaphragms with no interaction, and reversed improved anoxic tolerance in chronic hypoxic diaphragm. Five-min fatigue index values were: 10±2%, 28±2%, 10±3%, and 11±2%; control, chronic hypoxia, control+L-NNA, and hypoxia+L-NNA; mean±SEM; % of initial force; p=0.008 for hypoxic treatment, p=0.027 for drug treatment and p=0.013 for interaction, two-way ANOVA. Diaphragm SDH activity was unaffected by chronic hypoxia. Conclusion: The results of this study indicate that improved anoxic tolerance in chronic hypoxic rat respiratory muscle is NO-dependent. Our results may have implications for respiratory disorders that are characterized by chronic hypoxia

such as COPD. Acknowledgements: Supported by the Health Research Board (Ireland). [1]: McMorrow C, Fredsted A, Carberry J, O'Connell RA, Bradford A, Jones JFX, O'Halloran KD (2010). Chronic hypoxia increases rat diaphragm muscle endurance and Na+-K+ ATPase pump content. Eur Respir J, Epub ahead of print; PMID 21148231. 2011.

IN VIVO EVIDENCE FOR AN ACETAZOLAMIDE-AUGMENTED NITRITE REDUCTASE CAPACITY OF CARBONIC ANHYDRASE AND REDUCTION IN HYPOXIC PULMONARY VASOCONSTRICTION. Philipp A Pickerodt[1], Angela Fago[2], Micheal J Emery[3], Erik R Swenson[4]. [1]Department of Anesthesiology and Intensive Care Medicine Charité – Universitätsmedizin Berlin, [2]Department of Biological Sciences Aarhus University, [3]VA Puget Sound Health Care System, [4]Division of Pulmonary and Intensive Care Medicine University of Washington and VA Puget Sound Health Care System. *Email: philipp.pickerodt@charite.de*. Introduction: The carbonic anhydrase (CA) inhibitor acetazolamide (ACZ) reduces hypoxic pulmonary vasoconstriction (HPV) in isolated lungs, and in animals and humans by an unknown mechanism not dependent upon CA because other potent structurally different CA-inhibiting sulfonamides are without effect. Recently, Aamand et al. have shown a novel nitrite reductase activity of CA surprisingly augmented by ACZ5. We hypothesized that ACZ may reduce HPV by this mechanism of NO generation. Methods: White New Zealand rabbits (3.0 ± 0.2 kg; n=4) were anesthetized and ventilated. Exhaled NO (NOex) was measured by an ozone-based chemiluminiscense analyzer. Catheters were inserted into the right ventricle for measurements of right ventricular systolic pressures (RVSP) and the carotid artery for systemic blood pressure monitoring and arterial blood gas analysis. To stimulate nitrite reduction to NO three 20 min hypoxic challenges (FIO_2 = 0.12) were performed. To inhibit all NO production from NO synthases, L-NAME (10 mg/kg i.v.) was given. The i.v. dosages of sodium nitrite ($NaNO_2$) and acetazolamide were 250 µmol/kg and 20 mg/kg, respectively. Results: During hypoxia NOex decreased by 13% and RVSP increased from 14 ± 1 to 20 ± 0.3 mmHg. L-NAME decreased NOex by 20.8 ± 0.7 parts per billion. $NaNO_2$ given before the second hypoxic challenge prevented the increase in RVSP (Δ RVSP: 1 ± 1.9 mmHg) and caused a 63% peak increase in NOex. Although the peak rise of NOex with $NaNO_2$ during the third hypoxic challenge with ACZ (64%) was similar, there was a slower decrease in NOex during the remainder of the hypoxic challenge. At 20 min, NOex was still 41% elevated above baseline with ACZ versus a complete return to baseline without ACZ. Conclusion: Our results implicate CA as a functional nitrite reductase in vivo and suggest this may be one mechanism of HPV reduction by ACZ. Acknowledgements: P.A.P. and A.F. contributed equally to this work. 2011.

IN VIVO OXYHEMOGLOBIN DISSOCIATION CURVES (ODC) IN ACUTE AND CHRONIC ACCLIMATIZATION TO HIGH ALTITUDE. Dahlia Y Balaban, David Preiss, Alexandra Mardimae, Alex Vesely, Marat Slessarev, Richard Greene, Gustavo Zubieta, James Duffin, Joseph A Fisher. University of Toronto, University of British Columbia, University of Toronto, University of New Mexico, Clinica

IPPA. *Email: dahlia.balaban@utoronto.ca.* In vitro methods of studying the ODC precisely control variables that influence the position of the ODC but may not reflect metabolic changes that can occur during hypoxia in vivo. It would therefore be advantageous to use in vivo methods to compare the ODC in acute and chronic acclimatization to altitude. We provided conditions for measuring the ODC in vivo by prospectively targeting end-tidal PCO_2 ($PETCO_2$) and PO_2 ($PETO_2$) using a specialized gas blender (RespirAct™, Thornhill Research Inc., Toronto, Canada) and a sequential rebreathing circuit. At 3600 m, we studied 6 lowlanders (2 females) within 2 weeks of arrival at altitude and 8 healthy, chronically acclimatized Bolivian highlanders (3 females). We maintained $PETCO_2$ at each subject's resting level while targeting $PETO_2$s of 100, 70, 60, 50, 40 and 35 mm Hg, in steps lasting 2 min. During the last 30 s of each step an arterial sample was drawn and oxyhemoglobin saturation was recorded from a pulse oximeter (Onyx II, Nonin, Plymouth MN). There was no difference between baseline PaO_2, $PaCO_2$, pH, Hct, or BE between groups. During the tests, at each PaO_2 level, $PaCO_2$ remained constant and was the same in both groups (RMANOVA p=0.664). While pH remained constant within a group during testing (RMANOVA p=0.344), the pHs of the two groups tended to diverge, with the highlanders becoming more acidemic with progressive hypoxia and the lowlanders becoming more alkalemic (RMANOVA p=0.013). Linearization of the data via the Hill equation did not reveal any significant differences between the slopes (p = 0.91), y-intercepts (p = 0.85), or P50s (p = 0.81) of the two groups. The in vivo ODCs of those with chronic and acute acclimatization to hypoxia were similar, though their pH responses to progressive hypoxia differed. We cannot offer an explanation for this unexpected finding. We thank CIHR and Thornhill Research Inc. for the generous support that made this research possible. 2009.

INCIDENCE OF HIGH ALTITUDE PULMONARY EDEMA IN SUBJECTS WITH INCREASED HYPOXIC PULMONARY VASCULAR RESPONSE. Christoph Dehnert, Fabian Scheurlen, Derliz Mereles, Ekkehard Grunig, Peter Bartsch. Medical Clinic, Thorax Clinic, and Medical Clinic, University Hospital Heidelberg. *Email: christoph.dehnert@med.uni-heidelberg.de.* High altitude pulmonary edema (HAPE) develops in 6% of otherwise healthy mountaineers after rapid ascent to 4559m and in 62% of HAPE-susceptible mountaineers. HAPE-susceptibility is characterized by increased hypoxic pulmonary vascular response (HPVR) leading to systolic pulmonary artery pressures (PASP) <40mmHg during acute exposure to hypoxia (FIO_2=12%). Furthermore, it has been shown that about 10% in the population have increased HPVR of the same degree during hypoxic exposure. Purpose: To evaluate the incidence of HAPE after rapid ascent to 4559m in individuals with HPVR <40mmHg. Based on previous data we expected an incidence of HAPE of about 60%. Methods: Screening 186 healthy individuals we found 18 with echocardiographically determined PASP over 40mmHg after 2h exposure to FIO_2=12% (~4500m) consistently in two independent exposures. 13 of them (average PASP 23±5mmHg in normoxia, 49±4mmHg in hypoxia) ascended with one overnight stay at 3611m to 4559m within 24h where they stayed for 2 days. PASP was repeatedly determined at altitude and chest x-ray was taken before

subjects descended or to confirm the diagnosis of HAPE if HAPE was clinically suspected. Results: PASP at altitude was 46±8mmHg 4h after arrival at 4559m, 46±8mmHg in the morning of the second day and 46±7mmHg before descend. In only 3 subjects clinically or radiographically assured HAPE developed. HAPE was unequivocally diagnosed on clinical basis in one subject already at 3611m and in the 2 others at 4559m. Questionable radiographic signs of HAPE were found in 3 additional asymptomatic subjects at 4559m. Thus, the incidence of clinically relevant HAPE was 23%. Conclusion: In this small sample the incidence of HAPE in subjects with increased HPVR is considerably lower than observed with reexposure of HAPE-susceptible individuals. If this study can be confirmed in a large group of subjects this would suggest that other factors in addition to increased HPVR are necessary for the development of HAPE. 2009.

INCREASED NIGHT-TIME EPO/SEPOR RATIO IN ANDEAN HIGHLANDERS WITH CHRONIC MOUNTAIN SICKNESS. Francisco Villafuerte[1], Romulo Figueroa-Mujica[1], Cecilia Anza-Ramírez[1], Gustavo Vizcardo-Galindo[1], Noemí Corante[1], Daniela Salazar[2], Roberto Accinelli[2], Fabiola León-Velarde[1]. [1]Laboratorio de Fisiología Comparada. Facultad de Ciencias y Filosofía. Universidad Peruana Cayetano Heredia, [2]Facultad de Medicina. Universidad Peruana Cayetano Heredia. *Email: francisco.villafuerte@upch.pe*. Introduction: Excessive erythrocytosis (EE) is the main sign of Chronic Mountain Sickness (CMS), a highly prevalent syndrome in Andean highlanders. Although chronic hypoxemia represents its underlying stimulus, the fundamental pathophysiological mechanism is still debatable. Sleep-disordered breathing and altered erythropoietin (Epo) circadian rhythm have been proposed as possible factors associated to accentuated hypoxemia and EE. Studies during sleep in CMS patients consistently find lower average pulse O2 saturation (SpO_2) values and also a lower SpO_2 nadir compared to healthy highlanders (HH). However, there is controversy on whether the resulting morning Epo concentration is elevated in these patients and contributes to explain the occurrence of EE. We have recently shown that the soluble Epo receptor (sEpoR), an endogenous Epo antagonist, is decreased in CMS patients leading to increased available circulating Epo concentration (increased Epo/sEpoR). The aim of the present study was to characterize the concentration profile of sEpoR and serum Epo during night-time, and their relationship with SpO_2 and hematocrit (hct) in CMS. Methods: Blood samples were taken every 4h (6pm-6am) for Epo and sEpoR measurements, and SpO_2 monitored at 6pm and continuously from 10pm-6am in age-matched CMS patients (n=18) and HH (n=19) with normal iron status from Cerro de Pasco, Peru (4340m). Results: As expected, hct and CMS Score were significantly higher in the CMS group, while basal SpO_2 was lower. Average and nadir SpO_2 during sleep were notably lower in CMS patients compared to HH. There were no differences in sEpoR or serum Epo within each group at each time-point. CMS group showed higher Epo and lower sEpoR throughout the study period, but differences were statistically significant only at 10pm. Interestingly, the Epo/sEpoR was significantly higher in the CMS group from 10pm-6am. Also, morning sEpoR and Epo/sEpoR correlated negatively with average SpO_2 during sleep. Conclusions: In

conclusion, our results suggest that the contribution of low SpO_2 to erythropoiesis during sleep in CMS is better explained by its relation to the Epo/sEpoR ratio rather than only to Epo concentration. Acknowledgements: Wellcome Trust Grant:097275/Z/11/Z. 2015.

INCREASING CEREBRAL BLOOD FLOW WITHOUT CHANGING BLOOD GAS TENSIONS REDUCED CENTRAL SLEEP APNEA SEVERITY AT 5050M. KR Burgess[1,2], SJE Lucas[3], KME Burgess[1,2], K Sprecher[1] ,J Donnelly[3] , AS Basnet[4] & PN Ainslie[5]. [1]Peninsula Health Care Pty Ltd., Sydney, Australia, [2]Univ Sydney, Sydney, Australia. [3]Univ Otago, Dunedin, New Zealand, [4]Good Samaritan Hosp., Phoenix, USA, [5]Univ British Columbia, Kelowna, Canada. *EMAIL: krburgess@optusnet.com.au* INTRODUCTION: Exposure to very high altitudes causes an universal increase in central sleep apnea (CSA) mediated by alterations in ventilatory control and possibly cerebral blood flow (CBF) regulation. We have previously shown that increasing CBF by IV acetazolamide (acet) reduced CSA severity by ~ 50%; however, this effect was likely mediated by a concomitant rise in $PaCO_2$ of ~ 3 mmHg. AIM: To study the effects of increasing CBF on CSA without altering $PaCO_2$. METHODS: Arterial blood gas samples were collected and ventilatory responses to hypercapnia (HCVR) and hypoxia (HVR) were measured (by rebreathing and steady state techniques) during wakefulness. CBF in the internal carotid and vertebral arteries, and velocities in the middle and posterior cerebral arteries were measured by transcranial Doppler technique. CSA was recorded during sleep by full polysomnography. CBF was increased by one injection of iv acet (10mg/kg) plus IV infusion of dobutamine (dob) (2-5ug/kg/min) during sleep at 5050m at Lobuje, Nepal (The Pyramid). RESULTS: Preliminary analysis of n=5 (4M, 1F) ages 31±10 yrs, showed acet/dob increased global CBF by 15% ($P<0.01$). HCVR declined from 6.8±3.7 to 5.3±2.2 l/min/mmHg (NS). CSA index fell from 131±24 to 9±5 events / hour of sleep. ($P<0.001$). HVR, $PaCO_2$, pH and PaO_2 were all unchanged. CONCLUSION: Pharmacologically increasing CBF without alteration of arterial blood gas tensions markedly reduced the severity of central sleep apnea at high altitude. ACKNOWLEDGEMENTS: Supported by Peninsula Health Care Pty Ltd, NSERC, CRC and Lottery Health NZ. Research conducted under the memorandum between Nepal Health Research Council and EVK2-CNR. 2015.

INCREASING CEREBRAL BLOOD FLOW WITHOUT CHANGING BLOOD GAS TENSIONS REDUCED CENTRAL SLEEP APNEA AT 5050M. Keith Burgess[1], Samuel Lucas[2], Katie Burgess[1], Katherine Sprecher[1], Joseph Donnelly[2], Aparna Basnet[3], Philip Ainslie[4]. [1]Peninsula Health Care Pty Ltd. Sydney. Australia, [2]University of Otago. Dunedin. New Zealand, [3]Good Samaritan Hospital. Phoenix. USA., [4]University of British Columbia. Kelowna. Canada. *Email: krburgess@optusnet.com.au.* Introduction: Exposure to very high altitudes causes a universal increase in central sleep apnea (CSA) mediated by alterations in ventilatory control and possibly cerebral blood flow (CBF) regulation. We have previously shown that increasing CBF by iv acetazolamide (acet) reduced CSA severity by ~ 50%; however, this effect was confounded by a concomitant rise in $PaCO_2$ of ~ 3 mmHg. Aim:

To study the effects of increasing CBF on CSA without altering $PaCO_2$. Methods: Following ~1 week of living at 5050m (The Pyramid, Lobuje, Nepal), 12 subjects (9M,3F; aged 32±7 y) completed awake ventilatory response testing pre and post injection of either iv acet (10mg/kg) combined with dobutamine (dob) (2-5ug/kg/min) or placebo injections / i.v (order randomized). Immediately following pharmacological / placebo, CBF measurements and ventilatory response testing were made, and subjects underwent full polysomnography monitored sleep while the pharmacological (dob) intervention was maintained. Arterial blood gas samples were collected and ventilatory responses to hypercapnia (HCVR) and hypoxia (HVR) were measured by rebreathing and steady state techniques. Duplex ultrasound of blood flow in the internal carotid and vertebral arteries was used to estimate global CBF. The CSA index was compared between trials from the initial 3-4 hrs of sleep. Results: IV acet/dob increased global CBF by 34% compared to placebo ($P<0.001$). HCVR declined from 5.9±2.7 to 4.2±2.8 l/min/mmHg ($P=0.03$). CSA index fell from 129±58 to 44±37 events /hour of sleep ($P<0.001$). HVR, $PaCO_2$, PaO_2 and pH were all unchanged. Conclusion: Pharmacologically increasing CBF without alteration of arterial blood gas tensions markedly reduced the severity of central sleep apnea at high altitude, possibly by reducing HCVR. Acknowledgements: Supported by Peninsula Health Care Pty Ltd, NSERC, CRC and Lottery Health NZ. Research conducted under the memorandum between Nepal Health Research Council and EVK2-CNR. 2015.

INCREASING RESPIRATORY DEAD SPACE IMPROVES SLEEP DISORDERED BREATHING AND HYPOXEMIA IN PATIENTS WITH CHRONIC MOUNTAIN SICKNESS. Emrush Rexhaj[1], Stefano Rimoldi[1], Pierre-Yves Jayet[2], Alban Lovis[2], Daniela Andries[2], Carlos Salinas Salmòn[3], Mercedes Villena[3], Yves Allemann[4], Raphael Heinzer[2], Urs Scherrer[1], Claudio Sartori[1]. [1]Department of Internal Medicine Lausanne-CHUV, Switzerland, [2]Pulmonary Department and Center for Investigation and Research in Sleep Lausanne-CHUV, Switzerland, [3]IBBA La Paz, Bolivia, [4]Department of Cardiology Bern-Inselspital, Switzerland. *Email: Emrush.Rexhaj@chuv.ch*. Introduction: Chronic mountain sickness (CMS) is a major public health problem characterized by chronic hypoxemia and erythrocytosis. The underlying mechanism is unknown. The only existing treatment is descent to low altitude, an option that for social reasons almost never exists. Sleep disordered breathing (SDB) may represent an underlying mechanism. We recently found that in mountaineers increasing the respiratory dead space markedly improves SDB. The aim of the present study was to assess the effects of this procedure on SDB in patients with CMS. Methods: In 17 male Bolivian high-altitude dwellers (56±9 y) suffering from CMS (haemoglobin >20g/L) full night sleep recordings were obtained in La Paz (3600 m). In random order, one night was spent with a 500 ml increase in dead space through a custom designed full face mask and the other night without it. Recordings were also obtained in 6 control subjects. Results: The major new findings were two-fold; a) CMS patients present markedly more severe SDB and hypoxemia ($P<.01$) than control subjects; and b) added dead space dramatically improved SDB in CMS patients, as evidenced by a

decrease of the apnea/hypopnea (P<.01), hypopnea (P=.01) and oxygen desaturation (P<.01) indexes, and an increase of the nocturnal oxygen saturation (P=.01). The procedure was easily accepted and well tolerated. Conclusion: Here, we show for the very first time that an increase in respiratory dead space through a fitted mask dramatically improves SDB in high-altitude dwellers suffering from CMS. We speculate that when used in the long-term, this procedure will improve erythrocytosis and pulmonary hypertension and offer an inexpensive and easily implementable treatment for this major public health problem. 2011.

INDIVIDUAL RESPONSES TO MAXIMAL EXERCISE DURING ACUTE HYPOXIA USING HEART RATE VARIABILITY TO ASSESS AUTONOMIC FUNCTION. Patrick Neary, Vladislav Bukhman, Les Ansley, Angus Hunter, Justin Skowno, Alan St. Clair Gibson, Timothy D Noakes. University of Regina, Northumbria University, University of Stirling, Red Cross Memorial Children's Hospital, Northumbria University, University of Cape Town. *Email: patrick.neary@uregina.ca.* This study investigated the physiological stress to acute hypoxia during maximal aerobic exercise using statistical, linear and non-linear measures of heart rate variability (HRV) to assess autonomic nervous system (ANS) function. Seven male cyclists (VO_2max=64 ± 5 mL/kg/min) performed incremental exercise to exhaustion on separate days on their own bicycles mounted on a Computrainer Pro under hypoxia [H] (FIO_2=15% ~2700m) and normoxia [N] inside a normobaric pressure chamber. HRV included time domain (SDNN, RMSSD), frequency domain (HF, LF, VLF, LF/HF), linear stress index (SI), combined regulatory system activity factor (RAF), Sample Entropy, and Emotion based on spectral analysis. These variables reflect the complexity, regularity and predictability of the physiological regulatory systems under different environments. Group results showed significant differences between H and N for power output (278 vs 349 W) and test duration (549 vs762 sec), respectively, but few group differences were found between trials for the HRV parameters; this was likely related to individual responses of each cyclist to hypoxia. However, all subjects demonstrated significant differences between trials for most HRV parameters for the exercise portion of the VO_2max test. Of the 11 HRV parameters assessed, 6-10 parameters were significantly different between H vs N. The most common variables that were significantly different were HF, LF/HF, VLF, Emotion, and SI. Overall, 4 of the 7 cyclists experienced higher levels of neuro-autonomic stress during the hypoxic exercise trial. These results suggest that linear and non-linear HRV parameters can be used to assess the ANS during acute exposure to hypoxia during maximal exercise. Furthermore, these data support the need to report individual subject responses, as group data may not truly reflect physiological differences between exercise conditions. Funding: NSERC (JPN); Kingston University Promising Young Researcher Award (LA). 2009.

INDIVIDUAL SUSCEPTIBILITY TO HAPE/IPE IS RELATED TO VARIATIONS IN PULMONARY LYMPHATICS. Eric Carter[1], John Mayo, Martin MacInnis[1], Donald McKenzie[3], Michael Koehle[3]. [1]School of Kinesiology, Univ British Columbia, Vancouver, BC, Canada, [2]Dept Radiology, Univ British Columbia,

Vancouver, BC, Canada, [3]Faculty of Medicine, Univ British Columbia, Vancouver, BC, Canada. *EMAIL: eric.a.carter@me.com* INTRODUCTION: High-altitude pulmonary edema (HAPE) and immersion pulmonary edema (IPE) are potentially life-threatening conditions that affect athletes, including high-altitude climbers, long-distance swimmers, and underwater divers. The objectives of this study were to measure lung density (before and after exercise) and to quantify the pulmonary lymphatic network in individuals susceptible and resistant to HAPE/IPE. METHODS: Eighteen male (n = 10) and female (n = 8) subjects were recruited. Based on medical histories, nine subjects were susceptible to HAPE/IPE, and nine were resistant. Subjects were matched for gender, age, height, weight, and cold-water diving or high-altitude trekking experiences. Lung mass and density (at three slice locations) were determined using computed tomography at rest and after intense exercise. Lung mass and density were calculated from x-ray attenuation values. Two blinded investigators counted interlobular septal lines according to criteria established by the research group. Data are presented as mean (standard deviation). RESULTS: Susceptible subjects had a lower lung density [0.19 (0.035) g ml-1 vs. 0.22 (0.029) g ml-1; p = 0.08), a significantly lower lung mass (132.1 (24.16) g vs. 156.1 (19.19) g; p=0.03] at rest, and significantly fewer interlobular septa [17 (4.5) vs. 23(7.1); p=0.04] compared to resistant subjects. Intense exercise did not affect density (p=0.52) or mass (p=0.82). CONCLUSION: Subjects susceptible to HAPE/IPE had lower lung density, significantly lower lung mass, and fewer interlobular septa than subjects resistant to HAPE/IPE, suggesting a smaller pulmonary lymphatic network in those susceptible to HAPE/IPE. The observed differences in lymphatics could represent either predisposing factors to, or sequelae of, HAPE and IPE. ACKNOWLEDGEMENTS: Funding provided by the Canadian Academy of Sport and Exercise Medicine, the Natural Science and Engineering Research Council, the Canadian Foundation for Innovation, and the B.C. Sports Medicine Research Foundation. The authors wish to thank the Dept Radiology and the UBC Thoracic Imaging Group at Vancouver General Hospital. 2015.

INFLUENCE OF BETA-BLOCKADE ON MAXIMAL APNEA DURATION AND CARDIOVASCULAR FUNCTION IN ELITE BREATH-HOLD DIVERS. Ryan Hoiland[1], Philip Ainslie[1], Anthony Bain[1], David MacLeod[2], Mike Stembridge[3], Ivan Drvis[4], Dennis Madden[5], Otto Barak[5,6], Doug MacLeod[6], Zeljko Dujic[5]. [1]Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia–Okanagan, Kelowna, British Columbia, Canada, [2]Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA, [3]Cardiff Metropolitan University, Cardiff School of Sport, Cardiff, United Kingdom, [4]University of Zagreb School of Kinesiology, Zagreb, Croatia, [5]Department of Integrative Physiology, University of Split School of Medicine, Split, Croatia, [6]Department of Physiology, University of Novi Sad School of Medicine, Novi Sad, Serbia, [7]Emory University, Atlanta, GA, USA. *Email: ryanleohoiland@gmail.com.* Introduction: The precise mechanism(s) responsible for breath-hold (BH) breaking point remain to be elucidated; however, anecdotal reports indicate that β-adrenergic blockade improves BH duration in elite BH divers. We

hypothesized β-blockade would result in a lower heart rate throughout the BH, thus lowering cardiac output to metabolically active tissues preserving O_2 and ultimately prolonging BH duration. Methods: In nine competitive BH divers (age: 29±9years; personal best static BH: 394±61sec; years competing: 4±3years) we used a single blinded randomized placebo controlled study to assess the effect cardiac specific β-blockade (150μg·kg^{-1}·min^{-1}, esmolol or volume matched saline) on maximum BH duration. Cerebral blood flow (CBF; duplex ultrasound), blood pressure, heart rate, stroke volume, and cardiac output (Finometer) were continuously measured throughout the BH. Following adequate rest peripheral chemosensitivity data was collected using end-tidal forcing to match hypoxia experienced at the end of BH. Results: β-blockade increased BH duration by10.5±9.7% (324±61 vs. 356±57sec; P<0.01) despite no change in peripheral chemosensitivity (0.49±0.40 vs. 0.35±0.47 L•min^{-1}•-%SpO2^{-1}; P=0.27). While heart rate was only reduced at baseline (65±9 vs. 54±13bpm; P=0.02) and during the first 10% of the BH (92±26 vs. 77±18bpm; P=0.01) and mean arterial pressure was unaltered (P>0.05 at all stages), cardiac output was reduced at all time points during the last 50% of BH with esmolol (all P<0.01 vs. placebo). Esmolol also reduced rate pressure product (RPP) at baseline, 40%, and 60 to 100% of BH duration (P<0.03 vs. placebo). There was no change in CBF after β-blockade except for reductions at 90% (988±238 vs. 882±217mL/min; P=0.02) and 100% (1041±213 vs. 928±241mL/min; P=0.03) of BH. Conclusion: In summary, β-adrenergic blockade likely improves BH time via a reduced cardiac output to metabolically active tissues and reduced myocardial oxygen consumption (i.e., RPP). Acknowledgements: Funded by a NSERC Discovery Grant and Canada Research Chair (PNA). 2015.

INFLUENCE OF CEREBRAL BLOOD FLOW ON CENTRAL SLEEP APNEA AT HIGH ALTITUDE. Phil N Ainslie, James D Cotter, Andrew Dawson, Jui-Lin Fan, Rebekah Lucas, Samuel Lucas, Karen Peebles, Kelly Bilson, Marianne Swart, Kate Thomas, Keith Burgess. University of Otago, Peninsula Sleep Laboratory. *Email: philip.ainslie@stonebow.otago.ac.nz.* We tested the hypothesis that, at high altitude (HA), reductions in cerebrovascular reactivity to CO_2 would exacerbate development of central sleep apnea (CSA). We also anticipated that elevations in CBFv would attenuate CSA. In a placebo-controlled, randomized design (n=12), indomethacin (100 mg PO) and acetazolamide (10 mg/kg i.v.) were used in both wakefulness and prior to sleep to alter CBFv and cerebrovascular reactivity to CO_2. These measures and blood gases were obtained before and during each intervention at sea level (SL) and HA (5050 m; ~6-d acclimatisation). Sleep, including CBFv monitoring, was studied using full polysomnography. Prior to interventions at HA, whilst CBFv-CO_2 reactivity was reduced (-36 ± 69%, P=0.06), baseline CBFv was comparable to SL. Indomethacin reduced (P<0.05) CBFv at SL and HA to a similar extent (-23 ± 16% and -22 ± 8%, respectively), but reduced CBFv-CO_2 reactivity more at HA (-47 ± 27% vs. -88 ± 62% respectively; P<0.01) and led to periodic breathing in 10 participants during wakefulness. Acetazolamide at HA raised $PaCO_2$ (+3 mm Hg; P<0.05) and increased CBFv (compared to effect at SL: 15 ± 8% vs. 31 ± 6%,; P<0.05). Acetazolamide, relative to Indomethacin, halved the frequency of

CSA (54 ± 46 vs. 101 ± 28 events/h; P<0.01) and elevated CBFv more during NREM sleep. These results indicate that reductions in CBFv and CBFv-CO_2 reactivity play a key role in the pathogenesis of CSA at altitude. Whilst acetazolamide-induced elevations in CBFv may also confer additional benefits against development of CSA, we cannot dissociate this effect from its other known prophylactic benefits. 2009.

INFLUENCE OF HYPERCAPNIA AND SKIN TEMPERATURE ON HUMAN VENTILATION DURING LIGHT EXERCISE. Jesse G Grenier, Michael L Walsh, Miriam E Clegg, Matthew D White. Simon Fraser University. *Email: mwhite1@sfu.ca.* Hyperthermia potentiates the influence of carbon dioxide (CO_2) on human ventilation (VE). It remains to be resolved, if, and how, peripheral and core temperatures contribute to this elevated ventilatory response to CO_2. PURPOSE: To examine the influences of mean skin temperature (TSK) and end-tidal CO_2 ($PETCO_2$) on VE during submaximal exercise. It was hypothesized TSK and $PETCO_2$ would positively interact in their influence on VE. METHODS: Five males and 3 females who were 1.77±0.12 m tall (mean ± SD), 75.9±15.8 kg in weight and 22.0±2.2 years of age volunteered for the study that was approved by the SFU Office of Research Ethics. On a single day each volunteer performed three ~1 h exercise trials with the relative humidity (RH) held at 31.5±9.5 %. The ambient temperature (TAMB) for each 1 h trial was one of 25, 30, or 35°C. With the volunteer breathing eucapnic air, each trial started with a 5 min rest period at a TAMB of 23.4±1.7°C and a RH 28±9.4 %. Subsequent cycle ergometer exercise at 50 W was until TES stabilized to the same value of ~37.1±0.4°C in each of the 3 trials. Once TES stabilized in each trial, each volunteer breathed hypercapnic air twice for ~5 min at either ~+4 mm Hg or ~+7.5 mm Hg from the preceding eucapnic period. The 2 hypercapnic periods were separated by 5 min of eucapnic breathing. RESULTS: The significantly (p<0.05) different increases of $PETCO_2$ of +4.20±0.49 mm Hg and +7.40±0.51 mm Hg from the preceding eucapnic state gave proportionately larger increases in VE of 10.9±3.6 L/min and 15.2±3.6 L/min (p=0.001) from the preceding steady state exercise ventilation of ~ 28 L/min. The magnitude of this ventilatory response was not influenced by varying the TSK to 3 significantly different levels (p <0.001) of 33.2 ±1.2°C, 34.5±0.8°C and 36.4±0.5°C. CONCLUSION: The data support that peripheral temperature has no effect on ventilatory responses to CO_2 during a light exercise with a normothermic core temperature. Funding Sources: NSERC and CFI. 2009.

INFLUENCE OF HYPOXIA ON PERFORMANCE DURING INTERMITTENT FOREARM EXERCISE OF MAXIMAL INTENSITY. Norbert Maassen, Andreas Hillrichs, Shushakov Vladimir. Medical School Hannover. *Email: Norbert.Maassen@mh-hannover.de.* After exercise of high intensity oxygen is consumed to restore the energy stores inside the muscle (oxygen debt). The recharge is dependant on blood flow and oxygen pressure. Thus we investigated the influence of changes in position and of hypoxia on the performance of a small muscle group during intermittent exercise of high intensity. Methods: 7 male subjects performed 10 bouts (EB) of forearm exercise with the maximal contraction frequency possible

(15 s exercise and 45 s rest) under 3 conditions (C: horizontal arm position, V: vertical forearm Position VH: vertical position combined with hypoxia; 11% oxygen). Blood was drawn from a cubital vein of the exercising arm and from an arterialized hand vein. Voluntary and evoked EMG were recorded via surface electrodes from the working flexor muscles. Results: HBO_2 was reduced to less than 80% in arterialized blood under H. Power was significantly ($p<0.001$) reduced at the beginning of the first 2 EB under V and VH. Hypoxia had no additional effect. During all following EB power was not different, neither at the beginning nor at the end. Blood flow, VO_2, and Lactate release were not significantly influenced by the different conditions. Time courses of venous [Na], [K], [Hb], [Prot] and osmolality were not different under either condition. There was a small reduction of the m-Wave under V and VH ($p<0.069$) during the late EB. Propagation velocity did not change differently under the 3 conditions. The same held true for median frequency and the EMGrms. The change in position but not hypoxia reduced power at the beginning of exercise. No accumulating effect of the oxygen debt could be seen during the progress of exercise. The decrease in power was neither related to changes in excitability of the muscles nor to changes in central command under these conditions. 2009.

INFLUENCE OF INHALED AMILORIDE ON LUNG FLUID CLEARANCE IN RESPONSE TO NORMOBARIC HYPOXIA. Courtney M. Wheatley[1], Sarah E. Baker[1], Bryan Taylor[1], Robert Wentz[1], Manda Keller-Ross[1], Eric Snyder[1], Bruce Johnson[1]. [1]Mayo Clinic. *Email: Wheatley.Courtney@mayo.edu.* Introduction: Epithelial sodium channels (ENaC) play a critical role in the body's ability to respond to the potential threat of pulmonary edema by mediating efflux of sodium and water from the airspace to the interstitial space for subsequent clearance via the pulmonary lymphatics. Methods: Twenty-three subjects (female=55%, age=28±5yrs, BMI=24±3kg/m2, VO2MAX= 128±23%pred) participated in this study. We investigated the role of ENaC on lung fluid clearance in response to normobaric hypoxia through ENaC inhibition with inhaled amiloride (A; 1.5mg in 5ml normal saline) vs inhaled placebo (P). Changes in lung fluid were assessed pre and post 12 hours of hypoxic exposure (FiO2= 12.5%) on two randomize visits by chest CT scan for lung tissue volume (Vtis), exhaled breath condensate sodium concentration (EBCNa) to look at sodium movement, and the diffusing capacity for carbon monoxide and nitric oxide (DLCO and DLNO, respectively) to determine pulmonary-capillary blood volume (VC). Extravascular lung water (ELW) was derived as Vtis – VC. Results: In response to normobaric hypoxia, there was a significant reduction in ELW, an increase in VC, and a small insignificant change in DLCO for both treatments (ELW: -8.5±3.8 vs. -7.9±5.2% change from baseline*; VC: 53.6±28.9 vs 54.0±52.2% change*; DLCO: 9.45±29.3 vs 9.95 ±23.8% change, mean±SD, A vs P respectively, *$p<0.05$). There was no difference in symptoms (Lake Louise Score: 1.0±1.2 for both A and P) nor the percentage of time spent with a SpO_2 below 80% between treatments (18.3±16.1 vs 15.8±15.3%, A vs P, $p=0.76$). There was a trend for an increase in EBCNa and a greater net urine output with A, suggesting successful ENaC inhibition (EBCNa: 6.2±68.1 vs

-9.9±44.4%change, p=0.40; Fluid Input/Output: -272.8±319.6 vs -136.3±732.4mL, p=0.44, A vs P). Conclusion: Inhaled amiloride had no impact on fluid clearance in response to acute normobaric hypoxia, suggesting ENaC do not play a critical role and highlighting the importance of the pulmonary lymphatics in the lung fluid clearance seen during normobaric hypoxia. Acknowledgements: Funding support: NIH HL71478 and HL108962. 2015.

INFLUENCE OF ISOCAPNIC HYPOXIA ON ESOPHAGEAL TEMPERATURE THRESHOLDS FOR EXERCISE VENTILATION. Michael J Rogers[1], Lauren J. Rietche[1], Matthew D White[1]. [1]Simon Fraser University, Burnaby, BC, Canada. *Email: matt@sfu.ca.* Introduction: The effect of isocapnic hypoxia was assessed for its effect on esophageal temperature (T_{ES}) thresholds for exercise ventilation Methods: Five participants, on two separate days, performed incremental exercise tests on an electrically braked, seated, cycle ergometer from rest to the point of exhaustion. In each exercise trial the inhalant was either isocapnic hypoxic (IH) gas mixture with a 12% O_2 or isocapnic normoxic air (IN). End-tidal partial pressure of CO_2 ($PETCO_2$) clamped at a hypercapnic ~50 mm Hg in both trials. Esophageal and skin temperature were assessed with thermocouples and middle cerebral artery blood velocity was assessed with a transcranial Doppler. Means comparisons were made with paired t-tests and the level of significance was set at 0.05. Results: Results indicated the peak power of 243.1 (44.8) W during IH exercise was significantly lower ($p<0.05$) than the peak power of 296.5 (56.7) W during IN exercise. As well, T_{ES} thresholds for exercise ventilation were significantly lower ($p<0.05$) at 36.5 (0.6)°C in IH exercise relative to those of 37.4 (0.2)°C during IN exercise. There was also a significant decrease ($p<0.05$) of middle cerebral artery blood velocity from 55.6 cm/s in the IN exercise to 42.6 cm/s during the IH exercise. Conclusion: The results support isocapnic hypoxic significantly lowered esophageal temperature thresholds for exercise ventilation relative to the same thresholds during exercise with isocapnic normoxia. Acknowledgements: Supported by NSERC and CFI. 2015.

INFLUENCE OF NORMOBARIC HYPOXIA ON OBESITY-DOES DOSE MATTER? Uta Ferrari[1], Ueding Gesa[1], Stuhlmann Judith[1], Lippl Florian[2], Huber Rudolf[1], Fischer Rainald[1]. [1]Pneumology, Medizinische Klinik Innenstadt, University of Munich, Germany, [2]Gastroenterology, Medizinische Klinik Innenstadt, University of Munich, Germany. *Email: rfischer@med.lmu.de*. Introduction: In a previous study we showed that long term exposure over one week of hypobaric hypoxia (2650m) decreased body weight and blood pressure in subjects with metabolic syndrome. The objective of this study was to examine if higher altitude (for logistic reasons normobaric hypoxia) with lower exposure time reduces body weight and blood pressure in a similar way. Methods: We included 27 patients, 15 male obese patients in our study (age 55.7 ± 5.7 years, BMI 35.4 ± 4.5 kg/m2) and 12 female (age 52 ± 5.52 years, BMI 33.53 ± 3.95 kg/m2). Body weight, waist circumference, basal metabolic rate (BMR), nutrition protocols, and objective activity parameters as well as metabolic and cardiovascular parameters, leptin, were determined at low

altitude (LA) (Munich 530 m), at the beginning, after 2 weeks sham, and after 4 weeks exposition time. The exposition of normobaric hypoxia was equivalent to high altitude of maximal 4500 m, with an exposition time of 1440min (2 hours three times a week). Statistical analysis was performed with SPSS (version 17), under consideration of a level of 0.05 as significant. Results: The participants showed a SpO_2 with a mean of 95% during the sham period and 85.6% during the exposure time (maximal corresponds to a height of 4500 meter of high altitude). There was a significant influence on red blood parameters like MCH, MCHC and number of erythrocytes. No significant influence could be detected for body weight (34.56 ± 4.26 to 34.3± 4.47 kg/m2), and blood pressure (systolic 140.0 ± 18.5 to 133.4 ± 13.5 mmHg, diastolic 88.7 ± 9.5 to 86.6 ± 9.3), neither for fat components, leptin decreased over time without being significant, whereas the sham period influenced it similar to exposure time. Conclusion: This study presents that exposure of normobaric hypoxia equivalent up to 4500m over a time of 1440min is not sufficient to influence body weight or blood pressure. First signs of acclimatisation were detected in red blood parameters. We hypothesize that longer exposition time to hypoxia is necessary for significant effects. To validate the pathophysiology of normobaric hypoxia on obesity or metabolic syndrome, further research is required. Acknowledgements: This study was supported in part by the German Society of Mountain and Expedition Medicine (BExMed). 2011.

INHALED SODIUM NITRITE IN ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) IN PIGS. Philipp A Pickerodt[1], Philipp Lother[1], Roland CE Francis[1], Thilo Busch[2], Willehad Boemke[1], Erik R Swenson[3]. [1]Department of Anesthesiology and Intensive Care Medicine Campus Charité Mitte and Campus Virchow-Klinikum, Charité – Universitätsmedizin Berlin, [2]Department of Anesthesiology, Universität Leipzig, [3]Division of Pulmonary and Critical Care Medicine, University of Washington, and Pulmonary and Critical Care Medicine Section, Veterans Affairs Puget Sound Health Care System. INTRODUCTION: Nitrite (NO_2^-) has protective effects in multiple organ injuries including lung injury via nitric oxide (NO) dependent mechanisms. We hypothesized that the protective effects of $NaNO_2^-$ in lung injury may be associated with a reduction of pulmonary artery pressure (PAP) and resistance (PVR). METHODS: Lung injury was induced in anesthetized pigs (n=4) by lung lavages, followed by a 4h observation period (Timecontrol; n=1) or hourly inhalation of $NaNO_2^-$ for 20 minutes at 15, 30, 45 mg/min (n=2) or 45, 75, 90 mg/min (n=1). Systemic and pulmonary hemodynamics, exhaled NO (NO_{ex}), gas exchange, lung compliance and lung edema formation were measured. RESULTS: Arterial oxygen tension (paO_2) was within the normal range (442-597 mmHg; fraction of inspired oxygen = 1.0) at baseline and decreased to 66-93 mmHg after induction of lung injury. Mean pulmonary arterial pressure (MPAP) increased from 9-17 to 22-35 mmHg and PVR from 58-182 to 192-391 dyn x sec^{-1} x cm^{-5} after induction of ARDS. After lung injury, MPAP (29-35 mm Hg) and PVR (367-469 dyn x sec^{-1} x cm^{-5}) remained high while baseline NO_{ex} (3-12 ppb) did not change in the control animal. Following inhalation of $NaNO_2^-$, NO_{ex} increased over time to 119/167 ppb (15 mg/min), 153/256 ppb (30 mg/min),

168/249/1300 ppb (45 mg/min), 340 ppb (75 mg/min) and 212 ppb (90 mg/min). MPAP decreased by 10/14 % (15 mg/min), 8/14 % (30 mg/min), 19/20/29 % (45 mg/min), 27% (75 mg/min) and 25 % (90 mg/min) in response to $NaNO_2^-$. CONCLUSION: In these pilot experiments inhaled $NaNO_2^-$ decreased MPAP in lung injured pigs. The parallel increase in NO_{ex} with falling pulmonary artery pressure and vascular resistance is consistent with lung tissue and/or blood nitrite reductase generation of vasodilating NO at the level of the pulmonary vasculature. ACKNOWLEDGEMENTS: Supported by a Grant from the Deutsche Forschungsgemeinschaft (DFG; PI 795 /2-1) to Drs. Pickerodt and Boemke. 2015.

INHIBITION OF ALVEOLAR REABSORPTION AND ALVEOLAR INFLAMMATION INCREASE RIGHT VENTRICULAR SYSTOLIC PRESSURE. Bodo Flörchinger[1], Julian Gross[1], Marc M. Berger[2], Peter Bärtsch[1], Heimo Mairbäurl[1]. [1]Medical Clinic VII, Sports Medicine, Univ Heidelberg, [2]Dept. Anesthesiology, Univ Heidelberg, Germany. *EMAIL: heimo.mairbaeurl@med.uni-heidelberg.de* INTRODUCTION: Exaggerated hypoxic pulmonary vasoconstriction is a major reason for high altitude pulmonary edema (HAPE). The mechanism causing the increased pressure response is unclear. Impaired alveolar fluid balance by inhibition of alveolar reabsorption is a possible pathophysiologic mechanism in HAPE, although stimulation of reabsorption did not decrease pulmonary arterial pressure (PAP) in hypoxia {Sartori et al. NEJM 2002}. However, we reason that an increased alveolar fluid load would impair oxygen diffusion, causing hypoxemia, which might lead to pulmonary arterial vasoconstriction that adds to the physiological hypoxic pulmonary vasoconstriction to cause an exaggerated pressure response. METHODS: To test this hypothesis, anesthetized rats were ventilated with normoxic (N; 21%O_2) and hypoxic (H; 13.5%O_2) gasses for up to 2 hours. We also applied aerosols containing amiloride (A) to cause about 50% inhibition of alveolar reabsorption, and LPS to initiate mild inflammation and increase alveolar permeability. Right ventricular systolic pressure (RVP) was recorded as the indicator of PAP. RESULTS: Rats were stable for 2h in N and N+A, but became unstable (increased heart rate, decreased systolic blood pressure) after 90 min (N+LPS), 85 min (H), 60 min (H+A) and 80 min H+LPS. After equilibration, RVP was about 30 mmHg. RVP increased in H (44 mmHg). N+A caused a 10 mmHg increase. RVP was not affected significantly in N+LPS but increased significantly in H+LPS. Wet-to-dry-weight ratios (WD) as indicators of pulmonary edema were increased in all treatment conditions. Amiloride and LPS decreased arterial PO_2 in normoxia. In none of these parameters the effects of amiloride and LPS added to changes in hypoxia. Changes were less pronounced when exposure to the different treatments was shorter. CONCLUSION: Our results indicate that short-term inhibition of alveolar reabsorption and mild alveolar inflammation causes edema which subsequently leads to systemic hypoxia and pulmonary arterial vasoconstriction at a degree similar to that caused by alveolar hypoxia. The lack of additivity may indicate that, at least in short-term experiments, inhibition of alveolar reabsorption and mild alveolar inflammation do not contribute to exaggerated hypoxic pulmonary hypertension as it is found in HAPE. 2015.

INITIAL ORTHOSTATIC HYPOTENSION AT HIGH ALTITUDE. Kate N Thomas, Samuel J Lucas, Keith R Burgess, James D Cotter, Jui-Lin Fan, Karen Peebles, Rebekah A Lucas, Philip N Ainslie. University of Otago, University of Syndney. *Email: kate.thomas@otago.ac.nz.* This study evaluated lowlanders' responses to the initial orthostatic challenge of standing upright at sea level and following 14 days at 5050 m. We hypothesized that high altitude would compromise maintenance of mean arterial pressure (MAP), leading to marked reductions in cerebral perfusion. Ten healthy volunteers were instructed to rapidly rise from supine rest and stand for 3 min. Beat-to-beat blood pressure (Finometer), middle cerebral artery blood velocity (MCAv, Transcranial Doppler), end-tidal PCO_2 and PO_2, and heart rate (ECG) were recorded continuously. After 14 days at altitude, baseline MAP and MCAv were not different to sea-level, while HR was elevated 18 b·min^{-1} ($P<0.05$) and end-tidal PCO_2 and PO_2 were reduced, as expected. Neither the magnitude of initial (<15 s) responses to standing nor the time course of initial recovery differed at altitude compared with sea level ($P>0.05$), except that the nadir of MAP was 2 s earlier at altitude ($P<0.05$). At the end of the 3-min stand, MAP was restored to supine baseline values at sea level (-3±12 mm Hg) but not at altitude (-24±19 mm Hg; $P<0.05$), in conjunction with a more complete recovery of HR at sea level (+13 ±10 b·min^{-1}, $P<0.05$) than at altitude (+23 ±10 b·min-1, $P<0.05$). The reduction in MCAv at 3 min was, however, comparable at sea level and altitude (both -16%). These data indicate that initial cardiovascular and cerebrovascular responses to standing are similar at sea level and high altitude. Following steady-state (i.e. after 3 min) adjustments to standing, MCAv was well maintained despite marked hypotension and tachycardia, potentially indicating some adaptive process in cerebral autoregulation. 2009.

INTERLEUKIN-1 BETA HAS OPPOSITE EFFECTS ON HYPOXIA-INDUCIBLE FACTOR-1 PATHWAY IN NORMOXIC AND HYPOXIC GLIOBLASTOMA CELLS. Sun Wenwen[1], Depping Reinhard[1], Jelkmann Wolfgang[1]. [1]Institute Physiology, Univ Luebeck *EMAIL: wenwen.sun@medizin.uni-luebeck.de* INTRODUCTION: Glioblastomas are one of the most aggressive brain tumors in adults. Advanced glioblastomas typically contain hypoxic areas due to the expansive growth. The transcriptional responses to hypoxia are mainly mediated by the transcription factor hypoxia-inducible factor-1 (HIF-1). HIF-1 consists of an oxygen-labile alpha-subunit and a stable beta-subunit. Besides the role in adaptation to hypoxia, HIF-1 affects many processes with distinct influences on tumor progression including glucose metabolism, proliferation and apoptosis. Interleukin-1beta is a cytokine that is often found in glioblastomas. It has been reported that interleukin-1beta induces apoptosis in glioblastoma cells. Since HIF-1 plays an important role in regulating apoptosis, we aimed to investigate whether interleukin-1beta influences the HIF-1 pathway, thereby inducing apoptosis of glioblastoma cells. METHODS: Glioblastoma cells U87MG were treated with interleukin-1b under normoxic and hypoxic conditions, respectively. Apoptosis was studied using TUNEL assay. Protein levels of the labile alpha-subunit (HIF-1alpha) were estimated by immunoblotting. RESULTS: We confirmed that interleukin-1beta

increases apoptosis of U87MG. The pro-apoptotic effect of interleukin-1beta under hypoxia is much more marked than under normoxia. Interestingly, HIF-1alpha is increased by interleukin-1beta in normoxia, but decreased by interleukin-1beta in hypoxia, indicating that decreased HIF-1alpha may be responsible for the strong pro-apoptotic effect of interleukin-1beta in hypoxic cells. CONCLUSION: In conclusion, the pro-apoptotic effect of interleukin-1beta is much stronger under hypoxia than under normoxia. The apoptosis regulator HIF-1 is affected differently by interleukin-1beta under normoxia and hypoxia. It is necessary to further study the role of HIF-1 in interleukin-1beta-induced apoptosis. ACKNOWLEDGEMENTS: The project [W.S.] is supported by the faculty of medicine of the Univ Luebeck (F249599). 2015.

INTERMITTENT HYPOXIA AND ACUTE MOUNTAIN SICKNESS. Kai K Schommer, Neele Wiesegart, Peter Bartsch. University Hospital Heidelberg. *Email: kai.schommer@gmx.de.* In a placebo-controlled, double-blind study we tested the efficacy of a commercially offered acclimatization program performed in normobaric hypoxia for prevention of acute mountain sickness (AMS). It consisted of exercising on a bicycle ergometer for 70 min 3 times/week for 3 weeks at a workload equivalent to 60% of VO_2max. During the first week FIO_2 was kept at 0.16 (equivalent to the PO_2 at 2500 masl) and increased to 0.15 (3000 masl) and 0.14 (3500 masl) in weeks 2 and 3, respectively. 4 passive exposures of 90 min ($Fi0_2$=0.12, 4500masl) took place in the 4th week. 40 healthy, non-smoking, low-altitude residents (22 male; mean age 32.8 years, range 20 - 55) were randomised to undergo this program either in hypoxia (HG) or in normoxia (NG). The training took place in two different centres at below 120 masl. Five days after the last session subjects ascended in 2 days to 4559 masl. They were taken to 2900m by cable car and continued on foot with an overnight stay at 3611masl. The study endpoint AMS was assessed in the morning after the first night at 4559 masl and was defined as a Lake Louise score › 5 and an AMS-C score ›0.70. The AMS incidence (70% vs. 60% p = 0.74), the corresponding LL scores (7.1±4.3 (SD) vs. 5.9±3.4, p = 0.34) and the AMS-C scores (1.5±1.2 vs.0.9±0.8, p = 0.25) were all not significantly different between the NG and HG, respectively. In the morning at 3611 masl, AMS incidence in HG was significant lower (5.6%) than in NG (24.2%, p = 0.01) when analyzed separately (Chi square test, Holm-Bonferroni adjusted p). SpO_2 at 3611 masl, heart rate during ascent, ascent time, and arterial blood gases at 4559 masl were all not significantly different between groups. We conclude that the tested training procedure does not reduce AMS incidence after rapid ascent to 4559m and there is no evidence that such a preparation induces ventilatory acclimatization. It may, however, partially prevent AMS up to 3600 masl. 2009.

INTERPRETING MODIFIED REBREATHING RESPONSES. James Duffin, Dahlia Balaban, Richard Greene, Alexandra Mardimae, David Preiss, Marat Slessarev, Alex Vesely, Joseph Fisher. University of Toronto, New Mexico Highlands University, University of British Columbia, University of Toronto. *Email: j.duffin@utoronto.ca.* Our objective was to show how experimental data obtained during

modified rebreathing tests may be further interpreted by fitting a set of standardised respiratory chemoreflex model equations incorporating the effects of acid-base state (1). We hypothesised that the model parameters would provide insight into the characteristics of the respiratory chemoreflex control system. In modified rebreathing tests (2), following a period of voluntary hyperventilation, subjects rebreathe from a bag pre-filled with a hypoxic or hyperoxic gas mixture; during rebreathing isoxia is maintained by adding O_2 to the rebreathing bag under computer control. Test examples were selected from experiments on lowlanders at sea level and after acclimation at altitude, as well as from highlanders. These were fitted using a LabVIEW (National Instruments) program written for the purpose. Results: We obtained both expected and unexpected findings. As expected we found that peripheral chemoreflex reactivity was lacking in highlanders and enhanced in lowlanders at altitude compared to sea level. An unexpected finding was the ability to detect sea level subjects with enhanced peripheral chemoreflex reactivity; peripheral thresholds exceeding central thresholds and peripheral CO_2 sensitivity incompletely suppressed in hyperoxia (2). We concluded that this interpretation method provided insights into the contributions of the central and peripheral chemoreceptors to ventilation responses and the effects of acid-base state, that were not obvious from the rebreathing test results alone. Supported by a CIHR scholarship to Balaban, D. and Thornhill Research Inc. References: 1. Duffin J. Measuring the ventilatory response to hypoxia. J Physiol 584: 285-293, 2007. 2. Duffin J, Mohan RM, Vasiliou P, Stephenson R, and Mahamed S. A model of the chemoreflex control of breathing in humans: model parameters measurement. Respir Physiol 120: 13-26, 2000. 2009.

INTRA-INDIVIDUAL CORRELATIONS BETWEEN CENTRAL AND PERIPHERAL CHEMOREFLEX MAGNITUDE IN HUMANS. Jamie Pfoh[1], Maria Abrosimova[1], Lindsey Boulet[2], Michael Tymko[2], Anthony Bain[2], Philip Ainslie[2], Amy Varner[1], Liliana Cordona[1], Trevor Day[1]. [1]Mount Royal University, [2]University of British Columbia Okanagan. *Email: tday@mtroyal.ca.* Introduction: Central (brainstem) and peripheral (carotid body) respiratory chemoreceptors modulate blood gases through separate but interacting chemoreflexes, responding to differing chemostimuli and temporal domains. However, central and peripheral chemoreceptors are both derived embryologically from the neuroectoderm, which may suggest a similar intra-individual phenotype. Our objective was to quantify the central chemoreflex (CCR; hyperoxic hypercapnia) and peripheral chemoreflex (PCR; transient hypoxia and hypercapnia), and perform intra-individual correlations of the resulting responses. We examined the hypothesis that those with large CCR responses would also have large PCR responses. Methods: We tested CCR and PCR chemoreflexes in 20 healthy adults (25yrs; BMI 24kg/m2; 8 males). The CCR was tested via hyperoxic rebreathing, with linear regression beyond the ventilatory recruitment threshold (42±0.6 [mean±SD] Torr CO_2) quantifying the sensitivity (2.6±0.3 L/min/Torr CO_2; n=17). The PCR was tested from a mean of five trials each of a transient three-breath 100% N2 test (1.04±0.2 L/min/-% SpO_2; n=20) and a transient single-breath 13% CO_2 in air (1.09±0.2 L/min/Torr CO_2; n=19). Results: The CCR magnitudes were not correlated with either the variability

in the PCR to hypoxia (r=0.16; P=0.54; n=17) or hypercapnia (r=-0.37; P=0.162; n=16). However, the variability in the PCR to hypoxia and hypercapnia were correlated (r=0.65; P=0.003; n=19). Conclusion: Our data suggest that the similar proximal embroyological origin of central and peripheral respiratory chemoreceptor tissues (i.e., neuroectoderm) does not affect intra-individual phenotype. However, the more distal embryological differentiation of the central and peripheral chemoreceptors (neural tube and crest, respectively) may predict the intra-individual PCR responses to hypoxia and hypercapnia. Thus, either (a) the chemoreflex variability is coded for at the more distal tissue level or (b) differential developmental and epigenetic factors account for the variability of responses between respiratory chemosensitive tissues. Acknowledgements: Alberta Innovates Health Solutions Summer Studentship, Faculty of Science and Technology Innovation Grant, MRU Internal Research Grant. 2015.

INTRAPULMONARY SHUNT DURING SUPINE EXERCISE AT SEA LEVEL AND AT 5050M IN HEALTHY HUMANS. Trevor Day[1], Glen Foster[2], Michael Stembridge[3], Michael Tymko[1], Akke Bakker[4], Philip Ainslie[5], Andrew Lovering[6]. [1]Mount Royal Univ, Calgary, AB, Canada, [2]Univ British Columbia, Vancouver, and Okanagan, Kelowna, BC, Canada, [3]Cardiff Metropolitan Univ, Cardiff, UK, [4]Univ Twente, Enschede, Netherlands, [5]Univ British Columbia Okanagan, Kelowna, BC, Canada, [6]Univ Oregon, Eugene, OR, USA. *EMAIL: tday@mtroyal.ca*
INTRODUCTION: Intrapulmonary arteriovenous anastomoses (IPAVA) bypass the alveolar-capillary interface and can lead to intrapulmonary shunting when patent. Blood flow through IPAVAs can be induced during exercise (EX) at sea level (SL) and at rest in acute normobaric hypoxia, potentially due to increases in cardiac output and/or pulmonary artery pressure. The extent to which blood flows through IPAVAs at rest or during EX at high-altitude (HA) is unknown. We hypothesized that blood flow through IPAVAs would be exacerbated during EX at HA (5050m; Patm~413 mmHg). METHODS: Using a custom-made, supine cycle ergometer, seven healthy adult subjects (age=33.1±7.8yrs; no patent foramen ovale) underwent an incremental exercise test to exhaustion (VO_2peak) at SL (~350m; Patm~730 mmHg) to obtain relative peak power outputs. Echocardiography was used to determine cardiac output (Q; L/min) and pulmonary artery systolic pressure (PASP; mmHg) and agitated saline contrast was used to determine blood flow through IPAVAs (bubble score; 0-5) during rest and EX at SL (25, 50, 75% of VO_2peak) and HA (25, 50% of VO_2peak). RESULTS: The principle findings were: (1) Q did not increase from SL-rest (3.9±0.47) to HA-rest (4.5±0.49; P=0.382), but did increase from resting values during both SL-EX and HA-EX (all: P<0.001); (2) PASP increased from SL-rest (19.2±0.67) to HA-rest (33.7±2.8; P=0.001) and increased from resting values during both SL-EX (P=0.019) and HA-EX (P=0.003); (3) bubble scores increased from SL-rest (0) to HA-rest (1±0.31; P=0.027) and increased from resting values during SL-EX (P=0.002), but were unchanged during HA-EX (P=0.192). CONCLUSION: Despite elevations in Q and PASP during HA-EX, blood flow through IPAVAs did not increase, suggesting remodeling of pulmonary blood flow at HA. ACKNOWLEDGEMENTS: Supported by HSFC, MSFHR,

NSERC, CRC and APS Giles F. Filley Memorial Award. Research conducted under the memorandum between Nepal Health Research Council and EV-K2-CNR. 2015.

INTRAPULMONARY SHUNT VESSELS ARE RECRUITED WITH HYPOXIA AND POSTURAL CHANGES. Melissa L Bates, William G Schrage, Marlowe W Eldridge. The University of Wisconsin. *Email: mlbates@pediatrics.wisc.edu.* We tested the hypothesis that dormant intrapulmonary shunt vessels (IPAVs) are recruited under conditions that redirect flow toward the apex of the lung. We studied four healthy human participants (3M, 1F) under normoxia and hypoxia (10% and 8% O_2) using agitated saline contrast echocardiography (4-chamber apical view). In normoxia and hypoxia, participants were studied in three different positions: seated upright, supine, and supine with 15 degrees head-down tilt. In each position under normoxia, all participants had normal contrast echocardiograms and displayed no evidence of intracardiac or intrapulmonary shunting (presence of contrast bubbles in the left heart after > 3 cardiac cycles). Breathing 10% O_2, 2 of 4 participants while supine and 3 of 4 participants while head-down demonstrated intrapulmonary shunting. Breathing 8% O_2, 2 of 4 participants while upright, 3 of 4 while supine, and 3 of 3 while head-down demonstrated intrapulmonary shunting. We recorded arterial oxygen saturation (SpO_2) for two participants and found SpO_2 > 97% for both in each position under normoxia. Breathing 10% O_2, both participants experienced a 13% decline in SpO_2 and intrapulmonary shunting while head-down compared to upright. Breathing 8%, large declines in SpO_2 were also associated with changes in posture and intrapulmonary shunting. These data are not explained by ventilation/perfusion mismatch as changes in posture in normoxia did not alter SpO_2. Hypoxia and postural changes, both of which redirect blood flow apically, open IPAVs and support our hypothesis that IPAVs are located in the lung apex. 2009.

IS ELEVATED INTRAOCULAR PRESSURE A MARKER OF ACUTE MOUNTAIN SICKNESS? Ryan Paterson[1], N. Stuart Harris[2], Jason Haukoos[1], Tracy Cushing[1]. [1]Denver Health Medical Center, [2]Massachusetts General Hospital. *Email: rpaterson@hotmail.com*. Introduction: Acute Mountain Sickness (AMS) is common and can lead to high altitude cerebral edema (HACE) if not properly recognized. Intra-ocular pressure (IOP) has been suggested as a conduit of elevated intracranial pressure and a reasonable diagnostic approach to evaluate patients for AMS in remote settings. This study was designed to determine the association between IOP and AMS. Methods: Subjects were recruited from a convenience sample of travelers in the Kumbu region of Nepal, elevation 14,410ft (4,392m). Study participation involved completion of a 21-item questionnaire to assess for AMS by the Lake Louise Score (LLS), followed by three IOP measurements in each eye. Investigators were blinded to the LLS. Subjects with a history of ocular surgery were excluded. Three IOP measurements per eye were made using an applanation tonometer (Tono-Pen®, Reichart Technologies) and averaged across both eyes. Multivariable logistic regression analysis was used to estimate the association between IOP and AMS while adjusting for age, ascent or descent, and use of acet-

azolamide. Results: 161 subjects were enrolled with a median age of 36 (IQR: 29-45) years; 60% were male, 75% were ascending, and 64% were taking acetazolamide; additionally, 38%, (95% CI: 31%-47%) were diagnosed with AMS (LLS ≥ 3). The median IOP was 21 (IQR 18-24) mmHg. The logistic regression model demonstrated no association between IOP and AMS (odds ratio [OR] 1.0, 95% CI: 0.9 - 1.1). Age (OR 1.0, 95% CI: 0.9-1.0) and use of acetazolamide (OR 1.4, 95% CI: 0.6-2.6) were also not associated with AMS, while ascent (OR 0.4, 95% CI: 0.2-0.9) was negatively associated with AMS. Conclusion: IOP is not associated with, and appears to have little utility in, the diagnosis of AMS. Other approaches to easily and accurately diagnose AMS are needed. 2011.

IS HYPOXIA A HALLMARK OF NEUROLOGICAL DISORDERS? Jeff F Dunn[1], Runze Yang[1]. [1]Hotchkiss Brain Institute, Faculty of Medicine, University of Calgary. *Email: dunnj@ucalgary.ca.* Introduction: Oxygen is intricately linked to brain function, and so low oxygen conditions, or hypoxia, may be present in many neurological disorders. This presentation discusses the link between tissue oxygenation and neurological disease. Methods: A review and thought paper Results: It may be obvious that if blood flow is restricted, there will be hypoxia. In an ischemic stroke t has been shown that the severity of hypoxia is related to stroke outcome. Levels of oxygenation in a specific brain region is related to viability. If blood flow is not restricted, but there is excess metabolic demand, there should be hypoxia. In the event of a seizure, increases in energy demand caused by increases in neuronal activity cannot be compensated by increases in blood flow, causing hypoxia and increasing levels of HIF-1a. In a kindling model of epilepsy, it was found that HIF-1a is critical in regulating subsequent onset of seizures. Application of a HIF-1a agonist will reduce the frequency of seizures, while application of a HIF-1a antagonist will significantly increase the frequency of seizures. This suggests that oxygen plays a crucial role in regulating seizures. Chronic hypoxia is also implicated in the development of Alzheimer's disease. Hypoxia increases mRNA and protein levels of amyloid precursor protein as well as b-amyloid (Ab), two crucial proteins involved in Alzheimer's disease. In addition, presence of hypoxia will impede degradation of Ab, further exacerbating the condition. Hypoxia appears to be associated with neuroinflammation, and is present in conditions such as multiple sclerosis. Using PO2 implants in an experimental autoimmune encephalomyelitis model of MS, we show that onset of motor deficits coincided with the decrease in PO2, suggesting hypoxia is related to inflammation. Conclusion: Hypoxia appears to be implicated in many neurological conditions, and examining hypoxia may provide novel insights into the underlying cause, the progression and the treatment response. Acknowledgements: National Engineering and Research Council, MS Society. 2015.

IS IT POSSIBLE THAT ELDERLY PERSONS WITH LOWER-NORMAL PAO_2 LIVE LONGER THAN THOSE WITH HIGHER-NORMAL PAO_2? Jon A Hardie[1], Eirunn W Saure[2]. [1]Dept Clinical Science, Univ Bergen, Norway, [2]Haukeland Univ Hospital, Bergen, Norway. *EMAIL: jon.hardie@med.uib.no* INTRODUCTION:

Reference value studies show that PaO_2 in healthy persons falls with increasing age until about 70 years, after which it seems to plateau. This plateau may be the result of a healthy-survivor bias in the reference study. If so, reference subjects with low-normal PaO_2 would likely have higher risk of death than those with high-normal PaO_2. To investigate this we analyzed survival data from 2 reference value studies on PaO_2 among Norwegian elderly over 67 yrs. METHODS: In a population based study on respiratory health and pulmonary function among elderly Norwegians we performed arterial blood gas sampling. In a pilot study we tested 43 persons (recruited) and in the main study 146 persons (random population sample). All healthy non-smokers with no respiratory symptoms or disorders. Age 67-100 years. Arterial blood samples were taken from the radial artery with the subject supine for over 15 minutes. Testing was performed in 1998-2000 and the subjects where followed until October 2007. RESULTS: For the pilot sample and main study sample the median PaO_2 was 9.5kPa and 10.0kPa, respectively. There were 8 of 43 from the pilot study and 58 of 146 from the main study who died during follow-up. The mean PaO_2 among those who died and those who survived was 10.4kPa and 9.7kPa in the pilot study and 10.3kPa and 9.8kPa in the main study. Multivariate Cox regression adjusting for sex and age showed a hazard ratio (HR) for death of 0.08 (95% CI: 0.01 - 0.83) in the pilot study and 0.55 (95% CI: 0.32-0.95) in the main study for subjects with PaO_2 below the median. Combining the two samples in one Cox regression (n=189) gave HR 0.51 (95% CI: 0.31 – 0.85). CONCLUSION: There is no sign of the survival selection bias within the healthy sample that was tested. On the contrary, it seems that elderly persons with lower-normal PaO_2 have almost 50% lower risk of dying during 8 years of follow-up compared to those with higher-normal PaO_2. 2015.

IS REDUCED CAROTID FLOW OR DISTENSIBILITY RELATED TO ENHANCED CHEMOSENSITIVITY IN HEALTH AND HEART FAILURE?. Michael K. Stickland[1], M. Sean McMurtry[1], Ian Paterson[1], Justin A. Ezekowitz[1], Mark J. Haykowsky[2], Jason R.B. Dyck[3], Heather Edgell[1]. [1]Dept of Medicine, Univ Alberta, [2]Rehabilitation Medicine, Univ Alberta, [3]Dept of Pediatrics, Univ Alberta. *EMAIL: michael.stickland@ualberta.ca* INTRODUCTION: Enhanced chemosensitivity is predictive of mortality, and recent animal work has shown that hypoperfusion of the carotid chemoreceptor contributes to enhanced chemosensitivity. METHODS: To translate this work to the human, we investigated whether resting carotid blood flow or distensibility was related to chemonsensitivity as evaluated by the ventilatory response to hypoxia at rest (delta VE/delta SpO_2), and the ventilatory response to exercise (VE/VCO_2 slope) during a standard cardiopulmonary exercise test (CPET), in heart failure patients (HF; n=8) and risk-matched controls (n=10). Participants were recruited from the Heart Function Clinic at the Univ Alberta hospital, the Alberta HEART collaborative study, or the general population. CPETs and resting measurements were done on separate testing days as part of a larger study. Ventilation, blood pressure, pulse-oximetry and carotid flow/distensibility were measured at rest. Carotid flow was normalized to body surface area. Ventilation and oxygen saturation were measured during a step drop to 85% SpO_2, and delta VE/

delta SpO_2 was calculated as the change in each variable from baseline to 85% SpO_2. RESULTS: Interestingly, there were no between-group differences in chemosensitivity and carotid flow/distensibility, and therefore HF and controls were grouped together for regression analysis. Carotid flow was related to VE/VCO_2 (r=-0.491, p=0.045) but not delta VE/delta SpO_2 (r=-0.236, p=0.438), indicating that low carotid flow was associated with enhanced ventilatory response to exercise. The relationship between carotid distensibility and delta VE/delta SpO_2 was r=-0.516, p=0.071, while carotid distensibility was unrelated to VE/VCO_2 (r=-0.013, p=0.961), suggesting that low carotid distensibility may be related to enhanced chemosensitivity. CONCLUSION: Consistent with recent animal work, these preliminary results indicate that low carotid blood flow or carotid artery distensibility is related to enhanced chemosensitivity. ACKNOWLEDGEMENTS: Funded by the Heart and Stroke Foundation of Canada and the Canadian Institutes of Health Research. 2015.

IS RETINAL VESSEL DIAMETER A PREDICTIVE MEASURE FOR ACUTE MOUNTAIN SICKNESS OR HIGH ALTITUDE HEADACHE? Gabriel Willmann[1], Andreas Schatz[1], Manuel Dominik Fischer[1], Kai Schommer[2], Eberhart Zrenner[1], Karl-Ulrich Bartz-Schmidt[1], Florian Gekeler[1]. [1]Centre for Ophthalmology, Univ Tübingen, Tübingen, Germany, [2]Dept Sports Medicine, Medical Clinic, Univ Hospital Heidelberg, Heidelberg, Germany. *EMAIL: Gabriel.Willmann@googlemail.com* INTRODUCTION: This study aimed to quantify the impact of high altitude on retinal vessel diameter and to assess possible correlations to symptoms of acute mountain sickness (AMS) and high altitude headache (HAH). This work is related to the Tübingen High Altitude Ophthalmology (THAO) study METHODS: VesselMap 1 analyzer (Imedos Systems, Germany) was used to quantify changes of retinal vessel diameter within one diopter distance of the papilla in 18 healthy subjects during acute high altitude exposure to 4559 m compared to baseline recordings (341 m) using infrared fundus images obtained from a Spectralis® device (Heidelberg Engineering, Germany). Intra-individual differences were calculated using ANOVA with a significance level of $p < 0.05$. Pearson's correlation was used to assess a possible linkage between retinal vessel diameter and scores of AMS and HAH. RESULTS: Analysis of intra-individual differences revealed a significantly ($p < 0.05$) increased mean arterial (MAD; increased MADaltitude = 13.6 µm) and venous diameter (MVD; increased MVDaltitude = 26.7 µm) at high altitude in healthy subjects. Average arterial and vein diameters at baseline were: MADbaseline = 122.72±14.78 µm; MVDbaseline = 148.02±15.32; mean±sd) Changes were completely reversible upon descend. Pearson's coefficient showed neither a correlation between increased retinal vessel diameter and AMS (MAD vs. AMS-c score: r = 0.02, p = 0.95; MVD vs. AMS-c score: r = -0.17, p = 0.51) nor with HAH (MAD vs. headacheAMS-c score: r = -0.17, p = 0.50; MVD vs. headacheAMS-c score: r = -0.10, p = 0.71). CONCLUSION: A significant increase in central retinal vessels for both arteries and veins occurs in response to high altitude exposure in healthy subjects. The lack of correlation between retinal vessel diameter and symptoms of AMS or HAH is of special interest as a greater increase in retinal veins due to

restricted cerebral venous outflow has recently been suggested to be associated with headache burden in response to high altitude. Our findings do not support this concept, but do not rule out that restricted cerebral venous outflow may account for HAH. ACKNOWLEDGEMENTS: Wilderness Medical Society (WMS)and Heidelberg Engineering. 2015.

IS THE CARDIORESPIRATORY RESPONSE TO HYPOXIA ALTERED IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE? Michael Stickland[1], Norah Vogan[1], Bill MacGarvey[1], Eric Wong[1], Mohit Bhutani[1]. [1]Department of Medicine, University of Alberta, Edmonton, Canada. *Email: michael.stickland@ualberta.ca*. Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by smoking-induced lung damage, chronic inflammation, and acute exacerbations of the disease which can cause episodic hypoxemia. COPD is associated with increased cardiovascular morbidity and mortality; however the underlying mechanisms remain unknown. Cardiovascular diseases such as heart failure and hypertension are associated with an altered response to hypoxia, with the ventilatory response to hypoxia being predictive of mortality in heart failure. The purpose of this study was to examine the cardiorespiratory response to incremental hypoxia to determine if responses are similarly altered in COPD. Methods: Ten mild/moderate non-hypoxemic COPD patients (FEV1 = 58% pred, Age= 68.6 yrs) and nine age-matched healthy controls (FEV1=104% pred, Age=69.5 yrs), with no nocturnal hypoxemia or sleep-disordered breathing as measured by an ambulatory overnight sleep study, breathed progressive hypoxic gas at rest while in the supine position (target SpO2 = 90% and 85%). Minute ventilation, heart rate, brachial arterial blood pressure, and arterial stiffness (assessed by pulse wave velocity), were evaluated on room air, then at the two levels of hypoxia. Results: At baseline on room air, COPD patients had increased minute ventilation and pulse wave velocity, while heart rate and mean arterial pressure were not different between groups. Similar increases in minute ventilation, and heart rate were observed in both groups with hypoxia, while mean arterial pressure was unchanged. With incremental hypoxia, COPD patients demonstrated a reduction in arterial stiffness (-5.8%), while in contrast, age-matched controls showed an increase in arterial stiffness with hypoxia (+15%). Conclusion: These preliminary findings suggest that the vascular response to hypoxia is altered in mild to moderate COPD patients. Acknowledgements: Funding: Canadian Institutes of Health Research. 2011.

ISCHEMIC CONDITIONING BLUNTS FLOW-MEDIATED BRACHIAL ARTERY DILATION AT HIGH ALTITUDE. Paresh Giri[1], Brendan Matus[1], Douglas Rodgers[1], Moussa Saleh[1], Lin Xiaohui[2], Gary Foster[2], James Anholm[2]. [1]Loma Linda University Medical Center, [2]VA Loma Linda Healthcare System. *Email: pareshcgiri@gmail.com*. Introduction: Many factors affect flow-mediated vascular reactivity including nitric oxide (NO), an important modulator of physiologic responses to high altitude (HA) exposure. Flow mediated dilation (FMD) of the brachial artery is useful for assessing endothelial function and impaired FMD correlates with adverse cardiovascular events. Ischemic pre-conditioning of the

extremity (IPC) induces systemic cardio-protective effects in part via NO. The purpose of this study was to evaluate the hitherto unknown effects of IPC and HA on FMD. Methods: FMD was measured in 12 healthy runners at baseline and after 5 days of daily IPC or sham-IPC at sea level (SL). Ascent to altitude occurred over 6 h after which runners completed a 12.8km competitive run to 4342m, rested 60 min at altitude, followed by FMD. Subjects completed the procedures twice separated by 6 weeks in a randomized cross-over design. IPC was administered using a cuff around the thigh inflated to 200mmHg for 5 min, deflated for 5 min and this sequence repeated for 4 cycles. The cuff was inflated to 40mmHg for sham-IPC. Brachial artery diameter was measured using duplex ultrasonography with a linear array transducer before and after 5 min of brachial artery occlusion.The effects of HA and IPC on FMD were evaluated using repeated-measures ANOVA. Results: At SL, FMD was 15.5% ± 7.0% (mean ± SD) without IPC and 9.8% ± 6.1% with IPC. At HA, FMD was 11.5% ± 9.5% without IPC and 6.0% ± 5.1% with IPC. IPC attenuated the FMD by 36.5% at SL and 60.7% at HA ($p < 0.05$) while altitude exposure reduced FMD by 25.5% ($p < 0.05$). These responses were largely due to pre-occlusion brachial artery dilation caused by IPC and HA (ANOVA $p < 0.05$ for both IPC and HA). Conclusion: Flow mediated dilation is significantly blunted by both ischemic pre-conditioning of the extremity and by rapid ascent to high altitude due predominantly to larger pre-occlusion artery diameters likely induced by vasodilators released by IPC and altitude exposure. 2011.

ISCHEMIC PRECONDITIONING REDUCES PULMONARY ARTERY PRESSURE AT HIGH ALTITUDE. Paresh Giri[1], Douglas Rodgers[1], Brendan Matus[1], Moussa Saleh[1], Terry Michael[1], Lin Xiaohui[2], James Anholm[2], Gary Foster[2]. [1]Loma Linda University Medical Center, [2]VA Loma Linda Healthcare System. *Email: pareshcgiri@gmail.com*. Introduction: Hypoxic pulmonary vasoconstriction occurs at high altitude (HA) resulting in pulmonary hypertension. Ischemic preconditioning of the extremity (IPC) induces systemic cardio-protective effects via multiple mechanisms including increased nitric oxide production and HIF-1α activation. The purpose of this study was to evaluate the effects of IPC on pulmonary artery pressure (PAP) at HA. Methods: PAP was measured using transthoracic echocardiography in 13 healthy runners after 5 days of daily IPC or sham-IPC at sea-level (SL) and immediately after a 12.8km competitive run to the summit of White Mountain, CA, (altitude = 4342m). Each subject completed the course twice (once following IPC and once following sham-IPC) separated by 6 weeks in a randomized cross-over design. IPC was administered using a blood pressure cuff around the thigh inflated to 200mmHg for 5 minutes, deflated for 5 minutes, and repeated for a total of 4 cycles. The cuff was inflated to 40mmHg for sham-IPC. Pulmonary artery systolic pressure (PASP) was calculated from peak continuous-wave Doppler tricuspid regurgitation velocity and mean PAP (mPAP) from pulsed-wave Doppler right ventricular outflow tract acceleration time. Oxygen saturation (SaO_2) was measured continuously over 10 minutes (during echocardiography) and averaged. Results: At HA, IPC significantly reduced PASP (38 ± 8 mmHg sham-IPC vs. 36 ± 6 mmHg IPC, $p< 0.03$, $n=8$) and mPAP (24 ± 7 mmHg

sham-IPC vs. 17 ± 6 mmHg IPC, p<0.004, n=13). IPC did not significantly change PASP or mPAP at SL. IPC significantly improved average SaO_2 at HA (75 ± 9% sham-IPC vs. 80 ± 8% IPC, p <0.001). Conclusion: At high altitude, ischemic preconditioning of the extremity induces systemic effects that are associated with increased oxygen saturation and decreased pulmonary artery pressure. 2011.

JAPANESE SOCIETY OF MOUNTAIN MEDICINE HAVE STARTED THE PILOT STUDY OF THE HEALTH CHECK PROGRAM FOR THE MIDDLE-AGE MOUNTAINEERS WHO WERE GOING TO HIGH ALTITUDE. Tomonori T Harada, Hayato Andou, Rika Ide, Junko Inaji, Hiroshi Kajitani, Hiroshi Kaneko, Norihiro Kamikomaki, Keishi Kubo, Ichiro Kuwahira, Mayuko Konomi, Toshio Kobayashi, Shigeru Saitou, Gen Sasao, Kimiyasu Sekiguchi, Morimasa Takayama, Hiroaki Natsui, Takahumi Nishioka, Shiori Hashimoto, Masayuki Hanaoka, Shigeru Masuyama, Masako Horii. Nihon University School of Medicine, Japanese Society of Mountain Medicine. *Email: tharada@med.nihon-u.ac.jp.* It is reported that unexpected health problem and death during mountaineering among middle-aged and elderly mountaineer happened in the case of alpinism and trekking. Japanese Society of Mountain Medicine(JSMM) started the pilot study of "Health Evaluation Network for Mountaineers" to reduce these events on October 2006. In the pilot study, travelers who were going to join the high altitude mountaineering or trekking planed by the tour agencies, undergoes medical health check formatted by this network. The client is performed history taking, clinical examination, laboratory tests, electrocardiogram, spirometry, Chest X-ray. With these results, the doctor who is the member of JSMM judges the health status and risk. From October 2006 to March 2008, the health checks in 334(male; 184, female; 150, mean age; 61.5) clients were carried out. In the judgment of health status, 80(24.0%), 224(67.1%), 30(9.0%), 0(0.0%) clients were excellent, good, fair, poor, respectively. Also 266(79.6%), 65(19.5%), 3(0.9%) clients were judged low, intermediate, high risk, respectively. As the results of health check, 2 clients were abandoned to join the tour. Over more, 6 clients not included these 334 were excluded from this health check, as they need treatment for the patients, and they also did not join the tour. Whereas, the clients of 332 joined the tour, and most of the clients, 330 were finished tour without health trouble. In these 330, 5 were recommended to cancel the participation but 3 of 5 joined the tour with recognition of risk. On the other hand, 2 clients who were judged good-low, have became high altitude pulmonary edema(HAPE) during the tour. Total 8 clients of 340(2.4%) were canceled the tour as the result of health check. And except HAPE, major event was none. These suggest the significance of this pilot study. However some problems are presenting. Therefore, continuation and improvement of the pilot study is needed. 2009.

KINANTHROPOMETRICAL, PHYSIOLOGICAL AND HEMATOLOGICAL RESPONSES AFTER 6 PEAKS > 8.000 M WITHOUT SUPPLEMENTARY O_2. Gaizka Mejuto[1], Julio Calleja-Gonzalez[1], Jose Antonio Lekue[2], Jose Ignacio Emparanza[3], Nicolas Terrados[4]. [1]Univ the Basque Country, Vitoria-Gasteiz, Spain, [2]Fadura-Getxo Sport Performance Center, Bilbao, Spain, [3]Donostia Univ Hospital,

San Sebastian, Spain, [4]Regional Unit of Sport Medicine, Aviles, Spain. *EMAIL: gaizka.mejuto@ehu.es* INTRODUCTION: Summiting the highest peaks on Earth "by fair means" (no bottled O_2) can be achieved by only a few top level athletes. The aim of the study was to observe and analyze Pre-expedition (PRE) vs. Post-expedition (POST) kinanthropometrical, physiological and haematological responses at sea level in an elite climber. METHODS: This is a case report of a highly endurance-trained elite climber. All measurements were collected at sea level 7 ± 2 d PRE and 9 ± 5 d POST to 6 peaks above 8,000 m in Himalaya (8,153 ± 154 m). RESULTS: Differences were not observed either in kinanthropometry or physiology PRE vs. POST (W: PRE 72.3 ± 0.6 kg vs. POST 72.9 ± 0.7 kg; ∑6SF PRE 30.0 ± 0.7 mm vs. POST 31.4 ± 2.0 mm; F% PRE 6.0 ± 0.2 % vs. POST 6.0 ± 0.3 %; HRmax 184 ± 4 bpm vs. POST 183 ± 4 bpm; [La-1]max PRE 7.6 ± 3.0 mmol/L-1 vs. POST 6.5 ± 1.3 mmol/L-1; VO_2 max PRE 70.6 ± 3.1 ml•min-1•kg-1 vs. POST 70.9 ± 3.1 ml•min-1•kg-1) (NS). In hematology, all variables increased an average of 23% PRE vs. POST (Hct: PRE 41.5 ± 1.7 % vs. POST 51.7 ± 2.9 %; RBC: PRE 4.59 ± 0.22 •10ˆ12/L vs. POST 5.53 ± 0.38 •10ˆ12/L; [Hb]: PRE 14.3 ± 0.6 gr/dL-1 vs. POST 17.8 ± 1.4 gr/dL-1) ($p<0.01$). CONCLUSION: Exposures to extreme altitudes (>8,000 m) lead to an unavoidable increase in RBC, Hct and [Hb] as a result of acclimatization process. Interestingly, changes in body mass and aerobic capacity were not observed as it could be expected. We hypothesized that the high fitness level of the climber combined with his experience in mountaineering and accurately designed food intake could in part explain these data. ACKNOWLEDGEMENTS: CPT Fadura-Getxo Medical and Scientific Staff. 2015.

LEFT VENTRICULAR DIASTOLIC FUNCTION IN SHERPA ADOLESCENTS RESIDING AT HIGH AND LOW ALTITUDE. Mike Stembridge[1], Philip Ainslie[2], Joseph Donnelly[3], Nicholas McLeod[4], Michael Hughes[1], Kami Sherpa[5], Rob Shave[1]. [1]Cardiff School of Sport, Cardiff Metropolitan University, Cardiff, UK, [2]Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia Okanagan Campus, Kelowna, Canada., [3]Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK., [4]University of North Carolina, Chapel Hill, NC USA, [5]Khunde Hospital, Khunde, Nepal. *Email: mstembridge@cardiffmet.ac.uk.* Introduction: Adult high altitude (HA) Sherpa demonstrate a decreased transmitral filling ratio (E/A), slower left ventricular (LV) diastolic relaxation and ultimately lower LV end-diastolic volume (EDV) relative to body size in comparison to lowlanders. Whether these characteristics of LV diastolic function are evident earlier in life or appear and progress with increasing HA exposure during adulthood is not known. It also remains to be determined whether diastolic function remodels in Sherpa after migration to lower altitudes. Methods: Therefore, the aim of this study was to compare ventricular function in adolescent (9-16 years) highland Sherpa (HLS; 3840 m) with age-matched Sherpa who had been residing at 1400 m for >5 years (LLS) and lowland Caucasian controls (LLC) born and living close to sea level. Sixty-seven participants (26 HLS, 11 LLS and 30 LLC) underwent a comprehensive two-dimensional, Doppler and speckle-tracking echocardiogram at their habitual altitude, and oxy-

gen saturation (SpO_2) was also collected. Results: SpO_2 was lower in HLS (91±2%) compared to LLS (96±1%) and LLC (98±1%). Only HLS exhibited a smaller relative LV EDV. However, both HLS and LLS demonstrated a longer isovolumic relaxation time (65±11 and 60±7 vs. 52±10 ms), slower early septal myocardial velocity (E'; 0.12±0.03 and 0.11±0.02 vs. 0.15±0.02) and lower peak LV untwisting velocity in comparison to LLC (92±26 and 100±45 vs. 130±43 °/s), but E/A was not different between groups. Conclusions: Much like their adult counterparts, HLS adolescents exhibit smaller ventricular volumes and slower LV diastolic relaxation. However, in this setting untwist velocity and EDV do not appear to be directly associated, as untwist velocity was lower in LLS but EDV and transmitral E/A were comparable to LLC. These findings suggest fundamental differences exist in the diastolic function of Sherpa that are present at an early age and may be retained after migration to lower altitudes. The presence of functional differences despite prolonged residence at low altitude may reveal the significance of hypoxic exposure during the developmental years on cardiac function. 2015.

LITERATURE REVIEW: ISCHEMIC STROKE AND HIGH ALTITUDE: IS HYPOXIA A RISK FACTOR? Esteban Ortiz, Jeff Dunn. University of Calgary. *Email: eortizpr@ucalgary.ca.* Despite our understanding of stroke, the risk factors involved and its treatment and prevention, stroke remains the second leading cause of death in men and women worldwide. Several risk factors have been strongly associated with higher incidences of stroke, such as hypertension or diabetes, while other non-traditional risk factors such as vitamin D deficiency or cardiac valvular thickness have recently been studied. The potential role of hypoxia or high altitude exposure as a risk factor has not yet been clearly established. We performed an evaluating review examining the potential relationship between high altitude exposure and the development of ischemic stroke. The literature is lacking in this specific area, and the available published papers were included. This review includes the relationship between acute and chronic high altitude exposure and the development of ischemic stroke, also includes a brief discussion of adaptations to hypoxia, which may be related with increased risk of ischemic stroke. This review suggests that several risks factors are involved in the development of ischemic stroke during high altitude exposure, such as polycythemia, increased platelet adhesiveness and greater risk to develop vascular thrombosis. These factors combined with co-adjuvant conditions such as dehydration, extreme cold and immobilization might lead to increase the risk to develop ischemic stroke. Interesting, young otherwise healthy men seem to be at higher risk to develop ischemic stroke when arrive above 4000 m without previous acclimatization, suggesting that some other risk factor, such as hypoxia are involved. 2009.

LIVE HIGH – TRAIN LOW DOES NOT INCREASE EXERCISE PERFORMANCE: FIRST DOUBLE-BLINDED, PLACEBO CONTROLLED STUDY. Christoph Siebenmann[1], Paul Robach[2], Peter Rasmussen[1], Robert Jacobs[3], Nordsborg Nikolai[4], Victor Diaz[3], Andreas Christ[5], Niels Vidiendal Olsen[6], Marco Maggiorini[5], Carsten Lundby[1]. [1]Center for Integrative Human Physiology, Institute

of Physiology, University of Zurich, Zurich, Switzerland, [2]Département Médical, Ecole Nationale de Ski et d'Alpinisme, Chamonix, France, [3]Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland, [4]Copenhagen Muscle Research Center, Rigshospitalet, Copenhagen, Denmark, [5]Intensive Care Unit, Department of Internal Medicine, University Hospital, Zurich, Switzerland, [6]6Department of Neuroanaesthesia, The Neuroscience Centre, Rigshospitalet, Copenhagen, Denmark. *Email: csiebenm@student.ethz.ch*. Introduction: The combination of living at altitude and training near sea-level (Live high-Train low, LHTL) may improve aerobic performance of endurance-athletes. However, to date no study can rule out a potential placebo-effect as at least part of the explanation. We therefore conducted a randomized, placebo-controlled and double-blinded study to investigate the effects of four weeks of LHTL on endurance performance. Methods: 16 endurance-athletes trained for eight weeks at low altitude (<1,200m). After a two weeks lead-in period subjects were randomly assigned to a Placebo-group (n=6) or LHTL-group (n=10). The following four weeks subjects spent 16h/day in rooms flushed with either normal air (Placebo-group) or a hypoxic gas mixture corresponding to an altitude of 3,000m (LHTL-group). Testing sessions were performed during the lead-in period, after three and four weeks of the LHTL-intervention and again one and two weeks later. Results: Questionnaires revealed that subjects were unaware of group assignment. Weekly training effort was similar between groups. Haemoglobin mass, maximal O_2-uptake and mean power output in a simulated 26.15km time-trial remained unchanged in both groups throughout the study. Exercise economy (i.e. O_2-uptake measured at 150 and 200 Watt) did not change consistently throughout the study and was never significantly different between groups. Conclusion: Four weeks of LHTL did not improve any of the measured components of endurance performance, suggesting that beneficial effects of LHTL in earlier studies may have been related, at least in part, to a placebo-effect. Acknowledgements: The research was supported by a grant from the Bundes Amt für Sport (BASPO) obtained by Carsten Lundby. 2011.

LONG DISTANCE TRANSPORT OF POLLUTION AND CLIMATE CHANGE IN THE HIGH HIMALAYA: IMPLICATIONS FOR MOUNTAINEERS AND INDIGENOUS COMMUNITIES. John Semple[1], GWK Moore[1]. [1]University of Toronto. *Email: john.semple@wchospital.ca*. Introduction: The Himalaya have warmed by ~ 0.6°C since the middle of the 19th century and this warming is predicted to continue. In addition, there is growing concern regarding the export and long-distance transport of pollution to these remote regions. Increasing levels of pollution in Southeast Asia have the potential for a dramatic impact on the health outcomes in populations who reside and work in the Everest region. Here we report on some of the implications of these changes. Methods: We access a 60 year-long meteorological dataset that has been validated against observations made at the South Col of Mount Everest. We also employ direct measurements of surface ozone, a tracer for pollution as well as the presence of stratospheric air, that we have collected along the Khumbu Valley since 2005. Results: The warming has resulted in a thickening of the atmosphere in the Mount Everest region that since 1948 has

resulted in an increase in the annual mean summit barometric pressure of ~1.5 mb. Ozone concentrations along the Khumbu Valley during the spring climbing season tend to increase with altitude and above 3500m, they typically exceed WHO exposure guidelines. Conclusion: The increase in summit barometric pressure is of a magnitude to be of physiological importance suggesting that it is becoming easier to climb Mount Everest without the use of supplementary oxygen. The ozone data shows contributions from both the stratosphere and the long distance transport of pollution. With global levels of background ozone increasing, unique communities in high-altitude areas, such as those in Nepal, are unexpectedly exposed to ozone concentrations that are similar to, if not higher than, those reported in industrialized cities. Acknowledgements: GWKM was supported by the Natural Sciences and Engineering Research Council of Canada. 2011.

LONG TERM VENTILATORY ADAPTATION AND VENTILATORY RESPONSE TO HYPOXIA IN PLATEAU PIKA (OCHOTONA CURZONIAE): ROLE OF NNOS AND DOPAMINE. Aurèlien Pichon, Bai Zhenzhong, Fabrice Favret, Han Shufeng, Guoen Jin, Dominique Marchant, Jean Paul Richalet, Ri-Li Ge. Lab'Réponses cellulaires et fonctionnelles à l'hypoxie', Research Centre for High Altitude Medicine. *Email: aurelien.pichon@orange.fr.* Aim: We assessed ventilatory pattern and ventilatory response to hypoxia (HVR) and hyperoxia in high altitude living "plateau pikas" and acclimatized and non acclimatized rats. We evaluated the role of nNOS and dopamine on this response by using S-methyl-L-thiocitrulline (SMTC) inhibitor and haloperidol (HAL) antagonist, respectively. Methods: Ventilation was measured using a whole body plethysmographic technique, in conscious pikas (n=9) and non acclimatized rats (n=7) at five different PIO_2 (56.3, 79.6, 111.1, 149.4 and 185.6 mmHg), and in acclimatized rats (n=9, 8 days at 4611m) at two different PIO_2 (56.3 and 79.6 mmH). The effects of NaCl, SMTC (10 mg/Kg ip) and HAL (1 mg/Kg ip) injections on ventilatory pattern were assessed in pikas for the same PIO_2. Results: We observed a main species effect with larger ventilation ($P<0.005$), tidal volume (Vt) ($P<0.005$), inspiratory time/total time (Ti/Tt, $P<0.01$) and lower expiratory time ($P<0.05$) in pikas than in non acclimatized rats. Pikas had also larger Vt and lower respiratory frequency when compared to acclimatized rats for low PIO_2. HVR of pikas and rats were not statistically different. In pikas, SMTC induced a significant increase in ventilation ($P<0.05$, main effect) and Vt ($P<0.05$, interaction) for a PIO_2 of 56.3 mmHg, but had no effect for a PIO_2 of 79.6 mmHg, i.e. the living altitude of pikas. In pikas, HAL injection had no effect on any ventilatory parameters. Conclusions: Long term ventilatory adaptation in pikas is mainly due to an improvement in alveolar ventilation and respiratory pattern (Vt and Ti/Tt), without change in HVR. The sensitivity to severe acute hypoxia in pikas seems to be regulated by a peripheral nNOS mechanism which could limit the energetic cost of ventilation in this well adapted animal. 2009.

LOW $PACO_2$ COMPOUNDS THE FALL IN CEREBRAL TISSUE OXYGENATION AND PARADOXICAL RISE IN $CMRO_2$ DURING ACUTE HYPOXIA. Zachary Smith[1], John Hunt, Jr[1], Ethan Li[1], Jia Guo[1], David Shin[1],

Richard Buxton[1], Miriam Scadeng[1], David Dubowitz[1]. [1]University of California San Diego, USA. *Email: zmsmith@ucsd.edu*. Introduction: We previously observed a paradoxical increase in cerebral O_2 metabolism ($CMRO_2$) during sustained hypoxia. Since CBF and $CMRO_2$ are both independently influenced by PaO_2 and by $PaCO_2$, we investigated the effect of $PaCO_2$ on $CMRO_2$ and how this impacts cerebral tissue oxygenation (PtO_2) during acute hypoxic conditions. Methods: 11 healthy subjects (age 27 +/-6 yrs) participated in 2 groups; one group (n=5) freely modulated their own end-tidal CO_2 during 12.5% O_2 conditions ("poikilocapnic hypoxia group"), while another group (n=6) experienced the same hypoxic conditions but with end tidal CO_2 clamped at normoxic levels ("isocapnic hypoxia group"). We made pulse oximeter measurements of SaO_2, and 3T MRI measurements of cerebral venous O_2 saturation, and cerebral blood flow (CBF) to calculate $CMRO_2$. We estimated PtO_2 in cerebral tissues from an O_2 diffusion model. Results: During normoxia: $CMRO_2$ was 1.58 +/-0.18 umol/g/min (poikilocapnic group - $ETCO_2$=33.9 +/-2.6 torr), and 1.69 +/-0.27 umol/g/min (isocapnic group - $ETCO_2$=36.4 +/-1.6 torr). Over 45 minutes of hypoxia: mean $CMRO_2$ rose 29% to 2.01 +/-0.45 umol/g/min during poikilocapnia, but only by 10% to 1.92 +/-0.43 umol/g/min during isocapnia. Estimated PtO_2 fell 73% from 25 to 9.2 +/-7.9 mmHg during poikilopcapnic hypoxia but only by 44% to 13.0 +/-2.4 mmHg during isocapnic hypoxia. Conclusion: The paradoxical rise in $CMRO_2$ we previously observed during sustained hypoxia is also present acutely. This phenomonen is partially mitigated if CO_2 remains elevated. High CO_2 also improves cerebral tissue oxygenation during acute hypoxic conditions. Low $PaCO_2$ increases neuronal firing and hence increases $CMRO_2$. This may account (in part) for the paradoxical $CMRO_2$ response during hypoxia. Low $PaCO_2$ also decreases CBF. This high $CMRO_2$ / low CBF may compound the PtO_2 decline in hypoxic conditions. $PaCO_2$ thus represents an important covariable in the cerebral response to hypoxia. 2011.

MAINTAINING EUHYDRATION AT HIGH ALTITUDE. Samuel J Oliver, Antony Wright, Marilena Leventi, Jamie H Macdonald. Bangor University, Medical Research Council. *Email: s.j.oliver@bangor.ac.uk.* The objectives of this study were to assess hydration status with valid markers during a 21 day expedition at high altitude and to investigate whether the addition of carbohydrate to water is an effective strategy to promote euhydration. Forty two participants consumed ad libitum a 10% solution carbohydrate energy drink (CHO: PSP22 Science in Sport) or an indistinguishable flavoured placebo (PLB) in a randomised double blind manner. Total body water (TBW) and fat free mass (by five compartment model), urine osmolality (Uosm) and saliva flow rate (Sfw) were assessed pre (960m), mid (5050m), and post expedition (2670m). Total fluid intake and urine output were determined at 1750 and 5050m. Using intention to treat design, hydration markers were analysed by mixed model analysis of variance (allocation x time). Hydration status at 5050m was also analysed by independent t-test after median split into high and low fluid intake groups (high >49mL·kg^{-1} body mass). Mid expedition allocation to CHO was associated with greater urine output and total fluid intake (CHO vs. PLB, 52 ± 18 vs. 44 ± 17mL·kg^{-1}). However, during the expedition there was no

difference between CHO and PLB for any hydration marker ($p > 0.229$). TBW was reduced (Pre 40.5 ± 7.4, Mid 39.0 ± 7.0, Post 39.3 ± 7.0L) but this was primarily attributable to a decrease in fat free mass. Furthermore, Uosm was unaltered (Pre 604 ± 240, Mid 531 ± 218, Post 510 ± 222mOsmol·kg^{-1}) and Sfw increased. Median split analysis supports the validity of Uosm and Sfw at altitude, as individuals consuming high fluid intakes had better hydration status. Contrary to popular belief, euhydration can be achieved at high altitude. Additionally, carbohydrate beverages were associated with greater fluid intakes and maybe considered an effective strategy to maintain high fluid intakes at high altitude. Supported by Ministry of Defence (Army) and Science in Sport. 2009.

MALLORY AND IRVINE ON MOUNT EVEREST: DID EXTREME WEATHER PLAY A ROLE IN THEIR DISAPPEARANCE? Kent Moore, John Semple, Dev Sikka. University of Toronto. *Email: gwk.moore@utoronto.ca.* Objective: The fate of Mallory and Irvine, who disappeared on Mount Everest during the 1924 British Expedition, is one of the most enduring mysteries of the 20th century. They were last seen at 12: 50 pm on June 8th high on Everest's Northeast Ridge before vanishing into the clouds. In the ensuing years, a vigourous debate has taken place regarding their climb. Although, it is generally recognized that weather plays an important role in the success or failure of every expedition on Everest, there has been little discussion into the nature of the weather during their climb, or the role it may have had in their disappearance. The objective of this study is to reconstruct the meteorological conditions during their attempt. Methods: We use hitherto unanalyzed meteorological data collected during the 1924 Expedition, sea-level pressure maps from the period, along with a modern reconstruction of the three-dimensional state of the atmosphere during the first half of the 20th century in this study. Results: We show that this attempt occurred during a period in which there was a drop in barometric pressure on Mount Everest of 18mb that was the result of meteorological conditions similar to those that occurred during the "Into Thin Air" storm of 1996 and other high impact weather events on Everest. Conclusions: Above 7000m, the available oxygen is barely sufficient to maintain the basal metabolic rate of a human being and we propose that this drop, which is equivalent to an increase by over 350m in the height of Mount Everest, was sufficient to push the climbers well beyond human physiological tolerances and most likely contributed to their demise. 2009.

MANAGEMENT OF FROSTBITE IN THE AUSTERE ENVIRONMENT: PORTABLE HYPERBARIC CHAMBER, LOCAL NERVE BLOCKS, THROMBOLYTICS AND PROSTACYCLIN. Emmanuel Cauchy[1], Chris Davis[2], Pasquier Mathieu[3], Peter Hackett[4]. [1]FREMMONT- Institut de Formation et de Recherche en Medecine de Montagne - Chamonix Hospital France, [2]University of Colorado Denver School of Medicine - Department of Emergency Medicine - Aurora, Colorado USA, [3]Emergency Service, Lausanne University Hospital, 1011 Lausanne, Switzerland, [4]University of Colorado Denver School of Medicine - Aurora, Colorado USA. *Email: cauchy@ifremmont.com.* Introduction: Even with today's advances in improved outdoor clothing and the medical management of

frostbite, victims may still suffer catastrophic amputations. Field treatment of frostbite remains challenging. Timely evacuation of frostbite victims within 48 hours is often impossible, and the ideal treatment should be even sooner, within 6 to 12 hrs. We submit that it remains possible to improve frostbite outcomes despite delayed evacuation using resource-limited treatment strategies delivered by both the layperson and the expedition or field-clinic physician. We propose a practical treatment pathway for the management of frostbite in the austere or remote environment. Methods: Recent articles have described the treatment of frostbite based on hospital care, with the use of thrombolytics and iloprost. To be effective, however, these treatments must be started within 24 hr after rewarming for rt-PA and within 48 hrs for iloprost. Synthesizing recent studies, we extend these new advances in management of frostbite to a resource-limited field context. Thrombolytics and iloprost, as well as local nerve blocks and portable recompression chambers should be considered to maximize chances for recovery and reduce amputations. Results: Basic care, including ibuprofen or aspirin administration can be administered in the field. Oxygen or portable hyperbaric chamber may be useful at very high altitudes by any rescuer. Other management options for experienced or trained physicians would be thrombolytics or prostacyclin infusion, and local-regional anesthesia. Particularly in light of predicted risk of complications, rt-PA should only be for severe and extended frostbite rewarmed within 12 hours. Rt-PA or prostacyclin could be considered in association with local-regional anesthesia. Conclusion: In cases of severe frostbite with delayed evacuation, the choice of treatment depends on three parameters: the severity of frostbite, the resources available, and the medical experience of the provider. An algorithm summarizes our proposal recommendations. 2015.

MARKERS OF CARDIOVASCULAR RISK IN KYRGYZ HIGHLANDERS WITH HIGH ALTITUDE PULMONARY HYPERTENSION. Michael Furian[1], Tsogyal D. Latshang[1], Sayaka Aeschbacher[1], Silvia Ulrich[1], Talant Sooronbaev[2], Erkin M. Mirrakhimov[3], Almaz Aldashev[3], Konrad E. Bloch[1]. [1]Pulmonary Division and Sleep Disorders Center, University Hospital of Zurich, Switzerland, [2]National Center for Cardiology and Internal Medicine, Bishkek, Kyrgyzstan, [3]Research Institute for Molecular Biology and Medicine, Bishkek, Kyrgyzstan. *Email: michael.furian@usz.ch.* Introduction: High altitude pulmonary hypertension (HAPH), a chronic altitude related illness, is associated with hypoxemia, dyspnea on exertion and reduced exercise performance. Since hypoxia enhances sympathetic tone, and pulmonary hypertension has been associated with cardiac repolarization disturbances we hypothesized that highlanders with HAPH may be at increased risk of cardiovascular disease compared to healthy highlanders (HH) and lowlanders (LL). Methods: We studied 34 highlanders with HAPH and 54 HH at Aksay, Kyrgyzstan (3250m), and 34 LL at Bishkek (760m). Mean pulmonary artery pressure (mPAP) measured by echocardiography was 34±3, 22±5, 16±4mmHg, respectively (mean±SD, $p<0.05$ all comparisons). During quiet rest, heart rate and the heart rate adjusted QT interval (QTc), both markers of increased cardiovascular

mortality (CVM), were derived from ECG, and the arterial stiffness index (SI), a marker for cardiovascular disease (CVD), was calculated from the photoplethysmogram derived by finger pulse oximetry. Multiple regression models were fitted to assess the independent effect of HAPH and high altitude residence on markers of CVD/CVM when controlled for known modifying factors. Results: SpO_2 in highlanders with HAPH, HH and LL was, mean±SD, 88±4, 92±2 and 95±2%, respectively ($p<0.05$ vs HAPH, both comparisons). QTc in highlanders with HAPH, HH and LL was 422±24, 405±27 and 400±28ms, respectively ($p<0.05$ vs HAPH, both comparisons); corresponding SI was 10.5±1.9, 8.4±2.6 and 8.5±2.0m/s, and heart rate was 75±8, 68±8 and 70±10/min ($p<0.05$ vs HAPH all comparisons). Regression analysis confirmed that HAPH was an independent predictor of increased QTc and SI even when controlled for age, gender, mean arterial blood pressure, body mass index, and SpO_2. Conclusion: Our data suggest that highlanders with HAPH but not healthy highlanders may be at increased risk of cardiovascular mortality and morbidity compared to healthy lowlanders. Acknowledgements: Grant: OPO Foundation, Zurich-Lung-League. 2015.

MAXIMUM BLOOD LACTATE RESPONSES ACUTELY AND AFTER TWO WEEKS OF MODERATE ALTITUDE EXPOSURE IN A LARGE MULTI-YEAR STUDY. John Davis. Alma College, Alma, Michigan. *Email: davisj@alma.edu.* Introduction: There has been a great deal of controversy associated with the Lactate Paradox since Edwards in 1936 proposed that maximum blood lactate responses are reduced after altitude acclimatization. Conversely, with acute ascent to altitude there are many reports that maximum blood lactate responses are unchanged. To our knowledge, no studies with a large subject pool have looked at maximum blood lactate after acute exposure, acclimatization and upon return to sea level in the same study. Therefore, the purpose of these studies was to determine the effect of moderate altitude exposure on maximum blood lactate acutely, after acclimatization, and upon return to sea level. Methods: Over the course of a 6-year period, thirty nine, active subjects (age = 23.3 ± 3.5 yrs, weight = 80.1 ± 17.5 kg, VO2max = 44.9 ± ml/kg/min) completed a graded-exercise test to exhaustion on a cycle ergometer at sea level (SL1), upon acute exposure to 3400 m (ALT1), two weeks following acclimatization at 3400 m (ALT2), and upon return to sea level (SL2). Workloads were increased every two minutes following a two-minute warmup until volitional fatigue. A venous blood sample was taken via an in-dwelling catheter without stasis for subsequent lactate determination. Lactate was measured every 2 minutes during the exercise and then every 2 minutes after for 10 minutes. Results: Maximum blood lactate was unchanged from SL1 to ALT 1 (11.87± 2.17 vs. 11.28 ±2.33 mmol/l). However, ALT2 (9.82± 1.70 mmol/l) was significantly lower than both SL1 and ALT1. Maximum blood lactate returned to SL1 values upon return to sea level (SL2 = 11.30± 2.77mmol/l). Conclusion: These data suggest that acute altitude exposure does not result in a reduction in maximum blood lactate. However, after two weeks of acclimatization maximum blood lactate is reduced and returns to pre-sea level values upon return to sea level. 2015.

MAXIMUM HEART RATES IN SEVERE HYPOXIA IN TIBETANS AND HAN CHINESE: EFFECT OF INHALED FUROSEMIDE AND ILOPROST. Bengt Kayser[1], Jui-Lin Fan[1], Liya Nan[2], Wang Liang Bang[2], Bianba[3], Tianyi Wu[2]. [1]ISMMS, Univ Geneva, Switzerland, [2]National Key Laboratory of High Altitude Medicine, High Altitude Medical Research Institute, Qinghai, P.R. China, [3]High Altitude Medical Research Center, Univ Tibet, Lhasa, Tibet, P.R. China. *EMAIL: bengt.kayser@unige.ch* INTRODUCTION: The mechanisms for the reduction of aerobic performance in conditions of severe hypoxia are still poorly understood. One unexplained finding is the reduction in peak heart rates during incremental maximal exercise tests. Naeije (2012) suggested that hypoxic pulmonary vasoconstriction (HPV) leads to pulmonary hypertension, right ventricular over-load, curtails cardiac output, and thus limits maximum oxygen transport and performance. Since Tibetans have less HPV and less performance decrement in hypoxia compared to non-Tibetans, interventions that influence HPV and its effects in non-Tibetans should not affect Tibetans. METHODS: We compared Tibetans to Han Chinese, both living at 2300m, during incremental treadmill running (Bruce protocol) at the equivalent of an altitude of 5000m in a hypobaric chamber, under placebo vs. inhaled furosemide (to decrease afferent traffic from pulmonary receptors) or inhaled iloprost (a synthetic analogue of prostacyclin PGI2 which decreases HPV). RESULTS: Tibetans reached similar maximum heart rates at 2300m and 5000m, independent of the intervention (peak heart rates > 200 bpm); their performance was decreased at 5000m but not influenced by the intervention. Han Chinese had lower heart rates at 5000m compared to 2300m and had a greater performance decrement than Tibetans; In Han Chinese furosemide partially restored maximum heart rate and iloprost improved maximum performance. The intervention thus made Han Chinese more similar to Tibetans with regard to peak aerobic performance in severe hypoxia. CONCLUSION: These findings suggest that HPV and right ventricular function play a role in limiting maximum aerobic performance in severe hypoxia. Since aerobic performance remains nevertheless reduced at 5000m in both Tibetans, and Han Chinese after intervention, additional mechanisms must be involved in the reduction of maximum aerobic exercise capacity in conditions of severe hypoxia. ACKNOWLEDGEMENTS: We thank the Sino-Swiss Science and Technology Cooperation for support. 2015.

MEASUREMENT OF RAT BRAIN PARTIAL PRESSURE OF OXYGEN IN ACUTE AND CHRONIC HYPOXIA WITH A NOVEL IMPLANTABLE PROBE. Esteban Ortiz, Sirajedin Natah, Jeff Dunn. University of Calgary. *Email: eortizpr@ucalgary.ca.* Hypoxia plays a key role in the genesis of several pathological conditions; however, little is yet known about brain oxygenation in unanesthetized, non-sedated, and unrestrained animal models during hypoxia. We have developed methodology to measure brain tissue partial pressure of oxygen (ptO_2) in awake and unrestrained rats. We measured brain ptO_2 during acute hypoxia before and after simulated high altitude acclimatization. A chronically implantable platinum-based fluorophore at the end of a fiber-optic probe (Oxylite systemÆ) was specially designed and surgically implanted in the parietal cortex of eight Wistar

rats (250- 400 g). After 4-6 days post-surgery, acute hypoxia was induced using different fractions of inspired oxygen (F_IO_2), while concurrently measuring cortical ptO_2. Chronic hypoxia was later induced in 6 of the 8 rats using a hypobaric chamber. After 3 weeks at Ω atmosphere (375 torr), brain ptO_2 was measured and compared with pre-acclimatization brain ptO_2. The results showed that acute hypoxia caused a rapid decline in brain ptO_2. There was a significant increase in brain ptO_2 after 3 weeks of acclimatization in every applied level of F_IO_2. Cortical brain ptO_2 increased by an average of 27% after acclimatization (33.4±1.17 mmHg to 39.2±1.46 mmHg; mean±SD, n=8 and 6 respectively $p \leq 0.001$) when breathing 0.19 F_IO_2. When measured a F_IO_2 of 0.08, the change with acclimation was (7.34±0.62 mmHg to 9.19±0.75 mmHg; mean±SD, n=8 and 6 respectively $p \leq 0.05$). We conclude that chronic hypoxia improves brain oxygenation and that it is possible to measure brain ptO_2 in awake unrestrained animals, using this novel methodology. This increment seen in brain ptO_2 could be explained by the fact that chronic hypoxia induces angiogenesis and polycythemia, thus increasing oxygen carrying capacity and delivery within the brain tissue. 2009.

MEASURING THE HYPOXIC VENTILATORY RESPONSE (HVR). James Duffin, Joseph Fisher, Anne Battisti. University of Toronto. *Email: j.duffin@utoronto.ca.* Duffin has proposed a regime to measure HVR consisting of 3 procedures: a) a modified rebreathing method (1) comparing isoxic hypoxic and hyperoxic rebreathing responses; b) isocapnic and c) poikilocapnic, isoxic hypoxic breathing responses. The latter 2 methods require maintenance of isocapnia and isoxia independent of ventilation; previously only possible with end-tidal forcing methods (3). Our objective was to illustrate performance of the latter 2 procedures using the new method of prospective end-tidal targeting using a sequential rebreathing circuit (2). We targeted end-tidal PO_2 and PCO_2 by providing pre-calculated flows of gas mixtures (RespirAct™, TRI, Toronto, Canada). When ventilation exceeds the flow supplied, the subject rebreathes previously exhaled gas so this circuit forms a self-regulating system where flows of rebreathed gas are proportional to increases in ventilation, thereby maintaining $PETO_2$ and $PETCO_2$ constant. Poikilocapnia and isocapnia were induced with the same system but for poikilocapnia we routed the rebreathed gas through a CO_2 absorber prior to its being inhaled. The measured responses were fitted to a set of equations describing the chemoreflexes and hypoxic ventilatory decline using custom software (LabVIEW, National Instruments). The results show example data with the fitted responses and the parameters obtained. We concluded that this method was able to provide the required conditions of isocapnic and poikilocapnic isoxic hypoxia. Supported by Thornhill Research Inc. 1. Duffin J. Measuring the ventilatory response to hypoxia. J Physiol 584: 285-293, 2007. 2. Slessarev M, Han J, Mardimae A, Prisman E, Preiss D, Volgyesi G, Ansel C, Duffin J, and Fisher JA. Prospective targeting and control of end-tidal CO_2 and O_2 concentrations. J Physiol 581: 1207-1219, 2007. 3. Steinback CD and Poulin MJ. Ventilatory responses to isocapnic and poikilocapnic hypoxia in humans. Respir Physiol Neurobiol 155: 104-113, 2007. 2009.

MEASURING THE OXYHEMOGLOBIN DISSOCIATION CURVE (ODC) IN VIVO. Dahlia Y Balaban, David Preiss, Alexandra Mardimae, Alex Vesely, Marat Slessarev, Richard Greene, Gustavo Zubieta, James Duffin, Joseph A Fisher. University of Toronto, University of British Columbia, University of New Mexico, Clinica IPPA, . *Email: dahlia.balaban@utoronto.ca.* In vitro methods of studying the ODC precisely control variables that influence the shape and position of the ODC, such as temperature, pH, and PCO_2. However, such in vitro manipulations of oxyhemoglobin saturation may not reflect metabolic changes that occur during hypoxia in vivo. Measurement of the ODC in vivo is difficult because it requires the maintenance of isocapnia during progressive levels of sustained hypoxia in breathing subjects. Furthermore, it is difficult to maintain alveolar PO_2 constant as minute ventilation increases. We used a specialized gas blender (RespirAct™, Thornhill Research Inc., Toronto, Canada) and a sequential rebreathing circuit with an expiratory gas reservoir to prospectively target end-tidal PCO_2 ($PETCO_2$) and PO_2 ($PETO_2$) in 6 subjects (2 females), to provide conditions for measuring the ODC in vivo. $PETCO_2$ was maintained at each subject's resting level. We then targeted $PETO_2$s of 100, 70, 60, 50, 40 and 35 mm Hg, in steps lasting 2 min. During the last 30 s of each step an arterial sample was drawn and oxyhemoglobin saturation was read from a pulse oximeter (Onyx II, Nonin, Plymouth MN). For all subjects, $PETO_2$ remained within ± 1.5 mmHg (SD) during at least the last 60 s of each step, while $PETCO_2$ remained within ± 0.5 mm Hg. During testing, PaO_2s spanned a range of 52.8 ± 7.5 mm Hg, yet $PaCO_2$ remained within ± 0.7 mm Hg. Thus, prospective end-tidal targeting can be used to induce isocapnic step changes in PaO_2 conditions required for in vivo measurement of the ODC. We thank the Canadian Institutes of Health Research and Thornhill Research Inc. for the generous support that made this research possible. 2009.

MECHANISMS OF SAO_2 SUPPORT BY THE HYPOXIC VENTILATORY RESPONSE AT ALTITUDE. David Preiss, Dahlia Balaban, Alexandra Mardimae, Marat Slessarev, Alex Vesely, Dick Greene, Joseph Fisher. Boston University, University of British Columbia, University of New Mexico, Thornhill Research. *Email: davidapreiss@gmail.com.* Introduction: The hypoxic ventilatory response (HVR) to altitude increases O_2 saturation of Hb (SaO_2) by two mechanisms: it increases alveolar PO_2 (PAO_2) (mechanism I) and it decreases arterial PCO_2 ($PaCO_2$), thereby shifting the oxyhemoglobin dissociation curve (ODC) leftward (mechanism II). Our aim was to calculate the relative contributions of these two mechanisms to increasing SaO_2 at altitude. Methods: We used the equations of Severinghaus and Kelman to predict the effects of independent ventilation-related changes in PAO_2 and $PaCO_2$ on SaO_2. We assumed a VO_2 and VCO_2 of 280 mL/min. We calculated SaO_2 for a range of minute ventilations (VE) from 5 to 30 L/min and range of ambient pressures (Pb) from 760 to 300 mmHg. We then generated an equation indicating the at each altitude (Pb) at which the contributions of the two mechanisms are equal. We further compared these values of VE at various altitudes with observed data from the literature to determine which mechanism might predominate in the field. Results: At VE = 0.00004*(Pb)-2.8212, the contributions of

increases in PAO_2 and decreases in $PaCO_2$ to SaO_2 are equal. At higher VE, the left shift of the ODC provides the greater increase in SaO_2 and vice versa for lower VE. Interestingly, the reported ventilatory responses to sub-acute acclimatization follow this equation closely. Conclusions: Our results imply that during subacute acclimatization - the HVR at altitude - the mechanisms supporting SaO_2 are divided about equally between increases in PAO_2 and leftward shifts of ODC. 2009.

MELATONIN AS A COUNTERMEASURE TO THE EFFECTS OF HIGH ALTITUDE. Christopher Jung[1], Paul Huske[1], Duncan Talome[2], Christian Dean[3], Diana Redwood[4], Peter Hackett[5], Loren Buck[1], Shea Lowery[1]. [1]Department of Biological Sciences, University of Alaska Anchorage, [2]Universidad Pontificia de Salamanca, [3]Methodist Charlton Medical Center, [4]Department of Psychology, University of Alaska Fairbanks, [5]Institute for Altitude Medicine. *Email: cmjung@uaa.alaska.edu.* Introduction: Hypoxic conditions have been reported to cause disrupted sleep, decrements in cognition and increases in systolic blood pressure (SBP). Melatonin has sedative, antioxidant and vasodilatory properties which have previously been reported to aid in preventing the negative effects of high altitude. However, melatonin has yet to be tested as a countermeasure to the effects of hypoxia. The aim of this study was to determine if melatonin would improve sleep, cognition and SBP at high altitude. Methods: A randomized placebo-controlled, double blind, crossover, within-subjects design was conducted at 4,300m on Mt. McKinley. Thirteen climbers (2 women), aged 34.0±9.7 (mean±SD) were studied on two consecutive nights. A wireless sleep recorder was used to quantify sleep quality, a computerized version of the Stroop task was used to assess cognitive performance and an automatic blood pressure monitor was used to measure SBP. Participants slept in their own tents while cognitive performance and blood pressure assessments were performed in a separate tent to minimize distractions. Drug administration was scheduled 90 min prior to the participants chosen bedtime and a cognitive performance test was scheduled the following day. Results: Compared to placebo, there were significant decreases ($p<0.05$) in wakefulness after sleep onset, sleep onset latency and SBP following melatonin administration. Additionally, cognitive performance (mean reaction time) was improved following the night of melatonin administration. Conclusion: Despite being in extreme conditions, the decrease in wakefulness following melatonin administration might in part play a role in the improved cognitive performance. Performing optimally at high altitude is of major importance for search and rescue and military operations. Additionally, the decrease in SBP might help prevent high altitude related coronary incidences. These results suggest that melatonin, a safe, natural and readily available over-the-counter dietary supplement, appears to act as a countermeasure to some of the effects of high altitude. Acknowledgements: Funded by Foundation and Faculty Development Grants from the University of Alaska Anchorage. Equipment support of Zeo, Inc., and Mountain Hardwear Inc. We would also like to acknowledge Zachary Worthington, Christopher Drake, PhD and Robert Niebert and Frontier Medical Pharmacy for their assistance. 2015.

METALLOTHIONEINS I+II MODULATE MTF-1 NUCLEAR LOCALIZATION DURING DELAYED ISOFLURANE PRECONDITIONING. Scott Edmands[1], Adam Hall[1]. [1]Smith College. *EMAIL: sedmands@smith.edu* INTRODUCTION: Delayed preconditioning is a conserved biological response to oxidative stress in which pre-exposure to sublethal stimuli induces cellular and molecular adaptations that can protect against subsequent ischemic insults. The current study examines the role of metallothionins I+II in modulating nuclear localization of metal-responsive transcription factor-1 (MTF-1) during isoflurane mediated preconditioning. METHODS: MTF-1 Translocation: Primary dissociated cultures of neurons and glia were derived from mouse (P2) cortex. Changes to nuclear MTF-1 levels were assessed by Western blot analysis following exposure to either the nitric oxide donor S-nitroso-N-acetyl-l,l-penicillamine (SNAP), the superoxide generator paraquat, or isoflurane in wild-type (WT) and MT-I + II knockout derived cultures. MTF-1 localization was also assessed following isoflurane-preconditioning in cells treated with AG or the superoxide dismutase mimetic manganese (III) tetrakis (4-benzoic acid) porphyrin chloride (MnTBAP). In parallel with the MTF-1 localization studies, protection studies were performed for each preconditioning regimen using lactate dehydrogenase (LDH) release assays to assess survival following OGD. RESULTS: MTF-1 Translocation: Preconditioning with SNAP, paraquat, and isoflurane produced significant levels of protection against OGD with concomitant nuclear translocation of MTF-1 in WT cultures. Treatment of isoflurane preconditioned cultures with AG or MnTBAP significantly reduced the level of protection and decreased levels of nuclear MTF-1. Knockout of MT-I + II abrogated preconditioning and decreased levels of nuclear MTF-1 levels for all agents tested. CONCLUSION: We conclude that MT-I + II are important for anesthetic mediated preconditioning, via activation of MTF-1and may play roles in determining transcriptional response of other genes involved in anesthetic mediated delayed preconditioning. ACKNOWLEDGEMENTS: This research was supported by a grant from the National Institutes of Health. 2015.

MICROCAPILLARY STRUCTURE OF SHERPAS AND NEPALIS AT HIGH ALTITUDE. Cynthia Beall[1], Buddha Basnyat[2], Maniraj Neupane[2]. [1]Case Western Reserve Univ, [2]Nepal International Clinic. *EMAIL: cmb2@case.edu* INTRODUCTION: Structural features of blood vessels could contribute to offsetting high-altitude hypoxia by increasing diameter and thus blood flow and oxygen delivery or by increasing density and thus shortening oxygen diffusion distance, for example. We hypothesized that Sherpas have wider or more dense microcapillaries than lowland Nepalis residing at the same high (3800m) altitude and that both differ from U.S. controls at low-altitude. METHODS: A portable CapiScope HVCS Handheld Video Capillaroscopy System (KK Technology, Honiton, England) provided non-invasive measurements of the sub-labial microvasculature of 72 Sherpa and 19 lowland Nepalis, resident for six months or longer, and 18 U.S. controls at low altitude. 43 Sherpa, 11 Nepalis, and 17 U.S. men and women each provided three readable 30-second video clips. Tortuous (curved) microcapillaries were assessed as the ratio of length to the straight line between the two ends. We report the average values quantified from four frames of each video. RESULTS: The

results somewhat supported the hypothesis. Average capillary diameter did not differ significantly among the three samples. The U.S. sample had significantly shorter vessels with fewer curves and, consequently, roughly half the tortuosity index of the other two. Lowland Nepalis at high altitude had the longest microcapillaries with the fewest curves; the Sherpa vessels trended toward shorter with fewer curves. The two high-altitude samples had the same amount of tortuosity. CONCLUSION: In summary, these results show that Sherpa and resident Nepalis at 3800m had similar sub-labial microcapillary diameters along with longer and more curved vessels than U.S. low-altitude controls. Hypothetically, this structure shortens diffusion distance to active tissue and improves oxygen delivery. ACKNOWLEDGEMENTS: Funded by NSF award 0924726 to CMB. 2015.

MILD HYPOXIA DURING MANNED SPACEFLIGHT: PAST AND FUTURE. James Wessel[1], Jason Norcross[1], Omar Bekdash[1], Johnny Conkin[2]. [1]Wyle Science, Technology and Engineering Group, Houston, TX, USA, [2]Universities Space Research Association, Houston, TX, USA. *Email: james.wessel-1@nasa.gov.* Introduction: Spacecraft for distant exploration will require a special atmospheric composition that balances flammability, mild hypoxia, and prevention of decompression sickness for crews undergoing extravehicular activities. A proposed atmosphere is 34% oxygen (O_2) and 66% nitrogen at 8.2 psia. This combination renders a mildly hypoxic atmosphere (ppO_2 = 144 mmHg, P_IO_2 = 128 mmHg, P_AO_2 = 88 mmHg), which is nearly equivalent to the shuttle 10.2-staged-prebreathe protocol (ppO_2 = 140 mmHg, P_IO_2 = 127 mmHg, PAO_2 = 87 mmHg). Methods: A retrospective data mining exercise was undertaken to determine when the shuttle cabin depressed to 10.2 psia and repressed to 14.7 psia, as well as ppO_2 and $ppCO_2$ values for each of the shuttle flights that utilized the 10.2-staged-prebreathe protocol. Results: Shuttle crewmembers experienced 124.5 days (744 man-days, adjusted for crew size) under the 10.2 staged condition. Length of exposure averaged about 3.25 days and the longest was just over 8 days. The ppO_2 averaged142.2±3.5 mmHg while depressurized to 10.2, and 163.7±6.4 mmHg at 14.7 psia before and after depressurization. The $ppCO_2$ averaged 1.91 ± 0.6 mmHg while depressurized, and 2.07 ± 0.8 mmHg at 14.7 psia. 26 missions had $ppCO_2$ over 4 mmHg with 7 missions above 7.6 mmHg. 5.51% of all $ppCO_2$ values were above 4 mmHg, and 0.063% were above 7.6 mmHg. The maximum recorded $ppCO_2$ was 11.02 mmHg. Conclusion: Terrestrial experience indicates that chronic exposure to a PIO_2 of 128 mmHg is well-tolerated by healthy humans. It is unclear how chronic mild hypoxia interacts with physiologic changes due to microgravity, as it has never been formally evaluated. Forward work will include examination of shuttle sleep logs and flight medical records for incidence of symptoms associated with hypoxia during 10.2-staged missions to be compared with non-depressurized missions and future ground-based studies of the exploration atmosphere. 2015.

NATURAL SELECTION OF GREATER BIRTH WEIGHT AT HIGH ALTITUDE. Megan J Wilson, Abigail Bigham, Mark Shriver, Enrique Vargas, Miriam Lopez, Colleen G Julian, Lorna G Moore. Altitude Research Center, University of Colorado Denver, Pennsylvania State University, Instituto Boliviano

de Biologia de Altura, Wake Forest University. *Email: megan.wilson@uchsc.edu.* Fetal growth is slowed at high altitude (HA) and preeclampsia more common. Populations of multi-generational residence at HA show a decrease in fetal growth at high altitude, but the decrease is less than that seen in relative newcomers to HA. There is debate as to whether decreased birth weight at HA has been selected for or against. Previously, direct tests of the genetic adaptations in pregnancy to HA have been unavailable. We explored the relationship between genes that show evidence of natural selection and their effect on phenotype by 1) identifying hypoxia-related gene regions that showed evidence of natural selection through the analysis of genome-wide SNP microarray data, and 2) searching for association between genotypes at candidate loci, circulating gene product (protein) levels, uterine artery (UA) blood flow and other pregnancy phenotypes at HA. In 50 multi-generational HA Andeans compared to low-altitude control populations (Amerindians and Han Chinese), five hypoxia-related gene regions showed differential expression of alleles in Andeans. In 55 multi-generational HA pregnant Andeans, CDH1 gene region SNP genotypes were associated with UA blood flow. Soluble CDH (sCDH1) correlated with CDH1 genotype and also with UA blood flow. Additionally, AMPKa1 genotype was strongly associated with gestational age at birth and ARNT2 was associated with birth weight. In all cases, alleles more frequently found in Andeans were positively associated with UA blood flow, gestational age at birth, or birth weight, suggesting evolution at HA may have selected for increased birth weight, as opposed to "small-but-healthy" babies, in adapted populations. 2009.

NEAR-INFRARED SPECTROSCOPY AND A VASCULAR OCCLUSION TEST TO STUDY MICROVASCULAR REACTIVITY AT HIGH ALTITUDE. Michael PW Grocott[1], Denny ZH Levett[1], Rick Bezemer[2], Hugh E Montgomery[1], Daniel S Martin[1]. [1]UCL Centre for Altitude, Space and Extreme Environment Medicine (CASE Medicine), UCL Institute of Child Health, London, UK, [2]Department of Translational Physiology, Academic Medical Center, University of Amsterdam, The Netherlands. *Email: mikegrocott@gmail.com*. Introduction: Hypoxaemia at high altitude results in adaptations in the systemic and peripheral circulations. Using near-infrared spectroscopy (NIRS), skeletal muscle oxygenation (StO_2) can be measured during a vascular occlusion test (VOT) to assess microvascular reactivity. The VOT comprises three minutes of non-invasive arterial occlusion with a blood pressure cuff (ischaemic period), followed by cuff release, which induces a period of recovery and hyperaemia. The rate of StO_2 decline during ischaemia (down slope) represents tissue oxygen consumption (VtO_2) whilst the rise during recovery (up slope) indicates microcirculatory function. We hypothesised that at altitude, changes in skeletal muscle metabolism and microcirculatory blood flow would lead to a reduction in NIRS-VOT down slope and up slope. Methods: Thenar eminence StO_2 was measured in 12 subjects during a NIRS-VOT at sea level, 4900m and 5600m (after 7 and 17 days at altitude respectively). Results: Resting StO2 was reduced at 4900m and 5600m (69.3 (±8.2) % ($p=0.001$) and 64.2 (±6.1) % ($p<0.001$) respectively) when compared to sea level (84.4 (±6.0) %). The rate of StO_2 reduction during ischaemia (down slope) was not different between altitudes. The rate of StO_2 recovery after ischaemia (up slope)

was significantly reduced at 4900m (2.4 (±0.4) %/sec) and 5600m (2.4 (±0.8) %/sec) compared to sea level (3.7 (±1.3) %/sec) (p=0.021 and p=0.032 respectively). Conclusion: The findings suggest that resting skeletal muscle VtO_2 remains unchanged at altitude but microvascular reactivity may be altered. Acknowledgements: The Caudwell Xtreme Everest Research Group. Mr. John Caudwell, BOC Medical (now part of Linde Gas Therapeutics), Eli Lilly, the London Clinic, Smiths Medical, Deltex Medical, and the Rolex Foundation (unrestricted grants), the Association of Anaesthetists of Great Britain and Ireland, the United Kingdom Intensive Care Foundation, and the Sir Halley Stewart Trust. Some of this work was undertaken at University College London Hospital–University College London Comprehensive Biomedical Research Centre, which received a proportion of funding from the United Kingdom Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme. 2011.

NEONATAL HYPOXIA IMPAIRS VENTILATORY ACCLIMATIZATION TO CHRONIC HYPOXIA IN ADULT RATS. Delphine Lumbroso, Vincent Joseph. Laval University Hopital Saint François d'Assise, Laval University, Hopital Saint François d'Assise, *Email: delphine.lumbroso.1@ulaval.ca.* INTRODUCTION: Chronic Mountain Sickness (CMS) is characterized by a severe arterial hypoxemia, excessive erythrocytosis and arterial pulmonary hypertension. However, the origins of this syndrome are poorly known. Our general hypothesis is that CMS has a neonatal origin. The goal of our study is to show that neonatal exposure to hypoxia impairs acclimatization to chronic hypoxia in adult rats. METHODS: Newborn rats were raised in hypoxia (12% O_2 - nHx rats) for 10 days following birth. Controls were raised in normoxia. Acclimatization to chronic hypoxia is evaluated in adult (2-3 month-old) nHx and control rats exposed for 14 days to chronic hypoxia ($10\%O_2$). Minute ventilation is measured using whole body plethysmography, hemoglobin [Hb] and hematocrit (Hct) levels and right ventricle hypertrophy (as a sign of pulmonary hypertension) are evaluated before and after acclimatization. RESULTS: In control adults, minute ventilation and respiratory frequency increased by 78% and 79% during acclimatization, while this response was blunted in nHx rats (+45% and +54%). Both [Hb] and Hct were higher in nHx compared to control groups ([Hb] = 23.8 ± 0.1 vs 22.5 ± 0.3 g/dL $p<0.01$; Hct = 74.2 ± 1.0 vs 70,6 ± 0.9 % p=0.03). Right-to-left ventricle weight ratio was higher in nHx vs. control (0.40 ± 0.08 vs 0.28 ± 0.01 $p<0.01$) indicating greater pulmonary hypertension. These effects of neonatal hypoxia are present also in females. CONCLUSION: These results demonstrate that neonatal hypoxia induces a blunted hypoxic ventilatory acclimatization, an excessive erythrocytosis and a pulmonary arterial hypertension. This study shows that neonatal hypoxia impairs acclimatization to hypoxia in adults, suggesting that CMS has neonatal origins. This study is supported by NSERC funding. 2009.

NEUROGLOBIN (NGB) MRNA UPREGULATION BY HYPOXIA IS MEDIATED BY HIF-1A VIA ITS DIRECT BINDING TO 3' HRE OF NGB. Jeanne M. Pistillo[1], Bee Chun Sun[1], Graham Molineux[1], Liqin Liu[1]. [1]Hematology/ Oncology, Amgen Inc. Thousand Oaks, CA 91390. *Email: jeannep@amgen.com.*

Introduction: Ngb is a monomeric globin that is expressed in vertebrate brain and other neuronal tissues and can reversibly bind O_2. Ngb has been shown to possess neuroprotective activity in models of hypoxia in vitro and ischemia in vivo. It has been reported that the level of Ngb mRNA is increased in response to hypoxia; however the mechanism involved is unclear. Methods: Using an siRNA knockdown strategy, we were able to demonstrate that HIF-1a, but not HIF-2a, is responsible for upregulating Ngb mRNA expression. To elucidate whether HIF-1a directly regulates Ngb mRNA expression, we searched the gene for hypoxia response element (HRE) consensus sequences and we identified HRE in both 3' and 5' UTR of Ngb. Electrophoretic Mobility Shift Assays (EMSA) and Chromatin Immunoprecitation (CHIP)-PCR assays confirmed that HIF-1a binds directly to both HRE. Further transient transfection study identified a minimal enhancer region for HIF-1a, which is located to the 3' UTR of Ngb. Results: HIF-1a possesses two transcriptional activation domains (N-TAD, C-TAD), whose respective transcriptional activity is modulated by HIF-Prolyl Hydroxlases (PHD) and Factor Inhibiting HIF (FIH). N-TAD and C-TAD differentially regulate HIF-dependent genes and allow discrimination by FIH. Thus two groups of hypoxia-governed genes have been proposed based on whether additional inactivation of FIH is required: the "FIH-inhibited" and the "non-FIH-inhibited" groups. Here we demonstrate that HIF-1a-dependent Ngb upregulation requires inactivation of both PHD and FIH, indicating that Ngb belongs to FIH-inhibited gene group. Conclusion: In summary, our studies provide the first evidence that Ngb mRNA upregulation by hypoxia is mediated by HIF-1a via its direct binding to the 3' HRE of Ngb. Furthermore, we show that both FIH and PHD activities need to be neutralized in order to induce HIF-1a-mediated Ngb expression. 2011.

NITRIC OXIDE CONTRIBUTES TO MICROVASCULAR ACCLIMATIZATION OF PROLONGED HYPOXIA. Norberto Gonzalez. Univ Kansas Medical Center. *EMAIL: ngonzale@kumc.edu* INTRODUCTION: The widespread systemic inflammation induced by alveolar hypoxia in rats and mice is not initiated by the low systemic PO_2 but by the release of Monocyte Chemoattractant Protein-1 (MCP-1) from hypoxia-activated alveolar macrophages. Circulating MCP-1 degranulates perivascular mast cells (MC), which release renin and generate Angiotensin II (AngII) to mediate the inflammation. Prolonged (>3 days) hypoxia leads to microvascular acclimatization: the inflammation resolves; more severe hypoxia or inflammatory agents produce no inflammation. These experiments tested the hypothesis that increased NO levels participate in the microvascular acclimatization. RESULTS: Mesentery intravital microscopy in non-acclimatized rats (NA) breathing 10%O_2 for 1 h showed the inflammatory markers typical of acute hypoxia: MC degranulation (MCD), leukocyte recruitment in post-capillary venules, and increased microvascular permeability. None of these markers were observed in rats acclimatized to PB 380 Torr for 5 days (5dHx). Topical AngII or MCP-1 produced mesenteric inflammation in NA, but not in 5dHx. However, either non-specific NO synthase (NOs) inhibition (L-NMMA), or selective inhibition of inducible NOs (iNOs) with 1400 W, produced inflammation in 5dHx in response to MCP-1 and AngII. MCP-1

produced degranulation in primary cultures of peritoneal MC from NA; MCD was prevented by the NO donor spermine NOnoate. MCP-1 did not produce degranulation in 5dHx MC, but induced MCD in the presence of L-NMMA or 1400 W. Western blots showed that iNOs is expressed in 5dHx MC, but not in Nx MC. CONCLUSION: Increased NO levels, mediated at least in part by expression of iNOs in MC contribute to the microvascular acclimatization of prolonged hypoxia and may play a role in the overall phenomenon of acclimatization. ACKNOWLEDGEMENTS: Supported by NIH HL 39443. 2015.

NO CHANGE TO PROTAN OR TRITAN COLOR VISION UPON EXPOSURE TO 3,500M. Daniel S Morris, Andrew J Davies, Nicholas S Kalson, Adam Booth, Chris Imray, Chris Hogg. Eye Care Center Vancouver General Hospital, Salford Royal Hospital, University of Manchester, Birmingham Medical Research Expedition Society, Moorfields Eye Hospital. *Email: dsm@doctors.org.uk.* Several studies have shown a deterioration in color vision (CV), in particular the tritan axis (TA), but also in the protan axis (PA), at altitudes above 4,000m. The ChromaTest software programme analyzes colour contrast thresholds (CCT) for both TA and PA, and has been validated for use in screening for diabetic retinopathy, where TA deterioration is an early indicator. The objective of this study was to contribute to the body of existing data, and attempt to find a threshold at which CV changes occur. We examined 42 eyes with ChromaTest (21 subjects, mean age 44, range 22-71), at sea level and within 12-36 hours of exposure to 3500m. This was done in a darkened room, with refractive error correction. Non-parametric paired data was examined using the Wilcoxon signed rank test. There was found to be no change to either the PA (p=0.409) or the TA (p=0.871) upon ascent. Within the PA 16 eyes had a lower CCT at high altitude, whilst 26 were higher. In the TA 20 eyes had a lower CCT and 22 were higher. At sea level, mean CCT for PA was 4.21 (SD 2.29) TA was 7.06 (SD 1.77). At 3,500m mean CCT for PA was 4.36 (SD 2.86) and TA was 6.93 (SD 2.39). This experiment revealed no changes to colour vision with exposure to high altitude. This may be because the hypoxia experienced at 3,500m is below the threshold that affects retinal cone dysfunction. Alternatively there may have been dysfunction which had resolved by the time of measurement. 2009.

NO EFFECT OF SAME DAY SUCCESSIVE EUCAPNIC HYPOXIA TESTS ON BREATHING PATTERN IN HUMANS. Matthew D White[1], Luisa V Giles[2], Michael S Koehle[2], Patrick LL McDonald[1], Kate A Henderson[1], [1]Simon Fraser Univ, [2]Univ British Columbia. *EMAIL: matt@sfu.ca* INTRODUCTION: Our preliminary results indicated there is a progressive increase in the frequency of breathing (FB) across repeated hypoxic ventilatory responses (HVR) tests on a single day. It was hypothesized that mean inspiratory flow (V_T/T_I) would increase and the inspiratory duty cycle (T_I/T_{TOT}) would decrease with repeated HVR tests on a single day. METHODS: Twelve individuals (height: 1.75±0.12 m, weight 74.5±14.0 kg; mean±SD) volunteered for the study that was approved by the SFU Office of Research Ethics. On a single day 3 successive 20 min eucapnic HVR tests were given with each test separated by a 120 min. Using an end tidal forcing system

hypoxia was administered in a single step to an end tidal O_2 of 45 mm Hg with end tidal CO_2 maintained at a eucapnic partial pressure. Analysis was with a repeated measure ANOVA with factors of Time (Rest, minutes 5, 10, 15, 20 and Peak) and Trials (1, 2 and 3) with the significance level set at 0.05. RESULTS: Results indicated a trend for an effect of Trial on FB (F=3.25, p=0.06) that increased from similar rates of ~17.3±3.8 breaths/min in Trials 1 and 2 to 19.4±5.3 breaths/min in Trial 3. This was explained by a progressive decrease in T_I (F=4.01, p=0.03) from 1.7±0.5 s in Trial 1 to 1.5±0.4 s in Trial 3. Likewise, expiratory time (T_E) progressively decreased from 2.7±1.0 s in Trial 1 to 2.4±1.0 s in Trial 3 (F=5.98, p=0.01). In contrast, both the V_T/T_I of ~0.84±0.4 L/s and the T_I/T_{TOT} of ~0.37±0.9 (unitless) were similar across the 3 trials. CONCLUSION: In conclusion, mean inspiratory flow and inspiratory duty cycle were not influenced by 3 successive eucapnic hypoxic ventilatory response tests on a single day. ACKNOWLEDGEMENTS: Supported by the Natural Sciences and Engineering Research Council of Canada and the Canadian Foundation for Innovation. 2015.

NONINVASIVE QUANTIFICATION OF HYPOXIA INDUCED ANGIOGENESIS USING MRI AND NEAR INFRARED SPECTROSCOPY. Jeff F Dunn[1], Runze Yang[1]. [1]Hotchkiss Brain Institute, Faculty of Medicine, Univ Calgary. *EMAIL: dunnj@ucalgary.ca* INTRODUCTION: The response to chronic hypoxia in the brain involves improved substrate delivery by increasing capillary density through angiogenesis. The gold standard for assessing angiogenesis is to quantify capillary density using morphometrics on histological samples, limiting research to animal models. Using such animal models, we have developed MRI and near-infrared (NIRS) based methods for quantifying angiogenesis that could be applied to humans. The historical method for quantifying broad-band NIRS is not appropriate for human use, since the calibration involves breathing anoxic gas to convert the hemoglobin to deoxyhemoglobin. We also present a new calibration method that can be used to quantify broadband data. METHODS: Rats were exposed to chronic hypoxia, and blood volume as a marker of angiogenesis was measured before and after 2-3 weeks of hypoxia acclimation. With MRI, we applied steady-state relaxation measurements in combination with infusion of a super paramagnetic plasma based contrast agent. This method is based on quantifying the relaxation parameter T2, before and after infusion of the agent. We used T2 instead of the more sensitive T2* since changes in T2 are more weighted to the microvasculature. This makes the method more sensitive to angiogenesis. With near-infrared spectroscopy (NIRS), we used a custom built broadband system to quantify the changes in total hemoglobin. By correcting for changes in blood hemoglobin, cerebral blood volume can be calculated. RESULTS: Angiogenesis could be detected in the brain of individual rats with increases ranging from 30% to 140%. CONCLUSION: This opens the door for using MRI or broadband NIRS, as well as other quantified NIRS methods, for use in studying angiogenesis in human brain. We showed that, using these methods, one can detect angiogenesis in the mammalian brain. This work paves the way to quantify angiogenesis in human brain. ACKNOWLEDGEMENTS: Funding by NIH, AIHS, NSERC. 2015.

NORMOBARIC HYPOXIC EXPOSURE DURING SLEEP DOES NOT REDUCE ACUTE MOUNTAIN SICKNESS DURING SUBSEQUENT EXPOSURE TO 4300 M ALTITUDE. Allen Cymerman, Charles S Fulco, Stephen R Muza, Beth A Beidleman, Rob Demes, Janet Staab, Leonard Elliott, Paul Rock. USARIEM. *Email: allen.cymerman@us.army.mil.* Does sleeping in normobaric hypoxia (NH) induce sufficient acclimatization to reduce Acute Mountain Sickness (AMS) symptoms caused by subsequent altitude exposure (HA, 4300m)? Two groups of sea-level residents with similar peak oxygen uptakes (~47 ml/kg/min), ages (~24 yr), body weights (~75 kg), resting end-tidal partial pressures of CO_2 ($PetCO_2$, ~39 mmHg), and resting arterial oxygen saturations (SaO_2) slept for ~6.5 hrs/night for 7 consecutive nights in portable rooms under singly-blinded NH (n=14) or SHAM (n=8) conditions. Ambient % O_2 for the NH group was progressively reduced by ~0.30% O_2 each night from ~16.2% O_2 (2200 m equivalent) on the 1st night to ~14.4% O_2 (3050 m) on the 7th night. The SHAM group remained at ›20.5% O_2 (150 m). Within 25 h of treatment end, all were exposed to HA (Pikes Peak, CO; 4300 m; 101 h). SaO_2 and $PetCO_2$ were measured at sea level before, within 1 h after the 7th treatment, and ‹ 2 h at HA. AMS was measured using the cerebral score of the Environmental Symptoms Questionnaire (›0.7 indicative of cerebral AMS). Over 7 nights during sleep, NH SaO_2 decreased ($p<0.05$) from 92±2 to 88±3% (mean±SD), but SHAM SaO_2 remained at 96±2%. Before and after treatment at SL, resting $PetCO_2$ decreased ($p<0.001$) for the NH group (39.1±3.0 to 34.9±2.6 mmHg) but not for the SHAM group (39.3±1.9 vs 38.3±3.2 mmHg). Within the first 2 hrs of HA, there were no differences between NH and SHAM groups in resting $PetCO_2$ (33.5±2.4 vs 33.3±2.8 mmHg), or SaO_2 (83±7 vs 83±3%). AMS symptom severity (1.47±1.40, SHAM vs 1.05±1.10, NH) peaked after 10 and13 h, respectively and AMS incidence (86% SHAM vs 64% NH peaked after 7 h; $p=0.61$). There were no between-group significant differences in either parameter. These results indicate that sleeping 7 nights in NH did not induce sufficient acclimatization to beneficially effect the incidence or severity of AMS. Funding: US Army MRMC ATO IV. MD.2006.01. Authors' views; not official U.S. Army or DoD policy. 2009.

NOVEL MECHANISMS FOR ANOXIA SURVIVAL IN CAENORHABDITIS ELEGANS. John LaMacchia[1], Mark Roth[1]. [1]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA. *EMAIL: jlamacch@fhcrc.org* INTRODUCTION: The ability of some animals to tolerate oxygen deprivation for extended periods of time is well recognized, yet our understanding of the molecular mechanisms for such tolerance remains incomplete. One proposed mechanism for the tolerance of anoxia (operationally defined as <0.001 $kPaO_2$) is the "channel arrest hypothesis," which states that cells can maintain energy homeostasis during oxygen-limiting conditions by suppressing transmembrane ion leak and thereby reducing demand for Na-K-ATPase activity. Although experimental evidence for channel arrest exists in the anoxia-tolerant turtle brain, the extent to which it is relevant in other organisms is unknown. METHODS: The soil-dwelling nematode Caenorhabditis elegans is capable of surviving anoxia for more than 24 h in culture. To better understand the molecular mechanisms of anoxia survival in C. ele-

gans, we have developed a sensitized anoxia lethality assay that incorporates a hyposmotic culture environment as a secondary stress. RESULTS: In the hyposmotic anoxia, wild type (N2 strain) survival after 24 h is reduced nearly 20-fold and animals display an "exploding" phenotype suggestive of cell swelling. Using forward and reverse genetic techniques, we have identified genes whose activity either enhances or suppresses survival in hyposmotic anoxia. CONCLUSION: It has been demonstrated previously that the Na-K-ATPase is activated not only by changes in ion concentrations but also by increases in cell volume. The functions of several genes we have identified in the hyposmotic anoxia lethality assay strongly suggest a role for volume-dependent, as opposed to ion-dependent, suppression of ATPase activity. Further studies will attempt to more clearly define this apparent variation on a channel arrest theme. ACKNOWLEDGEMENTS: This work is funded by DARPA and the ARCS Foundation (Seattle Chapter). 2015.

ON THE LIMIT AND FEELING THE PRESSURE AT HIGH-ALTITUDE: INTRACRANIAL HYPERTENSION AND ACUTE MOUNTAIN SICKNESS. Justin Lawley[1], Paul Mullins[2], Samuel Oliver[1], Alperin Noam[3], Lee Sang[3], Jamie Macdonald[1]. [1]Extremes Research Group, Bangor Univ, Bangor, Gwynedd, LL57 2AS, United Kingdom, [2]Bangor Imaging Center, Bangor Univ, Bangor, Gwynedd, LL57 2AS, United Kingdom, [3]Dept Radiology, Univ Miami, Miami, Florida, 33136, United States. *EMAIL: JustinLawley@texashealth.org* INTRODUCTION: Objective. Acute mountain sickness is thought to be the result of a predisposition to intracranial hypertension. This hypothesis was tested by measuring regional intracranial volumes, cerebral hemodynamics and pressure during exposure to high-altitude METHODS: Thirteen males (mean (SD): 26 (6) years) were exposed to high-altitude (12%O_2) and normoxia (21%O_2) in a cross over design. After 2 and 10 hrs of exposure, grey matter, white matter and cerebral spinal fluid volumes, arterial and venous blood flow, and intracranial pressure were determined using complementary imaging techniques. RESULTS: Compared to normoxia, brain volume was increased throughout high-altitude (2 hrs, Δ7 ml, 95%CI -15 to 30; 10 hrs, Δ59 ml, 95%CI -2 to 120), principally due to an increase in grey matter volume. However, despite high-altitude resulting in an initial increase in cerebral blood flow at 2 hrs this was not sustained at 10 hrs, suggesting a role for the cerebral venous system in elevating intracranial blood (and consequently grey matter) volume. Nevertheless there was no change in mean intracranial pressure (2 hrs, Δ2 mmHg, 95%CI -2 to 6; 10 hrs, Δ2 mmHg, 95%CI -2 to 6). However, the individuals with the highest intracranial pressures were the most ill. Consequently, there was a significant relationship between the change in intracranial pressure and acute mountain sickness severity (R2=0.71, P=0.02). Furthermore, when the group was dichotomized, in AMS+ intracranial pressure tended (p=0.09) to increase (Δ4.6, 95%CI -2.03 to +11.39) mmHg and in AMS- it tended to decrease (Δ1.77, 95%CI -5.20 to +1.65 mmHg). CONCLUSION: Observations of altered cerebral blood flow dynamics and an increased brain volume that leads to elevated intracranial pressure in the most ill individuals are consistent with the intracranial hypertension

hypothesis of acute mountain sickness. ACKNOWLEDGEMENTS: This study was funded by an unrestricted Bangor Univ 125 Anniversary Scholarship. 2015.

OPTIC NERVE SHEATH DIAMETER, HYPOBARIC HYPOXIA AND ACUTE MOUNTAIN SICKNESS: A PROSPECTIVE COHORT STUDY. Giacomo Strapazzon[1], Piergiorgio Lochner[2], Tomas Dal Cappello[1], Michael Pohl[1], Emily Procter[1], Hermann Brugger[1]. [1]EURAC Institute Mountain Emergency Medicine, Bolzano, Italy, [2]Dept Neurology, General Hospital of Merano, Merano, Italy. *EMAIL: giacomo.strapazzon@eurac.edu* INTRODUCTION: Visitors to high altitude undergo a variety of acute and long term physiologic changes. Reproducible data on high-altitude adaptations are often lacking due to different patterns of acclimatization between studies and other confounding factors. The aim of this study was to investigate the effects of hypobaric hypoxia on optic nerve sheath diameter (ONSD) in a group of 21 healthy lowlanders by B-scan ultrasound. METHODS: Study variables, including ONSD, oxygen saturation (SpO_2) and Lake Louise scoring system (AMS score), were assessed longitudinally at sea level (SL) before and during acute and subacute exposure (3, 9, 24, 48, 72 hours and 1 week) to hypobaric hypoxia at an altitude of 3,830 m (12,566 ft) without physical activity (HA) in the Eastern Alps. Ultrasound exams were performed and read by separate blinded observers. AMS score equal or above 3 in presence of headache defined acute mountain sickness. RESULTS: Mean ONSD was 5.42 mm (95% confidence interval (CI) 5.26-5.75 mm) at baseline and increased significantly to 6.10 mm (95% CI 5.86-6.34 mm) after 3 hours of HA exposure ($p<.0001$). ONSD showed a parabolic pattern within the first 72 hours of HA exposure (partial eta squared=.84, $p<.0001$), followed by a trend to a further increase after 1 week of HA exposure. ONSD was significantly correlated with SpO_2 (coefficient=-.49, $p<.001$). Nevertheless, ONSD increased similarity in AMS+ and AMS- subjects (p=ns), whereas SpO_2 increased differently in AMS+ and AMS- subjects ($p<.05$). CONCLUSION: Measurement of ONSD has a strong association with exposure to hypobaric hypoxia in relation to time and early acclimatization, but it is not associated with the development of acute mountain sickness symptoms in the absence of physical activity in the current prospective study at a constant altitude. Further data will be required to assess ONSD in relation to long-term acclimatization. 2015.

ORTHOSTATIC RESPONSES TO HIGH ALTITUDE EXPOSURE IN SEA LEVEL AND HIGH ALTITUDE RESIDENTS. John Davis[1], Jessica Thorington[1], Cory Schall[1], Dale Wagner[2]. [1]Alma College, Alma, MI, [2]Utah State University, Logan, UT. *Email: davisj@alma.edu*. Introduction: This study evaluated the influence of altitude of residence on orthostatic responses when exposed to very high altitude. Methods: Sixty-nine subjects participated in the study at the Carroll Hut refugio at Mount Chimborazo in Ecuador (15,900 ft). This is an optimal location because many Ecuadorians and tourists who reside at a variety of altitudes can easily drive to the refugio. Cardiovascular measurements included heart rate, systolic and diastolic blood pressure, and oxygen saturation. These measurements were done during a stand test that consisted of sitting for three minutes, lying supine for

three minutes, and then standing for three minutes. All measurements were done upon arrival at the refugio. Subjects were then divided into four groups based on their altitude of residence: Low altitude residents - LOW (0-5,000 ft), moderate altitude residents - MOD (5,000- 10,000 ft), high altitude residents - HIGH (>10,000 ft), and Non-Ecuadorians that resided at Sea Level - NE-SEA. Results: Average supine heart rates were significantly lower in HIGH (72.7bpm) than the MOD (87.2±14.1 bpm), LOW (80.6±22.3), and NEA-SEA(82.4±13.9) altitude groups ($P<0.05$). Upon standing, average heart rate was significantly lower in HIGH (73.3±22.0 bpm) than MOD (96.7±17.2), LOW (92.5±22.7), and NE-SEA(94.0±15.0). The highest altitude residents had supine and standing systolic blood pressure measurements (104.2±6.3 mm Hg and 102.6±8.5 mm Hg, respectively) that were significantly lower ($P<0.05$) when compared to those of the lowest altitude residents (131.8±14.0 mm Hg and 129.0±22.6, respectively). However, there was no statistical difference in oxygen saturation between the groups. Conclusion: Height of altitude residence plays an important role in orthostatic tolerance of individuals when acutely exposed high altitude. These differences demonstrate favorable adaptations to long-term high altitude residence. 2011.

OXYGEN SATURATION AT HIGH ALTITUDE IS HIGHER IN THE SEATED VS. THE SUPINE POSITION. Sophia Larson[1], Chandra Patel[1], Paresh Giri[1], James Anholm[2], Gary Foster[2]. [1]Loma Linda Univ School of Medicine, [2]VA Loma Linda HCS, Loma Linda Univ School of Medicine. *EMAIL: srlarson@llu.edu* INTRODUCTION: Oxygen saturation progressively falls with ascent to altitude, although the magnitude of desaturation is highly variable between individuals. The effect of positional changes on arterial oxygenation in hypoxic environments is unclear. Individuals with cardiac or pulmonary disease sometimes demonstrate profound positional changes in oxygen saturation, but it is unknown if healthy individuals without altitude illness also exhibit these changes following rapid ascent to altitude. Anecdotal observations at high altitude suggested decreased O_2 saturation in supine subjects, thus we analyzed pulse oximetry saturation (SpO_2) data collected during two recent studies from our laboratory. The purpose of this study was to determine whether SpO_2 was different in the upright seated position compared with the supine position under hypoxic conditions. METHODS: Oxygen saturation was recorded with a transcutaneous ear or finger electrode system in healthy, well-trained subjects following a rapid ascent over approximately 6 hours to 3800 m or 4344 m altitude. Subjects were tested within a few hours of arrival at altitude. Data was analyzed from 10 subjects aged 36 ± 11 years (mean ± SD). Saturation data in each position was continuously recorded and averaged over several minutes. Subjects were blinded to the saturation values during the periods of measurement. RESULTS: SpO_2 was 88 ± 7% upright and 77 ± 13% in the supine position, $p = 0.02$. None of the subjects had clinically significant pulmonary edema. CONCLUSION: The mechanisms for these positional changes in SpO_2 are unclear but deserve further study. When reporting pulse oximetry saturation data at altitude, body position should be reported. These findings may also have broader implications in other clinical situations associated with hypoxia. 2015.

OXYGEN SATURATION IN HELICOPTER AIR CREW DURING HYPOXIA TRAINING AT 16,000 FT. Trond-Eirik Strand[1], Jan Ove Owe[1]. [1]Institute of Aviation Medicine, Norwegian Armed Forces Medical. *Email: strtro@yahoo.no*. Introduction: RNoAF helicopter air crew occasionally fly above 10,000 ft, and therefore receive altitude chamber training at the Norwegian Armed Forces Institute of Aviation Medicine. We wanted to establish baseline values for the reduction in oxygen saturation (SpO_2) over a 10 min period after acute exposure to 16,000 ft without supplemental O_2. Reference data of SpO_2 under these conditions are limited in the literature. Methods: The chamber training profile consists of a 2 min climb from near sea level to 8,000 ft for a one min stop, followed by a 45 sec climb to 16,000 ft where the chamber is leveled off. The students breathe air pre-flight, during climb and for the first 8-10 min at 16,000 ft, followed by supplemental O_2 (continuous flow). Pulseoximetry is obtained by finger probes connected to pulse oximeters outside the chamber and data logging in a medical monitoring database. Results: A total of 131 students (whereof 36 medical personnel) were available for analysis, 103 had a 10 min hypoxia exposure at 16,000 ft, while the rest had 8 min. Mean age was 40 years (range 25-68), 95% were males. Mean SpO_2 at sea level was 97.7% (range 100-87%), after rapid climb to 16,000 ft the saturation decreased with time and leveled off after approximately 5 minutes. At 1, 5 and 10 minutes mean SpO_2 were 90.6%, 81.7% and 79.5%, respectively. A considerable individual variation was observed and at 10 minutes the 90 percentile was at 85.5% and the 10 percentile at 74.3%. Conclusion: These results could serve as reference data for SpO_2 after acute exposure to 16,000 ft without supplemental oxygen and demonstrate that SpO_2 is falling through the first 5-8 minutes. Even persons with the most favorable response to hyopxia demonstrate critically low levels after 8-9 minutes. 2011.

PAN-OMIC EVALUATION OF SKELETAL MUSCLE MITOCHONDRIAL ADAPTATIONS TO HIGH ALTITUDE HYPOXIA: INSIGHTS FROM ALTITUDEOMICS. A Chicco[1], C Le[1], E Gnaiger[2], H Dreyer[3], A Hocker[3], J Prenni[1], A D'alessandro[4], T Nemkov[5], AT Lovering[3], AW Subudhi[4], RC Roach[4]. [1]Colorado State University, Fort Collins, CO USA, [2]Medical University of Innsbruck, Austria, [3]University of Oregon, Eugene, OR, USA, [4]University of Colorado Denver, CO, USA, [5]University of Colorado Denver, CO, USA. *Email: Adam.Chicco@colostate.edu.* Introduction: High-altitude acclimatization is associated with improvements in aerobic exercise capacity in hypoxic conditions, despite evidence for reductions in muscle mitochondrial content. We sought to elucidate the mechanisms of skeletal muscle metabolic adaptation to high-altitude hypoxia by combining metabolomic and proteomic profiling with comprehensive evaluation of mitochondrial respiratory function, integrated with systemic physiological assessments obtained during the 2012 AltitudeOmics expedition. Methods: Vastus lateralis biopsies were obtained from 14 subjects (7M, 7F; 21 ± 2 years of age) in Eugene, Oregon (131m) and after 16 days atop Mt. Chacaltaya in the Bolivian Andes (5260m). Muscle fiber bundles were saponin-permeabilized for high resolution respirometry or snap-frozen for subsequent pan-omic analyses by LC/MS. Results: Respirometry studies

revealed significant elevations in the capacity and control of oxidative phosphorylation (OXPHOS) after high-altitude exposure, particularly using fatty acid (palmitoylcarnitine) versus carbohydrate (pyruvate) as substrates, with no significant change in uncoupled or total respiratory capacity. Muscle proteomics revealed a complex remodeling of metabolic enzyme expression, with significant induction or suppression of enzymes involved in glucose and lipid metabolism and mitochondrial OXPHOS. Notable changes included increases in long-chain fatty acid Co-A dehydrogenase (ACAD), with reductions in short/medium-chain ACAD, pyruvate dehydrogenase (E1), select TCA enzymes, and some OXPHOS complex subunits. Muscle metabolomics revealed marked elevations in free fatty acids, select changes in lactate and glycolytic intermediates, and elevated free amino acids, which were consistent with serum metabolomics, a persistent reduction in resting RER, and a loss of lean body mass in these subjects. Conclusions: Taken together, our studies suggest a remodeling of muscle metabolism during high-altitude acclimatization that enhances OXPHOS efficiency and fatty acid oxidation capacity despite reductions in mitochondrial protein expression. This likely compliments increases in glycolytic flux and protein catabolism to maximize the economy of muscle energy metabolism in high-altitude hypoxia. Acknowledgements: DMRDP Award W81XWH-11-2-0040. 2015.

PARADOXICAL INCREASE IN CEREBRAL O2 METABOLISM DURING SUSTAINED HIGH ALTITUDE HYPOXIA. Zachary Smith[1], John Hunt Jr.[1], Billy Hsu[1], Ethan Li[1], Miriam Scadeng[1], David Dubowitz[1]. [1]University of California San Diego, USA. *Email: zmsmith@ucsd.edu*. Introduction: A well-documented response in hypoxia-tolerant animals to hypoxic environments is a reduction of cerebral oxygen metabolism ($CMRO_2$), termed oxygen conformance. We investigated if a similar protective mechanism is present in the human brain during acclimatization to sustained high altitude hypoxia, and if this differs for subjects who suffer AMS compared with those who do not. Methods: 25 healthy subjects were recruited (F=12, M=13). 6 developed AMS (Lake Louise Scores (LLS) >=8). 7 were resistant to AMS (LLS <=4). Subjects with intermediate LLS were not included. We made pulse oximeter measurements of SaO_2, and MRI measurements of cerebral venous O_2 saturation, and cerebral blood flow (CBF). From these we calculated cerebral O_2 extraction fraction (OEF) and $CMRO_2$. Measurements were repeated during normoxia, and following 2- and 7-days sustained hypoxia at White Mountain Research Station (3,800m altitude). Data were analyzed by ANOVA (significant at $p < 0.05$) Results: During normoxia (n=15), CBF was 47 +/- 12 ml/100ml/min, OEF 0.38 +/- 0.06 and $CMRO_2$ 1.55 +/- 0.3 umol/g/min. In all subjects, after 2-days sustained hypoxia (n=13) there was increased CBF (65 +/- 18 ml/100ml/min, $p < 0.005$), OEF (0.53 +/- 0.06, $p < 0.0001$) and $CMRO_2$ (2.42 +/- 0.5 umol/g/min, $p < 0.0001$). By 7-days (n=9) CBF was partially normalized. There was no significant change for OEF or $CMRO_2$ between 2- vs. 7-days hypoxia, and no differences between AMS vs. no-AMS subjects. Conclusion: Sustained exposure to high altitude hypoxia causes a paradoxical increase in $CMRO_2$, despite the reduced availability of oxygen. The biological or evolutionary imperative for this response is unclear, but implies

that $CMRO_2$ may not be the central control point for regulating the oxygenation status in the brain. There is no clear relationship linking changes in oxygen metabolism with AMS symptoms. Acknowledgements: Supported by NIH R01 NS053934 (DD). 2011.

PATENT FORAMEN OVALE DOES NOT INCREASE AMS SUSCEPTIBILITY DURING ASCENT TO OR AFTER ARRIVAL AT 5050M. Kara Beasley[1], Trevor Day[2], Glen Foster[3], Michael Stembridge[4], Christopher Willie[5], Kurt Smith[5], Samuel Lucas[6], Philip Ainslie[5], Andrew Lovering[1]. [1]Univ Oregon, Eugene, OR, USA, [2]Mount Royal Univ, Calgary, AB, Canada, [3]Univ British Columbia, Vancouver, BC, Canada; Univ British Columbia Okanagan, Kelowna, BC, Canada, [4]Cardiff Metropolitan Univ, Cardiff, UK, [5]Univ British Columbia Okanagan, Kelowna, BC, Canada, [6]Dept Physiology, Univ Otago, Dunedin, New Zealand. *EMAIL: kbeasley@uoregon.edu* INTRODUCTION: Previous studies have shown that greater degrees of arterial hypoxemia after breathing hypoxic gas for 30-60min is either predictive of, or associated with, greater acute mountain sickness (AMS) susceptibility upon further ascent. At high altitude it remains unknown why some individuals develop a greater degree of arterial hypoxemia and AMS than others, although right-to-left shunt may play a role. A patent foramen ovale (PFO) is a source of right-to-left shunt present in 30% of the population, which may exacerbate arterial hypoxemia at high altitude and increase AMS susceptibility. Accordingly, we sought to determine if a PFO increases AMS susceptibility during a controlled ascent to, and following arrival at, 5050m. METHODS: At sea level, subjects were screened for PFO using transthoracic saline contrast echocardiography at rest and following a Valsalva maneuver. Subjects were divided into PFO+ (n=4) and PFO- (n=7). Subjects ascended to 5050m using the following ascent profile: 1400m(1week), 2840m(one night), 3420m(2nights), 3860m(2nights) and 4250m(1-3nights). All subjects took Diamox during the ascent, but not following arrival at 5050m. AMS susceptibility was determined (AMS+=AMS susceptible & AMS-=AMS resistant) using the Lake Louise Symptoms Questionnaire. RESULTS: No subjects developed AMS during the trek to 5050m. Upon reaching 5050m, 50% of PFO+ subjects and 43% PFO- subjects had developed AMS. Following the first night at 5050m 25% of PFO+ subjects and 29% of PFO- subjects were AMS+. CONCLUSION: In this study, PFO+ subjects were not more AMS susceptible either during a controlled ascent to 5050m while taking Diamox or after arrival at 5050m following an ascent profile designed to minimize the incidence of AMS. ACKNOWLEDGEMENTS: Supported by NSERC, CRC and APS Giles F. Filley Memorial Award. Research conducted under the memorandum between Nepal Health Research Council and EV-K2-CNR. 2015.

PATHOPHYSIOLOGY OF HIGH ALTITUDE ILLNESSES: BACK TO BASICS. Jean-Paul Richalet. Université Paris 13, Sorbonne Paris Cité, Laboratoire "Réponses cellulaires et fonctionnelles à l'hypoxie". *EMAIL: richalet@smbh.univ-paris13.fr* INTRODUCTION: High Altitude Illnesses (HAI) include AMS, HAPE and HACE. A common feature of these clinical entities is "edema": accumulation of water out of the vascular space. In AMS, interstitial edema under the skin leads

to peripheral edema, a common clinical feature of AMS, often disregarded since it has no clinical consequence. In HAPE, alveolar edema paidly follows interstitial edema when the capacity of the pulmonary lymphatics is overwhelmed. In HACE, accumulation of water in the fixed skull volume leads to compression of cerebral structures and neurological symptoms. Therefore, in all organs exposed to hypoxia, a leak of water from the vascular to the interstitial space means that the permeability of the endothelial cell is abnormally increased. METHODS: A review of the literature shows hundreds of clinical and experimental evidence that hypoxia is responsible for edema and permeability changes of the endothelium. RESULTS: In hypoxia, edema is found not only in the skin, the lungs and the brain but also in the cornea, the inner ear, the retina. Cultured endothelial cells exposed to levels of ambient PO_2 equivalent to in vivo conditions show a marked increase in permeability, which could be reversed by various agents, especially corticosteroids. Recent studies evidenced alterations of biophysical properties of endothelial cells in hypoxia involving the cell membrane and the cytoskeleton. Morphological changes of endothelial cells have been found in hypoxia, with fragmentation of proteoglycans of the basement membrane. CONCLUSION: Altogether, we suggest that the primary cause of HAI is hypoxia-induced increase in endothelial permeability. The very impressive effect of dexamethasone in AMS, HAPE and HACE is clearly in favor of this hypothesis. Then uneven pulmonary vasoconstriction or epithelial dysfunction will precipitate the development of HAPE, mechanical factors will favor the development of HACE, etc. Inflammation processes then come in a later stage and may aggravate the symptoms. 2015.

PATIENTS WITH MILD PARKINSON'S DISEASE HAVE REDUCED SYMPATHETIC RESPONSE TO HYPOXIA. Leigh Seccombe[1], Peter Rogers[1], Michael Hayes[2], Claude Farah[1], Elizabeth Veitch[1], Matthew Peters[1]. [1]Thoracic Medicine, Concord Hospital, Sydney, Australia, [2]Neurology, Concord Hospital, Sydney, Australia. *EMAIL: leigh.seccombe@sswahs.nsw.gov.au* INTRODUCTION: Braak's hypothesis emphasises early brainstem, non-motor involvement in Parkinson's disease (PD). Dopamine is a standard therapy in PD, which may also have an inhibitory effect on ventilation via the activation of specific receptors within the carotid body. We sought to compare to healthy normal subjects the hypoxic and hypercapnic ventilatory and sympathetic responses in patients with mild PD, before and after their standard dopaminergic therapy. METHODS: Patients with mild PD with no known respiratory disease were recruited. Ventilatory response to progressive poikilocapnic hypoxia and isoxic hypercapnia were assessed via closed circuit rebreathing methods. Pulse transit time (PTT) and heart rate (HR) were also measured as markers of hypoxic sympathetic response. Tests were performed before and after dopaminergic therapy (Rx) on the same day. Minute ventilation, PTT and HR response to changing pressure of end-tidal gases and calculated oxygen saturation were assessed using a Pearson's correlation and linear regression. Healthy normal subjects were recruited for comparison. RESULTS: 12 (2 female) mild PD [mean (SD) age 63 (5) yrs] and 24 (8 female) normal subjects [age 42 (15)] were studied. Baseline ventilation and cardiovascular parameters were similar prior to all

rebreathing tests. PTT and HR response to progressive hypoxia was reduced in mild PD compared to normal subjects ($p<0.002$). In the PD subjects, all hypoxic and hypercapnic rebreathing ventilatory and sympathetic parameters were not different before and after Rx. CONCLUSION: The sympathetic response to progressive hypoxia was reduced in mild PD which is consistent with early non-motor impairment in PD. Impairment of VR in more severe disease is unlikely to be related to standard dopamine therapy. 2015.

PAXILLIN, A PROTEIN INVOLVED IN PULMONARY ARTERIAL SMOOTH MUSCLE CELL FUNCTION IN PULMONARY HYPERTENSION IS REGULATED BY HYPOXIA. Christine Veith[1], Ralph Theo Schermuly[1], Hossein Ardeschir Ghofrani[1], Friedrich Grimminger[1], Werner Seeger[1], Grazyna Kwapiszewska[2], Norbert Weissmann[1]. [1]Universities of Giessen and Marburg Lung Center (UGMLC), member of the German Center for Lung Research (DZL), Excellence Cluster Cardio-Pulmonary System (ECCPS), Giessen, Germany, [2]Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria. *EMAIL: christine.veith@innere.med.uni-giessen.de* INTRODUCTION: Chronic exposure to hypoxia induces pronounced remodeling of the pulmonary vasculature leading to pulmonary hypertension (PH). The remodeling process entails increased migration and proliferation of pulmonary arterial smooth muscle cells (PASMC), processes regulated by cytoskeletal proteins. In our previous study the cytoskeletal protein paxillin was identified to be upregulated in PH and involved in cellular processes leading to pulmonary vascular remodeling. However, the molecular mechanisms leading to deregulation of paxillin are still not fully understood. METHODS: RESULTS: In this study we aim to examine the molecular mechanisms regulating paxillin expression in human PASMC using quantitative RT-PCR, western blotting and immunostaining. Hypoxic exposure (24h, $1\%O_2$) of PASMC led to elevated paxillin expression on both protein and mRNA levels. Hypoxic effects on paxillin expression were HIF-1α dependent as assessed by siRNA transfection, electrophoretic mobility shift assays and chromatin-immunoprecipitation analysis. In addition, a time dependent increase in paxillin tyrosine 31(Y31) and 118 (Y118) phosphorylation was detected following hypoxic exposure. Hypoxia stimulation also increased the nuclear localization of paxillin-phoshoY31 as indicated by immunofluorescence staining in human PASMC. CONCLUSION: Paxillin has previously been documented to be involved in proliferation and apoptosis, features characteristic of pulmonary vascular remodeling. This is however the first report that indicates molecular mechanisms leading to deregulation of paxillin in pulmonary vascular remodeling underlying PH. ACKNOWLEDGEMENTS: Deutsche Forschungsgemeinschaft (WE 1978/4-1); Excellence Cluster Cardio-Pulmonary System; EU FP6 "PULMOTENSION" (LSHM-CT-2005-018725); Graduate scholarship of the Justus-Liebig-Univ Giessen, Germany. 2015.

PERFORMANCE AND LIFE AT HIGH ALTITUDE: HYPOXIC VENTILATORY RESPONSE DIFFERENCES IN BAR-HEADED AND ANDEAN GEESE. Sabine Lague[1], Beverly Chua[1], Anthony Farrell[1], Kevin McCracken[2], Yuxiang Wang[3],

William Milsom[1]. [1]University of British Columbia, [2]University of Miami, [3]Queen's University. *Email: lague@zoology.ubc.ca.* Introduction: Two bird species that champion high altitude life are bar-headed geese (*Anser indicus*; BHG) and Andean geese (*Chloephaga melanoptera*; AG). BHG migrate biannually across the Himalayas at altitudes >5000m between the Tibetan/Mongolian plateau and low altitude wintering grounds in India. AG do not migrate but reside lifelong at high altitudes (4000-5500m) in the Peruvian Andes. AG are very evolutionary diverged from BHG; however, both species have independently evolved a high oxygen-affinity hemoglobin. Methods: To compare how these species match oxygen supply and demand during hypoxia, we characterized the hypoxic ventilatory response (HVR) and the hypoxic cardiovascular response (HCVR) of wild BHG (at 3200m in Tibet) and AG (at 4000m in Peru). All birds were cannulated (brachial artery and vein) and exposed to stepwise hypoxia (12%, 9%, 7%, and 5% inspired oxygen) while measuring cardiovascular (heart rate, cardiac output, blood pressure, blood oxygen content, hematocrit), respiratory (breathing frequency, tidal volume, total ventilation), and metabolic (oxygen consumption) variables. Results: Both species displayed similar HCVR patterns with comparable *in vivo* oxygen equilibrium curves, unaltered oxygen consumption rates, and 2-fold cardiac output increases in severe hypoxia. Interestingly, the HVR of BHG and AG were very different. While BHG exhibited 3.5-fold total ventilation increases during severe hypoxia, AG did not increase total ventilation. Rather, AG increased lung oxygen extraction by up to 90%. Conclusion: Thus, BHG and AG employ radically different methods of maintaining oxygen supply and demand. Intriguingly, aspects of the HVR pattern differences between BHG and AG parallel HVR differences exhibited by Tibetan and Andean highland human residents. The HVR differences between AG and BHG may correlate to duration of high altitude exposure (chronic versus transient) and activity level (sedentary versus migrating). Acknowledgements: Funding was provided by a NSERC Discovery Grant to W.K.M. and a NSERC Vanier Canada Graduate Scholarship and a Killam Doctoral Scholarship to S.L.L. 2015.

PERIPHERAL CHEMORECEPTOR REGULATION OF SYMPATHETIC ACTIVITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE. Craig D Steinback[1], Mohit Bhutani[2], Eric YL Wong[2], Kelvin E Jones[1], Tracey L Bryan[2], Michael K Stickland[2]. [1]Faculty of Physical Education and Recreation, Univ Alberta, [2]Division of Pulmonary Medicine, Dept Medicine, Univ Alberta. *EMAIL: craig.steinback@ualberta.ca* INTRODUCTION: Augmented sympathetic nerve activity (SNA) and cardiovascular morbidity are concomitant factors associated with chronic obstructive pulmonary disease (COPD) and may be due to peripheral chemoreceptor sensitization. Peripheral chemoreceptor inhibition has been previously shown to reduce SNA in heart failure and hypertension. Similarly, we hypothesized that chemoreceptor inhibition (by breathing hyperoxia) may reduce SNA in COPD. METHODS: Non-hypoxemic COPD patients (age: 69±5 yrs; FEV1: 65±18% predicted) who were free of cardiovascular disease and sleep apnea, were examined (n=7). SNA (microneurography) and cardiovascular function (heart rate (HR), ECG, blood pressure (BP), photoplethysmography) were continuously mea-

sured at rest, during hyperoxia (100% inspired O_2) and during hypoxia (target SpO_2 ~85%). RESULTS: At rest, patients were normotensive (systolic BP: 121±17 mmHg; diastolic BP: 79±9 mmHg; mean BP 93±11 mmHg) with a HR of 67±6 bpm. Hyperoxia caused a reduction in total SNA (11.7±4.8 to 10.2±4.7 a.u., $p<0.001$). The change in total SNA was due to variable changes in burst frequency and amplitude which were inversely related to one another ($r=-0.87$, $p<0.01$). Hyperoxia also caused a relative bradycardia (63±4, $p<0.01$ versus baseline) however, blood pressure remained unchanged. Subsequent hypoxia caused an increase in total SNA (13.7±5.0 a.u., $p<0.05$ versus baseline) via an augmented burst frequency (34±9 bursts/min at baseline to 43±8 bursts/min during hypoxia, $p<0.001$). The increase in SNA associated with hypoxia did not cause any change in BP. CONCLUSION: These data demonstrate that peripheral chemoreceptor inhibition via acute oxygen administration reduces sympathetic outflow in COPD patients, which is in contrast to previously published healthy studies that showed no reduction in sympathetic outflow with chemoreceptor inhibition. The increase in SNA associated with acute hypoxia also demonstrates a persistent sympathetic reserve in COPD patients. These preliminary findings demonstrate that the peripheral chemoreceptor contributes to resting sympathetic outflow in COPD. ACKNOWLEDGEMENTS: Funded by the Canadian Institutes of Health Research. 2015.

PERIPHERAL CHEMORECEPTOR RESPONSE AND HYPOXIC PULMONARY VASOCONSTRICTION. Tyler Albert[1], Erik Swenson[2]. [1]Department of Medicine, University of Washington, Seattle, Washington, USA, [2]Division of Pulmonary and Critical Care, VA Puget Sound Health Care System, Seattle, WA, USA, Department of Medicine, University of Washington, Seattle, Washington, USA. *Email: talbert@uw.edu*. Introduction: It has been shown in animal models that denervation of peripheral chemoreceptors, thereby diminishing hypoxic ventilatory response (HVR), enhances hypoxic pulmonary vasoconstriction (HPV). We hypothesized that individuals with high HVR should have less HPV in response to low alveolar oxygen (PAO_2). Methods: In 15 healthy men and women we measured the normobaric HVR (L min -1 $\%SaO_2$-1) during 15 minutes of step poikilocapnic hypoxia (0.21 to 0.12 FiO_2). On a different day we then measured HPV using echosonography, calculating pulmonary artery systolic pressures (PASP, mmHg) while the subjects were randomly breathing 0.21, 0.18, 0.15 and 0.12 FiO_2, each for periods of 15 minutes. Results: We recorded a sufficient range of HVRs (0.05-0.3, mean 0.134 L min -1 $\%SaO_2$-1), correlating with previously published normobaric, poikilocapnic results. Measured end-tidal CO_2 showed the expected decrease with increasing HVR (0-5.0, mean 2.44mmHg, $p<0.035$). HPV was measured using PASP at a common arterial oxygen saturation (SaO_2) of 85% (21.75-41.28, mean 28.51mmHg). We purposely chose this strategy to obtain a common PAO_2 stimulus, using SaO_2 as a surrogate for alveolar oxygenation. We found a significant inverse relationship between poikilocapnic HVR and HPV ($p<0.01$, R2 0.41). Conclusion: Previous studies of individuals who are susceptible to high altitude pulmonary edema (HAPE) have suggested both low HVR and high HPV to be important contributing factors.

Our study shows that these two processes are in fact inversely related, and this may lead to future therapeutic directions. We conclude that there is a direct correlation between peripheral chemoreceptor response and hypoxic pulmonary vasoconstriction in healthy subjects. While this had previously been suggested in the literature using animal models, it has never been shown in humans. Acknowledgements: This was an unfunded study. 2011.

PERIPHERAL FATIGUE IS NOT A CRITICALLY REGULATED VARIABLE DURING MAXIMAL, INTERMITTENT, DYNAMIC LEG EXTENSIONS. Christian Ryan[1], Billaut Francois[2], Bishop David[3], Girard Olivier[1]. [1]Aspetar – Qatar Orthopaedic and Sports Medicine Hospital, Doha, Qatar, [2]Institut national du sport du Québec, Montréal, [3]Victoria Univ - Institute Sport, Exercise and Active Living (ISEAL), Melbourne, Australia. *EMAIL: ryan.christian@aspetar.com* INTRODUCTION: The aim of this study was to explore the interplay of both central and peripheral mechanisms during multiple bouts of maximal intensity, intermittent, dynamic leg extension under varying levels of hypoxia severity. METHODS: On separate days, 14 healthy men performed four sets of 6 x 5 maximal intensity isokinetic leg extensions (lasting ~7-s) at 300°/s (15 and 100 s of passive rest between repetitions and sets, respectively) under normoxia (simulated altitude/fraction of inspired O_2: 0m/0.21), moderate (3000m/0.14), and severe normobaric hypoxia (5400m/0.10). Neuromuscular assessments (electromyography and measurements of voluntary and evoked contractions of the knee extensors) were conducted before and immediately after each exercise set. RESULTS: There was a main effect of condition on both the mean peak power during each set of exercise ($p < .05$) and the decrement in mean peak power across each set (normoxia; 2.9 ± 1.3%, moderate hypoxia; 5.2 ± 1.5%, severe hypoxia; 10.3 ± 2.0%; $p < .01$). Despite a main effect of time, the reduction in the electromyographic activity of the biceps femoris during the leg extensions was not different between conditions (normoxia 6.2 ± 5.4%, moderate hypoxia 11.1 ± 4.6%, severe hypoxia 13.0 ± 4.2%, all conditions compounded; $p < .05$). Maximal voluntary contraction torque, voluntary activation (twitch interpolation) and potentiated twitch torque all decreased with time ($p < .05$). The end-exercise percent reduction in potentiated twitch torque was greater in severe hypoxia compared to normoxia, (41.3 ± 3.0 % v 28.0 ± 3.2 %; $p < .05$). Before exercise, cerebral tissue saturation was lower in moderate and severe hypoxia compared to normoxia (normoxia; 90.87 ± 2.26, moderate hypoxia; 80.86 ± 3.58, severe hypoxia; and 65.10 ± 3.16; $p < .001$) with no change in any condition during the exercise. CONCLUSION: In conclusion, performance decrements during maximal, intermittent, dynamic leg extensions are exacerbated by hypoxia and result from both peripheral and central adjustments. However, central adjustments do not appear to limit excessive development of peripheral muscle fatigue. 2015.

PHARMACOLOGICAL NITRIC OXIDE BLOCKADE DECREASES CHEMOREFLEX-MEDIATED INCREASES IN SYMPATHETIC ACTIVITY IN HUMANS. Mikael Sander, Quentin Croft, Mads L Haslund, Carsten Lundby. National Hospital Copenhagen Denmark, University of Oxford, England. *Email:*

sanders@dadlnet.dk. Hypoxic exposure engages the chemoreflexes and causes increases in sympathetic activity, heart rate and ventilation. Nitric oxide (NO) has been implicated both as an inhibitory signaling molecule in peripheral chemoreception, and an excitatory mechanism in the nucleus tractus solitarius (NTS), strengthening the chemoreflex through positive modulation of glutamatergic neurotransmission. We hypothesized that NO-blockade would cause desensitization of the chemoreflex in humans. In healthy subjects (n=10), muscle sympathetic nervous activity (MSNA) by microneurography, intra-arterial pressure, heart rate (HR), ventilation (Cosmed) and arterial blood gasses were measured during normoxic and graded hypoxic exposure (F_IO_2=0.21, 0.13, 0.11, 0.10, 0.09, 0.08, and 0.07 (modified Altitrainer). Measurements were repeated after NOS-blockade by L-NAME (4 $mg \cdot kg^{-1}$ infused over 30 min). The L-NAME-induced increase in blood pressure was counteracted by co-infusion of nitroprusside before repeating the chemoreflex-protocol. PO_2 were 13,3; 7,4; 5,9; 5,1; 4,7; 4,5; 4,2 kPa and PCO_2 were 5,2; 5,2; 5,0; 4,7; 4,4; 4,2; 3,4 during the protocol before L-NAME with similar values after L-NAME. MSNA during the protocols were 13; 13; 16; 18; 21; 24; 34 before and 14; 14; 13; 15; 14; 19; 25 bursts·min-1 after L-NAME, $p<0.05$. HR-responses during F_IO_2=0.07 were 33 vs 22 $beats \cdot min^{-1}$, $p<0.05$, whereas ventilation was similar during the two tests. These data indicate that NO-blockade desensitizes the sympathetic chemoreflex both by increasing the threshold for responsiveness and by decreasing the response to severe hypoxia. We speculate this is caused by blockade of brainstem NO-synthesis and thereby weakening of glutamatergic neurotransmission in the NTS. Funding: Novo Nordisk Foundation. 2009.

PHARMACOLOGY OF HIF-PROLYL HYDROXYLASE INHIBITION. Todd W. Seeley. FibroGen, Inc. *Email: tseeley@Fibrogen.com.* All tissues require a sufficient supply of oxygen for survival. Lack of oxygen triggers a system of protective biology and physiology present in all aerobic organisms. A key regulatory component of the body's hypoxia response system is hypoxia-inducible factor (HIF), a transcription factor that regulates the expression of genes whose protein products are involved in a wide range of protective responses to hypoxia. These responses occur at the systemic level, such as erythropoiesis, and at the cellular level, such as alterations in metabolism that reduce oxygen demand and promote cell survival despite oxygen deprivation (cytoprotection). FibroGen is developing small molecules that selectively increase the activity of HIF. HIF is regulated by prolyl hydroxylation, an oxygen-and iron-dependent enzymatic reaction that triggers the degradation of the HIF-α subunit. In hypoxia, or in the presence of small molecule inhibitors of HIF prolyl hydroxylases, degradation is suppressed and HIF-α accumulates to high levels, enabling HIF-mediated transcription of hypoxia-responsive genes. Research conducted by FibroGen and others suggests that pharmacological manipulation of HIF biology will be effective in treating a wide range of diseases. In human clinical studies, proof of principle has been demonstrated for oral therapy of anemia in chronic kidney disease. In other studies, FibroGen is actively investigating the utility of these treatments in preserving organ function resulting from ischemic tissue damage. 2009.

PPARG IS INVOLVED IN HYPOXIA ADAPTATION. Jianming Shao[1], Xianglong Zhang[1], Zhe Xu[1], Qian Li[2], Jiayou Chu[2], Changqing Zeng[1]. [1]Beijing Institute Genomics, Chinese Academy of Sciences, China, [2]Institute Medical Biology, Chinese Academy of Medical Sciences, Kunming, China. *EMAIL: czeng@big.ac.cn* INTRODUCTION: To understand hypoxia adaptation mechanism in Tibetans, we compared cell proliferation rates and RNA Seq data of 83 genes between lymphoblastoid cell lines from two Tibetans and two HapMap individuals (each of Chinese and European). We also conducted RNA capturing followed by sequencing the transcripts of a set of selected 83 genes related to hypoxia, using samples from each cell line cultured at hypoxia (1%) and normoxia for 24hrs. METHODS: Cells were collected followed by mRNA purifying, RNA capture and library preparation, and paired-end sequencing with HiSeq-2000. After adaptor trimming, raw reads were mapped utilizing Tophat. Isoform reconstruction was done by Cufflinks, and gene expression quantification by DESeq. RESULTS: 1) At 1% of oxygen supply, both Tibetan and HapMap cell lines grow a little faster than that at normoxia. Further hypoxia treatment at 0.3% completely inhibited cell proliferation. If a hypoxic condition may differentiate Tibetan and HapMap cell lines it is in the process of fine tittering on oxygen concentrations. 2) Among the 83 mRNAs captured and sequenced, PPARG was found significantly enriched in Tibetan cell lines, in which 9 of 15 reported isoforms were resolved. However, the two HapMap cell lines produced hardly any detectable isoforms of PPARG in both normoxic and hypoxic conditions, suggesting the specific expression of this gene is in Tibetan cell lines only. On the other hand, in our genome wide association analysis, one SNP of PPARG, which showed very low LD with flanking SNPs, exhibited significant allele frequency difference ($p=0.0002$) between Han and Tibetan populations. Moreover, its allele frequency differentiates among Tibetan groups residing at various heights. A similar signal was also observed in genotypes of Andeans. CONCLUSION: Our results suggest the contribution of PPARG to hypoxia adaptation in highlanders. ACKNOWLEDGEMENTS: This study was supported by Natural Science Foundation of China. 2015.

PRACTICAL LIMITATIONS OF CURRENT NON-PHARMACOLOGICAL TREATMENT OF HIGH ALTITUDE PULMONARY OEDEMA (HAPE) AND A REVIEW OF ALTERNATIVES. Stephan Sanders, Zoe Smith, Catherine Williams, James Leedham, Medical Expeditions. Yale University and Medical Expeditions, Ngwelezane Hospital, Queen Alexandra Hospital. *Email: stephansanders@hotmail.com.* High Altitude Pulmonary Edema (HAPE) is a common cause of altitude related fatalities. Severe symptoms and logistical issues often necessitate stabilisation prior to descent; this includes portable hyperbaric chambers (PHC) and oxygen, alongside pharmacological treatment. Experience of managing a severe case of HAPE during the recent Medex research expedition prompted a review of the literature on some of the non-pharmacological treatments, including Continuous Positive Airways Pressure (CPAP), Expiratory Positive Airways Pressure and Bi-level Positive Airways Pressure (BIPAP). The review highlighted several factors that limit the usefulness of the PHC, including patient deterioration due to orthopnea,

the difficulties in treating an unconscious patient and as a barrier to patient assessment and communication. No studies directly compare the different types of portable hyperbaric chambers or suggest potential modifications to the current designs. Modern materials may allow modification of PHC design to enable an upright position and room for a second person to monitor and treat the patient. There are case studies, simulated studies and small trials on the use of CPAP/EPAP/BIPAP at altitude and in the treatment of HAPE. There are potentially adverse effects including barotrauma, reduced cardiac venous return and raised intracranial pressure. The potential of CPAP/EPAP/BIPAP as a lightweight and simple adjunct to treatment deserves further investigation. Respiratory rate, heart rate, pulse oximetry and minute volume should be assessed. Pulmonary artery pressure, cardiac output, arterial blood gas measurements, end-tidal CO_2 and cerebral blood flow are other potentially measurable parameters. Machine performance at altitude should also be considered. 2009.

PRECLINICAL INVESTIGATIONS OF THE HYPOXIA ACTIVATED RADIOSENSITIZER DCQ IN BREAST CANCER CELLS. Khaled Ghattass[1], Makhlouf Haddadin[2], Marwan El-Sabban[3], Hala Gali-Muhtasib[1]. [1]Biology Department, American University of Beirut, Lebanon, [2]Chemistry Department, American University of Beirut, Lebanon, [3]Human Morphology Department, American University of Beirut, Lebanon. *Email: kig01@aub.edu.lb*. Introduction: Eliminating the highly resistant hypoxic cells by hypoxia activated drugs is an exceptionally promising approach for treatment of aggressive tumors. 2-Benzoyl-6,7-dichloro-3-phenylquinoxaline 1,4-dioxide (DCQ) is a synthetic quinoxaline dioxide that targets tumor cells within their hypoxic microenvironment. Here, we present novel data showing that DCQ has antineoplastic effects in breast cancer cell lines. Methods: MCF-7 and MDA-MB-231 cells were treated with 1-7 μM of DCQ for different time intervals either under hypoxia or normoxia. MTT proliferation assay, colony formation assay, western blot, alkaline comet assay, Flow cytometry of PI stained DNA, ELISA of secreted VEGF, and DCFH assay were used to study DCQ effects. Results: DCQ decreased the viability of MCF-7 (IC50: 2-10 μM) and MDA-MB-231 (IC50: 2-3μM) cells more so under hypoxia and this was due to an increase in the preG1 population and the induction of apoptosis. DCQ also reduced colony forming ability in an oxygen concentration dependent manner in the two cell lines, however MCF-7 were more resistant. The mechanism of DCQ toxicity appeared to be different between the two cell lines. In MCF-7, cytotoxicity was correlated with an increase in phosphorylated p53 and cyclin D1 proteins, and reduction in hypoxia inducible factor 1α (HIF-1α), and in p21 and VEGF proteins. In MDA-MB-231, cytotoxicity correlated with the cleavage of caspases 3 and 9, and an increase in reactive oxygen species in addition to a significant increase in p21 protein. Conclusion: Our results show that DCQ is a promising drug that induces apoptosis through different mechanisms in breast cancer cell lines with different metastatic potential. Acknowledgements: This project was supported by the Lebanese National Council for Scientific Research and by the University Research Board of the American University of Beirut. 2011.

PRESERVED RIGHT-VENTRICULAR POWER DESPITE ELEVATED PULMONARY ARTERY PRESSURE IN HIGH ALTITUDE PULMONARY EDEMA SUSCEPTIBLE PERSONS DURING MODERATE EXERCISE AT 4559M. Katja Auinger[1], Christoph Siebenmann[1], Beat Kaufmann[2], Stephanie Kiencke[3], Bart De Boek[4], Christoph Dehnert[5], Michèle Schöb[1], Stefanie Zügel[6], Heiko Fruehauf[7], Thomas Lutz[8], Marco Maggiorini[1]. [1]Medical ICU and ZIHP University Hospital Zurich, [2]Cardiology, University Basel, [3]Cardiology, Spital Bruderholz, Basel, [4]Cardiology, University Hospital Bern, [5]Sports Medicine University Heidelberg, [6]Cardiology University of Heidelberg, [7]Gastroenterology University Hospital Zurich, [8]Institute of Veterinary Physiology and ZIHP, Univeristy of Zurich. *Email: klinmax@usz.uzh.ch*. Introduction: Excessively elevated pulmonary artery pressure (Ppa) is a hallmark of high altitude pulmonary edema susceptible (HAPEs) persons. However, whether elevated Ppa jeopardizes exercise performance at high altitude is still debated. We compared right ventricular performance during moderate exercise between HAPE resistant (HAPEr) and HAPEs before and after rapid ascent to 4559m within 24 hours. Methods: Ppa, cardiac index (CI), arterial hemoglobin oxygen saturation (HbO_2) and oxygen consumption (VO_2) were measured at rest, and during moderate exercise at 20% and 30% of VO_2max in 17 HAPEr and 8 HAPEs. Ppa was estimated by measuring tricuspid valve regurgitation jet velocity (dpTI). Right ventricular power (CPrv), oxygen delivery (DO_2) and oxygen extraction ratio (O_2ER) were calculated using standard formulas. Results: At low altitude at rest and during exercise dpTI, CI, CPrv, HbO_2, DO_2 and O_2ER were not different between HAPEr and HAPEs persons. Ascent to 4559m increased dpTI by 18 mmHg in HAPEr and 31 mmHg in HAPEs ($p = 0.03$), the difference being maintained during exercise. At rest ascent decreased HbO_2 by 21% ($p < 0.001$) and DO_2 by 209 ml/min/m2 ($p < 0.001$), and increased O_2ER by 106 ml/min ($p < 0.001$), the difference between HAPEr and HAPEs being not significant. Resting CPrv increased from low to high altitude by 0.12 Watt in HAPEr ($p < 0.01$) and by 0.25 Watt in HAPEs ($p < 0.01$). During exercise CPrv increased by 0.21 Watt ($p < 0.01$) and 0.18 Watt ($p < 0.05$) in HAPEr and HAPEs, respectively. Moreover, exercise decreased HbO_2 by 7% ($p < 0.001$) and increased DO_2 by 400 ml/min/m2 ($p < 0.001$) and O_2ER by 344 ml/min ($p < 0.001$). Changes during exercise were not different between groups. Conclusion: In HAPEs an increased CPrv compensate for excessively elevated Ppa at rest and during mild exercise at high altitude. The lack of difference in CPrv, DO_2 and O_2ER between HAPEr and HAPEs at high altitude suggests, that HAPEr and HAPEs while exercising at altitude behave similarly despite the significant differences in Ppa. Acknowledgements: Center of Integrative Physiology (ZIHP) University of Zurich, Harmann-Müller Stiftung, Fondazione Crivelli for funding. 2011.

PREVALENCE OF AIRFLOW OBSTRUCTION (AO) IN NEPAL RURAL POPULATION EXPOSED TO INDOOR BUT NOT OUTDOOR POLLUTION. Gaia Mandolesi[1], Luca Pomidori[1], Sanjay Nath Khanal[2], Atindra Sapkota[2], Sandeep Shrestha[2], Annalisa Cogo[1]. [1]Sport Biomedical Studies Center, University of Ferrara, Ferrara, Italy, [2]Department of Environmental Sciences and

Engeneering, University of Kathmandu, Kathmandu, Nepal. *Email: gaia_mandolesi@libero.it*. Introduction: Biomass fuel (BMF) is the major energy source in developing country as a cooking and heating fuel, often on open fire. Its use causes indoor air pollution which plays a main role in development of chronic obstructive respiratory disease (COPD). Aim: To evaluate the impact of BMF on the respiratory health of people living in a mountain village of Nepal not exposed to traffic and industrial pollution. Methods: All the dwellers with an age > 14 years were selected, 105 subjects (51F, 54M), aged 14-82 years were evaluated by spirometry (due to technical problems, the reversibility test was not performed), and a questionnaire about smoke habits, kitchen detail and energy sources. Results: 4.8% (2F, 2M) of subjects were smokers, 92.5% (49F, 51M) were never smokers. The prevalence of AO (ERS criterion, FEV1/FVC % of predicted value <88%) was 26.6% (17F, 11M): 78.5 % mild, 10.7% moderate, 10.7% moderately severe. All subjects with AO were no smokers, exposed to BMF, living in traditional houses (85% with chimney, but only <50% since >10 years); 61% were women working at home. Conclusion: A high percentage of AO is demonstrated in non smokers with a long term exposure to domestic BMF smoke. The percentage is higher in householder women living in traditional houses. In these subjects the probability of COPD is higher than expected in the general population (4-10%). To the best of our knowledge this is the first study on respiratory health in no smoker subjects with different degrees of indoor pollution exposure, living in an area free from outdoor pollution. In fact in the previous studies the subjects were also exposed to other risk factors such as cigarette smoking and/or traffic exhaust fumes. 2011.

PREVALENCE, TIME-COURSE AND OUTCOME OF ACUTE MOUNTAIN SICKNESS IN NON-ACCLIMATIZED CHILDREN AND ADULTS AFTER RAPID ASCENT TO 3450 M. Sophie Garcin, Herve Duplain, Stefano F Rimoldi, Thomas Stuber, Claudio Sartori, Urs Scherrer. University Hospital, Inselspital. *Email: garcins0@etu.unige.ch.* Increasing numbers of persons, including families with children, travel to high-altitude destinations. Acute Mountain Sickness (AMS) is the most frequent debilitating medical problem during high-altitude exposure, but there are no controlled studies on its prevalence, time-course and clinical outcome. Therefore, we performed serial assessments of AMS (Lake Louise Score) in 68 non-acclimatized Swiss children (age 11±2 years, 34 girls and 34 boys) and 38 Swiss adults (age 40±9 years, 31 women and 7 men) with no previous high-altitude experience 6, 18 and 42 hours after a 2.5 hour ascent to 3450 m. We found that the overall prevalence of AMS during first 3 days at high altitude was greater in adults than in children (47 vs. 26%, P<0.05). In both groups, the large majority of cases (94%) of AMS developed during the first 18 hours. Thereafter, its prevalence decreased dramatically, and in the morning after the 2nd night only 3% of the children and 5% of the adults still had symptoms of AMS. In none of the subjects AMS lasted for more than 48 hours. Among the participants who suffered from AMS, the severity of the disease was similar in children and adults, and only 2 (3%) of the 68 children and 3 (8%) of the 38 adults scored 3 (severe incapacitating symptoms) on one of the items of the questionnaire. In these subjects, the symptoms responded

well to symptomatic treatment. For the groups as a whole, there existed significant differences in the frequency of the symptoms making up for AMS between children and adults. Sleep disturbances and headache were more frequent and severe in adults than in children. Conversely, gastrointestinal symptoms were more frequent and severe in children than in adults. We conclude that after rapid ascent to 3450 m a minority of non-acclimatized persons suffer from AMS. In the affected subjects, AMS develops rapidly, the time-course is brief and the clinical symptoms are benign. Children appear to be less susceptible to AMS than adults. 2009.

PRIMARY DIAGNOSES AT A HIGH ALTITUDE MEDICAL HOSPITAL: SPRING 2010 SEASON AT PHERICHE, NEPAL HIMALAYA, 4200M. Barbara Jones[1], Buddha Basnyat[2], Colin Grissom[3]. [1]University of Utah, Division of Pulmonary and Critical Care Medicine, [2]Himalayan Rescue Association, [3]Intermountain Medical Center, Division of Pulmonary and Critical Care Medicine. *Email: barbara.jones@hsc.utah.edu*. Introduction: BACKGROUND: Acute mountain sickness (AMS), high altitude pulmonary edema (HAPE), and high altitude cerebral edema (HACE) are expected among trekkers in the high altitude region of the Nepal Himalayas. Additional medical problems occur, however, for which the medical provider at high altitude should be prepared. AIM: Review primary diagnoses, reason for admission, and medications used for patients presenting to the Pheriche Himalayan Rescue Association (HRA) Medical Aid Post during the Spring 2010 season. Methods: Retrospective case series. Results: 422 patients were evaluated at the Pheriche Aid Post between March 1st and May 15th, 2010. Of these, 265 (61%) were Nepalese, and 168 (39%) were foreigner travelers. Upper respiratory infection and gastroenteritis were common among all patients. Foreign patients had a significantly greater incidence of AMS and HAPE ($p<.01$), while Nepalese patients had a significantly greater incidence of cough/bronchitis ($p<.05$). Thirty patients were hospitalized; 11 were Nepalese and 19 were foreigners. Primary diagnoses at admission included HAPE (12), HACE (7), both HAPE/HACE (4), gastroenteritis (2), severe AMS (2), cerebrovascular accident (2), and pancreatitis (1). All hospitalized patients but one were evacuated to lower altitude or to tertiary care by ground or helicopter. Conclusion: Of the 422 patients evaluated at the Pheriche medical hospital during the spring 2010 season, the majority were Nepalese, who had significantly lower rates of altitude related illness compared to foreigners. Other common medical problems encountered were upper respiratory infections, gastroenteritis, gastritis, and cough/bronchitis. Medical providers to travelers and locals of the Nepal Himalayn region should be prepared to manage a wide variety of illnesses. Acknowledgements: Thank you to the Himalayan Rescue Association, Bhuwan Acharya for data analysis, and the University of Utah for financial support. 2011.

PRO-INFLAMMATORY AND PRO-APOPTOTIC GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS AT HIGH ALTITUDE (4559M): PROTECTIVE FOR ACUTE MOUNTAIN SICKNESS?. Stefanie Zügel[1], Dagmar Albers[2], Marco Maggiorini[3], Christoph Siebenmann[3], Katja Auinger[3], Peter Bärtsch[4], Ralf Kinscherf[1], Christoph Dehnert[5]. [1]Anatomy and Cell Biology,

Philipps-University Marburg, Germany, [2]Internal Medicine VII, Sports Medicine, University Hospital Heidelberg, Germany;, [3]Department of Internal Medicine, Intensive Care Unit, University Hospital Zürich, Switzerland, [4]Internal Medicine VII, Sports Medicine, University Hospital Heidelberg, Germany, [5]Internal Medicine VII, Sports Medicine, University Hospital Heidelberg, Germany / Internal Medicine II, Section of Sports and Rehabilitation Medicine, University Hospital Ulm, Germany. *Email: stefanie.zuegel@staff.uni-marburg.de*.
Introduction: Hypoxia-induced oxidative stress, which has been discussed to be involved in the pathophysiology of acute mountain sickness (AMS), may influence plasma redox status (REDST) and gene expression in circulating peripheral blood mononuclear cells (PBMCs) representing the innate and adaptive immune system. Thus, we were interested to investigate the effect of hypoxia on the expression of pro-inflammatory and pro-apoptotic genes in PBMCs and on plasma glutathione in individuals with (AMS+) or without (AMS-) acute mountain sickness. Methods: 39 mountaineers at low altitude and after arrival at 4559m without preacclimatization PBMCs were isolated via Ficoll density gradient. The mRNA expression of glycerinaldehyde-3-phosphat-dehydrogenase (GAPDH) and several pro-inflammatory [cyclooxygenase 1 and 2 (COX-1/2); interleukin 6 and 8 (IL-6/8); insulin-like growth factor-1 (IGF-1); tumor necrosis factor∝ (TNF-∝)] and pro-apoptotic genes [caspase-3 and forkhead box O3 (FOXO-3)] were quantitatively analyzed. In the plasma, total glutathione (GSH), oxidized glutathione (GSSG) and reduced glutathione (red. GSH) as well as glutathione REDST (red. GSH2/GSSG) were determined. Results: 14 mountaineers developed AMS within the first day after arrival at 4559m, whereas 25 did not. The mRNA expression of GAPDH in PBMCs increased in all mountaineers [+21% AMS- (p=0.05); +40% AMS+ (p=0.03)] at 4559m compared to low altitude conditions. In AMS-, COX-1 increased (+47%; p=0.02), and IGF-1 decreased (-76%; p=0.006), whereas no significant changes in mRNA expression were observed in AMS+. In PBMCs of AMS+, caspase 3 increased 40% (p=0.04) within 24h after arrival at high altitude. In comparison to low altitude, TNF-∝, IL-6/8, COX-2 and FOXO-3 in PBMCs of AMS- and AMS+ were unchanged in high altitude. However in AMS+ a reduced plasma glutathione REDST was observed, whereas in AMS- an increase of plasma glutathione REDST (32fold; p=0.04) was found, which might be due to an increase of red. GSH (+21%; p=0.07). Conclusion: AMS+ do not reveal hypoxia-induced alterations of distinct pro-inflammatory genes in PBMCs like AMS-. These findings, in addition with an induction of apoptotic processes in PBMCs of AMS+ in parallel with a decreased plasma glutathione REDST suggests an impaired adaption to hypoxia in subjects who developed AMS and possibly a protective effect of the observed pro-inflammatory processes with respect to AMS. 2011.

PROSPECTIVE TARGETING AND CONTROL OF END-TIDAL PCO_2 AND PO_2 DURING FMRI SCANNING IN HEALTHY SUBJECTS AND PATIENTS. Jay Han, Stephanie Dorner, Marat Slessarev, Alexandra Mardimae, Cathie Kessler, David J Mikulis, Joseph A Fisher. University of Toronto. *Email: Jay.han@uhn.on.ca.* Cerebrovascular reactivity (CVR) is defined as change in cerebral blood flow

(CBF) for a given vasoactive stimulus. Blood oxygen dependent (BOLD) MRI signal can be used as the surrogate for CBF, and changes in end-tidal PCO_2 ($PETCO_2$) as the stimulus--if end-tidal PO_2 ($PETO_2$) can be kept constant. We set out to develop and test a system that, for the first time, will provide a repeatable $PETCO_2$ stimulus to both healthy subjects and patients in an MR environment. We used a custom gas blender (RespirAct™, TRI, Toronto Canada) and a sequential rebreathing circuit to study CVR in a 3T MRI unit in 10 healthy subjects (ages 20-50) and 24 patients (ages 20-80) being investigated for neurological symptoms. The target end-tidal values consisted of normoxic (100mmHg) quasi-square wave changes in target $PETCO_2$'s between 40 and 50 mmHg of 45 - 130 s durations. We analyzed the $PETCO_2$ values of the last 30 seconds of each stage and the $PETO_2$ for the entire test. In healthy subjects the $PETCO_2$'s in mmHg (m ± SD) at each stage were: stage 1-(40.3 ± 0.8), 2-(49.6 ± 1.0), 3-(40.3 ± 1.0), 4-(50.0 ± 0.67), 5-(40.6 ± 1.2). In patients: stage 1- (40.2 ± 0.7), 2-(48.9 ± 2.2), 3-(40.8 ± 1.2), 4-(49.5 ± 1.1), 5-(41.2 ± 1.6). $PETO_2$ was 103.0 ± 2.7 in subjects and 103.5 ± 2.0 in patients. There were no differences at any stage in $PETCO_2$ or $PETO_2$ between healthy subjects and patients. We conclude that the prospective targeting of $PETCO_2$ and $PETO_2$ provides a highly repeatable stimulus to both healthy subjects and patients during MRI scanning. 2009.

PROTEINURIA ASSOCIATED WITH ALTITUDE EXPOSURE AND EXERCISE. John Davis[1], Colleen Wren[1], Shaquice Mullany[1], Maurie Luetkemeier[1]. [1]Alma College, Alma, Michigan. *EMAIL: davisj@alma.edu* INTRODUCTION: The purpose of this study was to examine adaptations in urinary protein at sea level, and periodically throughout 13 days of living at moderate altitude (3400 m). Furthermore, it was intended to determine changes in urinary protein on selected days following a maximal graded exercise test. METHODS: Participants included 15 college-age individuals (10 females, 5 males) who were chronically adapted to sea level. Resting urine samples were collected on the day before ascent to 3400m, on days 1, 3, 5, 7, 9, &13 following ascent, and one day following descent back to sea level. All participants performed maximal graded exercise test on the day before ascent, one day after ascent, on the 13th day of living at 3400m and one day after returning to sea level. Protein concentrations were determined using a variation of the Bradford method. RESULTS: Resting urinary protein increased from 275±166 mg/L at sea level to 478±292, 622±344, 476±173, 620±265, 610±368 and 377±360 mg/L on days 1, 3, 5, 7, 9, and 13 respectively, while living at altitude, and remained elevated at 674±424 mg/L upon return to sea level. Urinary protein decreased following maximal exercise from 1013mg/L at sea level to 667mg/L, and increased to 1261mg/L, on days 1 and 13 following ascent, to 1154mg/L on the day after returning to sea level. CONCLUSION: These data suggest that urinary protein increased following ascent to moderate altitude as well as with maximal exercise. When these variables are combined, they produce an additive effect on proteinuria. 2015.

PROVOKED PERIODIC BREATHING IN SIMULATED MODERATE AND HIGH ALTITUDE IN HEALTHY YOUNG ADULTS. Stephan Pramsohler[1], Nikolaus Netzer[1]. [1]Hermann Buhl Institute for Hypoxia and Sleepmedicine Res.

Univ. Innsbruck. *Email: nikinetzer@yahoo.com.* Introduction: It is common sense that periodic breathing increases linear with greater heights. Cut off points for periodic breathing at specific altitudes are not known and seem to be very individual. Our aim was to study the difference of breathing patterns in healthy adults between simulated 3500m and 4500m Methods: 12 male and 6 female subjects (mean age of 24.7 yrs) randomly assigned to two goups had a 12 channel PSG at either 3500m (n=9) or 4500m (n=9) simulated altitude (normobaric hypoxia). Results: We have seen a statistically significant higher decrease in total sleeping time (325:417min) and arterial oxygen saturation (76.8% : 84.8%) as well as an increase of the heart rate (78.6 : 69.2 bpm) at 4500m compared to 3500m. No statistically significant difference for AHI and arousal frequency could be seen, although there is a obvious difference in mean AHI (68.6/h : 44.1/h) for the different groups. Whereas at 3500m group the main respiratory event were central hypopneas, at 4500m central apneas prevail. Conclusion: Sleep at increasing simulated altitude at the cut off from moderate to high altitude goes along with decreasing SaO_2 at a higher HF and reduced TST, but AHI's are individually too different in a sample of nine individuals in each altitude group to show a statistically significant difference. 2015.

PULMONARY ARTERY PRESSURE AND RIGHT VENTRICULAR FUNCTION IN HEALTHY CHILDREN AND ADOLESCENTS AFTER RAPID ASCENT TO 3450 M. Stefano S Rimoldi, Thomas Stuber, Stefano de Marchi, Herve Duplain, Sophie Garcin, Claudio Sartori, Urs Scherrer, Yves Allemann. Inselspital Bern University Hospital Bern, Imperial College Healthcare NHS Trust, St. Mary's Hospital. *Email: stefano.rimoldi@insel.ch.* Objectives: High-altitude tourist destinations are reached by increasing numbers of children and adolescents after rapid ascent by mechanical transportation. The high-altitude induced increase of pulmonary-artery pressure in these non-acclimatized young persons is expected to be substantial and may have adverse effects on the right ventricular function, but there is no information. Methods: We performed echocardiographic assessments of pulmonary-artery pressure (Pap) and systolic right ventricular (RV) function in 120 healthy, non-acclimatized children and adolescents (56 female, mean±SD age 11±2.4 y, range 6-16 y) with no previous high-altitude experience, at low altitude (540m) and 24 hrs after rapid ascent to 3450 m. Results: As expected, the altitude-induced decrease in oxygen saturation (from 97±1.2 to 90±2.4%, P<0.0001) was accompanied by a more than two-fold increase of the systolic RV to right atrial pressure gradient (from 16±3 to 35±11 mm Hg, P<0.0001). Surprisingly, this dramatic increase in Pap did not have any adverse effects on RV function, but was associated with a significant increase in the systolic RV function parameters. Tricuspid annular plane systolic excursion (TAPSE) (20.1±1.8 vs. 20.8±2.5 mm, P=0.02), peak systolic tissue Doppler contraction velocity signal of the lateral tricuspid annulus (14.5±2.0 to 15.7±2.6 cm/s, P<0.0001) and isovolumic myocardial acceleration, a proxy of the right-ventricular contractile function (2.7±0.7 vs. 5.0±1.1 cm/s2, P<0.0001) were all significantly greater at high than at low altitude. Conclusions: These data provide the first evidence that in young healthy non-acclimatized children and adolescents, the dramatic increase of pulmonary-artery

pressure induced by rapid exposure to high altitude is associated with increased systolic right ventricular function. These findings contrast with observations in adults in whom systolic right ventricular function remains unchanged under these conditions. 2009.

PULMONARY DIFFUSION VARIABLES IN ANDEANS AND HIMALAYANS. Robert R Naeije, Claire C de Bisschop, Gil G Leurquin, Jean-Benoit J Martinot, Vitalie V Faoro, Hervè H Guènard. Free University of Brussels, Sport Science Faculty, Erasme University Hospital, St Elisabeth Hospital, University Hospital Bordeaux. *Email: rnaeije@ulb.ac.be.* Adaptation to high altitude (HA) chronic hypoxia depends on ethnic origin. The aim of the study was to investigate whether chronic hypoxia could induce differences in pulmonary diffusion at HA as a function of ethnic origin. Two groups of highlanders (4000m) from Himalaya (Him, n=11), and Bolivia (Bol, n=8) and two groups of acclimatized European lowlanders (Eur1, n=21 and Eur2, n=16) were studied. All of them volunteered to follow the protocol. CO and NO transfers were measured during single breath method with an automated material (Hypercompact Medisoft Dinant B). Lung capillary blood volume corrected for haemoglobin concentration (Vccor) and conductance for CO (Dm) were calculated. Measurements were made at 5000m altitude for Him and Eur1 and at 4000m for Bol and Eur2. Him were younger than Eur1 (25 yrs vs 39.5yrs; p=0.04). Mean age of the others groups were 39.5yrs (Bol) and 36.5yrs (Eur2). The groups were not different in height and in body mass index. Haemoglobin concentration was higher in Bol than in the two European groups (17.13g·dL^{-1} vs 14.48 and 14.03g·dL^{-1}; p<0.001) and Him (15.93g.dL^{-1}). All the pulmonary diffusing variables (TLCOcor, Dm, Vccor) were higher in highlander groups than in lowlanders groups (p<0.000). Mean TLCOcor, Dm and Vccor expressed in % of sea level reference were respectively 173%, 134% and 194% in Him; 153%,114% and 185% in Bol ; 100%, 92% and 93% in Eur1 and 112%, 89% and 124% in Eur2. The ratios Vc/VA and Dm/VA were higher in Him (28.2 and 15.8) and Bol (28.2 and 14.1) than in Eur1 (13.9 and 11.1) and Eur2 (20.1 and 11.8) . TLNO/TLCO and Dm/Vccor were higher in Eur1 than in the other groups. As reported previously haemoglobin concentration in Andeans is greater than that of Himalayans suggesting different adaptation to HA. Pulmonary diffusion variables are raised in the two HA groups (+50 to +100%) mainly Vccor, suggesting that chronic hypoxia might similarly stimulates pulmonary angiogenesis in Andeans and Himalayans. 2009.

PULMONARY HYPERTENSION IN CHRONIC HYPOXIA: CAUSE OR CONSEQUENCE OF LUNG EDEMA? GIUSEPPE MISEROCCHI, ILARIA RIVOLTA, VALERIA LUCCHINI, CRISTINA D'ORLANDO, FRANTISEK KOLAR. University Milano-Bicocca, SAN GERARDO HOSPITAL, Academy of Sciences. *Email: giuseppe.miserocchi@unimib.it.* Data from Wistar rats exposed to chronic normobaric hypoxia CNH for 1 month were compared to controls matched for age following different protocols: measurement of right ventricular systolic pressure (RVSP); lung excision after tying the trachea and fixation for morphometry; lung excision and processing for molecular biology; measurement of pulmo-

nary interstitial pressure (Pip) by micropuncture servonull technique. RVSP averaged 26 mmHg in control and increased in CNH by 30 up to 200%, thus displaying a large variability. In CNH rats lung regions were classified as "well adapted" (WA, nice and pink) or "mal-adapted" (MA, juicy, leaky, dark red), both typologies co-existing to a different extent in the same animal. The air/(tissue+water) volume ratio was 3.64 in C and WA but decreased to 0.43 in MA regions. Lung vascularization, estimated from red blood cells/tissue volume ratio (corrected for hematocrit) was 0.48 in C but sharply decreased to 0.16 in MA regions. mRNA analysis showed that, relative to C, HIF 1a transcript was unchanged in WA but increased by 33% in MA regions. Endot1 increased by 40% in WA and about 800% in MA. Pip averaged ñ 13.7±2.03, 5±1 and 15±3.2 cmH2O in C, WA and MA regions, as judged from in vivo-imaging through the pleural window. Thus, in MA regions the respiratory function appears greatly impaired by a parallel decrease in alveolar volume and vascularisation. The latter may reflect the increase in Endot1 but also the impairment of capillary patency due to the increase in Pip. We suggest that the degree of pulmonary hypertension might reflect two combined effects leading to an overall increase in pulmonary vascular resistance: 1) a decrease in vascular bed proportional to the extent of MA regions, 2) an increase in vasoconstriction in WA regions to protect pulmonary capillaries from an excessive increase in vascular pressure. 2009.

PULMONARY HYPERTENSION LIMITS EXERCISE CAPACITY IN HYPOXIC CONDITIONS. Robert R Naeije, Vitalie Faoro, Michel Lamotte, Kathleen Retailleau, Sandrine Huez, Claire de Bisschop, Saroj Neupane, Jean-Benoit Martinot. Free University of Brussels. *Email: rnaeije@ulb.ac.be.* We tested the hypothesis that hypoxic pulmonary hypertension limits exercise capacity in healthy subjects. An incremental cycle ergometer cardiopulmonary exercise test together with echocardiographic estimation of pulmonary vascular resistance were performed before and after the intake of sitaxsentan, a selective ETA blocker, 100 mg/day during 7 days, in normoxia (n=13), in acute normobaric hypoxia (one hour of breathing 12 % of oxygen in nitrogen, n=13) and at the "Pyramid hut", at the altitude of 5050 m in Nepal (n=22). The subjects were randomised to the intake of placebo or sitaxsentan and to alternated normobaric normoxic and hypoxic conditions following a cross-over design at sea level, and directly randomised in placebo or sitaxsentan after baseline measurements at 5050 m. Normobaric as well as hypobaric hypoxia increased pulmonary vascular resistance (PVR) and decreased maximum workload and oxygen uptake (VO_2max). In both acute and chronic hypoxia, sitaxsentan inhibited the hypoxia-induced increase in PVR (from 4.2 ± 0.3 mmHg/L/min to 3.2 ± 0.3 mmHg/L/min and 4.6 ± 0.3 mmHg/L/min to 3.3 ± 0.2 mmHg/L/min ($p<0.001$)) and increased VO_2max (from 32 ± 2 ml/min/kg to 36 ± 2 ml/min/kg ($p<0.01$) and 27 ± 1 ml/min/kg to 29 ± 1 ml/min/kg ($p<0.05$)) (mean ± SE). The hypoxia-induced decrease in VO_2max was restored by 30 % in acute normobaric hypoxia 10 % in chronic hypobaric hypoxia. Sitaxsentan-induced changes in PVR and VO_2max were correlated ($p = 0.01$). We conclude that ETA-mediated signalling in hypoxia contributes to pulmonary hypertension and partially limits exercise capacity in healthy subjects in

acute and chronic hypoxia. This study was supported by Encysive Pharmaceuticals, and carried out within the framework of the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology, Nepal and Italy, and thanks to contributions from the Italian National Research Council. 2009.

PULMONARY VASCULAR REGULATION AND SHUNTING: COMPARISON OF SEA-LEVEL INHABITANTS TO SHERPAS. Glen Foster[1,2], Philip Ainslie[2], Mike Stembridge[3], Trevor Day[4], Akke Bakker[5], Samuel Lucas[6], Nia Lewis[2], Keith Burgess[7], David MacLeod[8], Andrew Lovering[9]. [1]School of Kinesiology, Univ British Columbia, [2]School of Health and Exercise Science, Univ British Columbia, [3]School of Sport, Cardiff Metropolitan Univ, [4]Dept Biology, Mount Royal Univ, [5]MIRA Institute, Univ Twente, [6]Dept Physiology and School of Physical Education, Univ Otago, [7]Dept Medicine, Univ Sydney, [8]Dept Anesthesiology, Duke Univ, [9]Dept Human Physiology, Univ Oregon. *EMAIL: glen.foster@ubc.ca*
INTRODUCTION: We examined the hypothesis that pulmonary hemodynamics, intracardiac and intrapulmonary shunting differ in Sherpas permanently residing at high altitude (HA) when compared to sea-level (SL) inhabitants. We assessed the presence of intracardiac and intrapulmonary shunt, and measured pulmonary hemodynamics in SL inhabitants at SL (SL-SL; n=17; age=30±7yrs; birth altitude=185±277m), in a subset (n=8) of SL subjects during 3wks of acclimatization (SL-HA) to 5050m, and in Sherpas at 5050m (Sh-HA; n=14; age=34±15yrs; birth altitude=3931±425m). METHODS: Measures of pulse oximetry, heart rate (ECG), pulmonary artery systolic pressure (PASP; triscuspid regurgitation), and cardiac index (Q_i; pulsed wave Doppler) were made during 5min of rest at SL and HA, and during 5min of hyperoxia (FiO_2=1.0; Sh-HA only). Intracardiac and intrapulmonary shunt was evaluated by agitated saline contrast echocardiography during rest and on release of valsalva. RESULTS: The prevalence of intracardiac shunt was similar between SL-SL (41%) and Sh-HA (50%). Intrapulmonary shunt at rest was found in 4 out of 7 Sh-HA and 1 out of 8 SL-HA. Hyperoxia reversed intrapulmonary shunt in 1 out of 4 Sh-HA. PASP was similar between Sh-HA (30.0±6.0 mmHg) and SL-HA (32.7±4.2 mmHg; P=0.27), but greater than SL-SL (19.4±2.1 mmHg; P<0.001). In Sh-HA, Q_i was greater compared with SL-HA (2.24±0.49 vs. 1.91±0.17 l/min/m^2; P=0.04) but similar to SL-SL (2.21±0.40 l/min/m^2; P=0.85). Hyperoxia reduced PASP and Q_i in Sh-HA (-5mmHg; -0.53 l/min/m^2, respectively). CONCLUSION: In conclusion, Sh-HA has similar prevalence of intracardiac shunt and PASP compared to SL-SL and SL-HA, respectively. Despite elevated PASP and hypoxemia the incidence of intrapulmonary shunt in both Sh-HA and SL-HA was low compared with studies of acute hypoxia. ACKNOWLEDGEMENTS: Ev-K2-CNR Project (Nepal Academy of Science and Technology, Italian National Research Council), HSFC, MSFHR, NSERC, APS Giles F. Filley Memorial Award. 2015.

RADIOSENSITIZATION OF HYPOXIC BREAST CANCER CELLS WITH PHYTOCHEMICALS AND THEIR ANALOGUES INVOLVES REL PROTEIN REGULATION. Natarajan Aravindan[1], Joseph Balthazar[1], Sheeja Aravindan[1], Jamunarani Veeraraghavan[1], Terence Herman[1], Mohan Natarajan[2]. [1]University of

Oklahoma Health Sciences Center, [2]University of Texas Health Science Center at San Antonio. *Email: naravind@ouhsc.edu*. Introduction: Heterogeneously distributed hypoxic areas are a characteristic property of locally advanced breast cancers (BCa) and generally associated with therapeutic resistance, metastases, and poor patient survival. About 50% of locally advanced BCa, where radiotherapy is less effective are suggested to be due to hypoxic regions. In this study, we investigated the potential of bioactive phytochemicals in radiosensitizing hypoxic BCa cells. Methods: Hypoxic (O_2-2.5%; N2-92.5%; CO_2-5%) MCF-7 cells were exposed to 4Gy radiation (IR) alone or after pretreatment with Curcumin (CUR), curcumin analog EF24, neem leaf extract (NLE), Genistein (GEN), Resveratrol (RES) or raspberry extract (RSE). The cells were examined for inhibition of NFkB activity, transcriptional modulation of 88 NFkB signaling pathway genes, activation and cellular localization of radioresponsive NFkB related mediators, Nos3, Erk1/2, SOD2, Akt, p50, p53, p65, pIkBα, TNFα, IAP1, IAP2 and Survivin and, associated induction of cell death. Results: EMSA revealed that cells exposed to phytochemicals showed complete suppression of IR-induced NFkB. Relatively, cells exposed EF24 revealed a robust inhibition of IR-induced NFkB. QPCR profiling showed induced expression of 59 NFkB signaling pathway genes after IR. Conversely, 58, 55, 59, 58, 57 and 59 of IR-induced genes were completely inhibited with EF24, NLE, CUR, GEN, RES and RSE respectively. In addition, 20, 24, 22, 15, 9 and 17 of 29 IR-suppressed genes were further inhibited with EF24, NLE, CUR, GEN, RES and RSE respectively. Immunoblotting revealed a significant attenuating effect of IR-modulated radioresponsive Nos3, Erk1/2, SOD2, Akt, p50, p53, p65, pIkBα, TNFα, IAP1, IAP2 and Survivin with EF24, NLE, CUR, GEN, RES or RSE. More importantly, Annexin V-FITC staining showed a consistent and significant induction of IR-induced cell death with these phytochemicals. Notably, EF24 robustly conferred IR-induced cell death. Conclusion: Together, these data identifies the potential hypoxic cell radiosensitizers and further implies that the induced radiosensitization may be exerted by selectively targeting IR-induced NFkB signaling. 2011.

RAPID ASCENT OF KILIMANJARO BY THIRTY ALTITUDE NAIVE, ABLE AND DISABLED TREKKERS, WITHOUT EARLY MOUNTAIN MEDICAL INPUT: LESSONS LEARNED! Jack Kreindler, Laura Jackson, Hugh Montgomery. UCL Hospitals / Centre for Health & Human Performance, The University Hospital of Wales. *Email: jack@blueorange.net.* Charity ascents of Mt Kilimanjaro (5,985m) have become increasingly common, attracting diverse ages and abilities. This poses hazards. We discuss the risks, benefit and outcomes of a high profile, media driven, "first buddy-assisted climb" by disabled and able-bodied adults. Late involvement of altitude medicine experts revealed complex medical, legal and ethical issues. A full review was undertaken 12 weeks before departure. We identified inadequate consideration of: impact of ascent profile, altitude illness, effect of prolonged exertion, self-reporting of acute mountain sickness (AMS), uses of acetazolamide, psycho-social dynamics on team cohesion, and obtaining true informed consent. Historic data predicted >50% risk of failure for a day-6 summit. Despite warnings the itinerary was not changed so an aggressive preventative approach was taken: We

instigated psycho-social and bio-physical assessment including maximal cardiopulmonary exercise testing. The Lake Louise system was modified to emphasise early detection of AMS. Climbers were educated and their expectations corrected by professional guides and two accompanying mountain medics supported by the UK Diploma in Mountain Medicine. Summit numbers were better than predicted at 60% including 3 of the 4 disabled climbers with cognitive impairment. However, severe AMS forced two evacuations, milder AMS was widespread as were minor physical and emotional complaints that threatened the expedition. Anecdotally, psycho-social benefits were reported and successfully documented on film, but in future early mountain medical input and sensible ascent profiles would improve the risk benefit ratio. In response, we have developed an interactive education tool to aid non-expert organisers and those responsible for vulnerable mountaineers. Funding sources: The Centre for Health & Human Performance, London. 2009.

REDOX-DEPENDENT METABOLIC AND FUNCTIONAL REMODELLING IN MOUSE DIAPHRAGM FOLLOWING CHRONIC SUSTAINED HYPOXIA. Philip Lewis[1], David Sheehan[2], Ken O'Halloran[3]. [1]Department of Physiology, University College Cork, Ireland, [2]School of Biochemistry & Cell Biology, University College Cork, Ireland, [3]Department of Physiology, University College Cork. *Email: k.ohalloran@ucc.ie.* Introduction: Chronic sustained hypoxia (CH) is a feature of respiratory disease. CH induces mitochondrial remodelling, fibre-type transitions, atrophy and altered contractile and endurance properties in the diaphragm muscle. We hypothesize that reactive oxygen species are pivotal in diaphragm muscle adaptation to CH. Methods: Adult male C57BL6/J mice were exposed to normoxia or CH (FiO2=0.1) for 1, 3 or 6 weeks. Diaphragm samples were subsequently profiled using a redox proteomics approach followed by mass spectrometry. Redox modified metabolic enzyme activities were assessed. Diaphragm isotonic contractile performance was assessed ex vivo after 6 weeks of CH with or without chronic antioxidant supplementation. Results: Protein carbonyl and free thiol content in the diaphragm were increased and decreased respectively after 6 weeks of CH – indicative of protein oxidation. CH caused extensive remodelling of proteins key to contractile, metabolic and homeostatic functions. Several oxidative and glycolytic enzyme activities in the diaphragm were decreased by CH. Redox-sensitive chymotrypsin-like activity of the diaphragm was increased. Atrophy signalling was observed through decreased phospho-FOXO3a and phospho-mTOR. HIF-1alpha and phospho-p38 MAPK (but not phospho-Akt or phospho-ERK1/2) content was increased in CH diaphragm and this was attenuated by antioxidant treatment. CH exposure decreased force- and power-generating capacity of the diaphragm and this was prevented by antioxidant supplementation with N-acetyl cysteine. Conclusion: Redox remodelling is pivotal for diaphragm (mal)adaptation to CH. Atrophy signalling through p38MAPK-FOXO3a with resultant muscle weakness is a feature of CH. Antioxidant supplementation may be useful as an adjunctive therapy in respiratory-related diseases characterised by hypoxic stress. Acknowledgements: Funded by the Health Research Board (Ireland) and the University College Cork Strategic Research Fund. 2015.

REDUCED CEREBRAL OXYGEN AVAILABILITY LIMITS EXERCISE PERFORMANCE OF LOWLANDERS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) TRAVELLING TO 2590M. Michael Furian[1], Sara E. Hartmann[2], Tsogyal D. Latshang[1], Deborah Flueck[1], Phillipp M. Scheiwiller[1], Séverine Mueller-Mottet[1], Silvia Ulrich[1], Malcolm Kohler[1], Marc J. Poulin[2], Konrad E. Bloch[1]. [1]Pulmonary Division and Sleep Disorders Center, University Hospital of Zurich, [2]Dept of Physiology & Pharmacology and Hotchkiss Brain Institute, Faculty of Medicine, University of Calgary, Calgary, Canada. *Email: michael.furian@usz.ch.* Introduction: The aim of the current study was to quantify exercise performance of lowlanders with COPD during a stay at moderate altitude and to investigate whether reduced cerebral oxygen availability limits performance. Methods: 31 patients with COPD (mean±SD, FEV1 56±15%), underwent constant-load bicycle ergometry to exhaustion at 60% of maximal work rate (65±29Watts) in Zurich (490m) and Davos (2590m), after spending one night there. The order of altitude exposure was randomized. Pulmonary gas exchange, arterial blood gas analysis, cerebral tissue oxygenation (CTO) by near-infrared spectroscopy and middle cerebral artery peak blood flow velocity (Vpeak) by transcranial Doppler ultrasound were measured. The final 30sec of exercise were compared between altitudes. Results: At 2590m the median endurance time was significantly reduced to 205 sec (quartiles 139;297) compared to 500 sec (256;795) at 490m. Mean (±SD) arterial and cerebral tissue oxygen saturation at 2590m were lower (85±5%; 55±7%) than at 490m (93±4%; 61±7%), and $PaCO_2$ was also reduced (4.7±0.5kPa vs. 5.6±0.9kPa) ($p<0.05$ vs 490m, all comparisons) while minute ventilation at 2590m was increased (50±15L/min vs 44±14L/min, $P<0.05$). The exercise induced increase in Vpeak was similar at both altitudes (+23±20% vs +27±25%, P=ns) while Vpeak sensitivity to exercise-mediated hypoxia was reduced at 2590m (3±3 %/% desaturation) compared to 490m (9±21 %/% desaturation) ($p<0.05$). Conclusion: In lowlanders with COPD travelling to 2590m, exercise endurance was reduced by 60% compared to 490m. Our data suggest a role of reduced cerebral oxygen availability in limiting exercise performance at 2590m since the blood flow response to hypoxia during exercise was not enhanced (possibly related to hypocapnia) despite a greater degree of arterial and cerebral tissue deoxygenation at 2590m compared to 490m. Acknowledgements: Grant:Swiss National Science Foundation, Lung League Zurich. 2015.

REDUCED CEREBROVASCULAR REACTIVITY IN AREAS SUSCEPTIBLE TO HYPOXIA AND ISCHEMIA. Jay Han, Daniel Mandell, Julien Poublanc, Adrian Crawley, Alexandra Mardimae, Joseph A Fisher, David Mikulis. University of Toronto. *Email: Jay.han@uhn.on.ca.* Paradoxical reductions in blood flow in response to cerebral vasodilatory stimuli have been demonstrated in the periventricular white matter (PVWM) (where elderly patients with dementia and stroke develop leukoariosis) but not in sub-cortical white matter (SCWM) (1). We set out to administer a standard, repeatable stimulus and document values for normal cerebrovascular reactivity (CVR; change in cerebral blood flow (CBF) (for a given vasoactive stimulus) in white matter in 6 healthy subjects (age 20 - 50). We assessed CVR using a 3T MR to generate blood oxygen dependent (BOLD) signal as the

surrogate for CBF. We used a custom gas blender (RespirAct™, TRI, Toronto Canada) and a sequential rebreathing circuit to apply two ventilatory sequences consisting of normoxic (100 mmHg) near-square wave changes in target end-tidal PCO_2 ($PETCO_2$), first stepping between 30 and 40, and then 40 and 50 mmHg. Steps were of 45 - 120 s duration. In post hoc brain segmentation analysis, PVWM and SCWM regions of interest where identified from two axial sections above the head of the caudate nucleus. CVR was quantified as $\%f$ BOLD signal/f $PETCO_2$ and mapped voxel by voxel. Hypercapnic stimuli showed reduced CVR in PVWM compared to the SCWM (0.105 ± 0.034 vs. 0.145 ± 0.03 respectively, P = < 0.05; mean ± SD). Hypocapnic stimuli did not affect CVR (0.120 ± 0.042 vs 0.116 ± 0.027, P = > 0.05, in PVWM and SCWM, respectively). PVWM is supplied by the long penetrating arteries which lack distal collateralization. This may account for its reduced vascular reserve compared to that of the SCWM. The data supports the concept (1) that the PVWM is more susceptible to developing ischemic demyelination than the SCWM. (1) Mandell DM. et al. Stroke 2008 July;39(7):1993-8. 2009.

REDUCED SUBLINGUAL MICROCIRCULATORY BLOOD FLOW AT ALTITUDE. Daniel Martin, Peter Goedhart, Andre Vercueil, Denny Levett, Can Ince, Mike Grocott, for the Caudwell Xtreme Everest Investigator Group. University College London, Academic Medical Center, University of Amsterdam, King's College Hospital. *Email: dan.s.martin@gmail.com.* Objective: We set out to measure blood flow within the sublingual (SL) microcirculation in a group of healthy volunteers ascending to and remaining at high altitude (5300m). Methods: A light emitting microscope probe, side-stream darkfield imaging (SDF), was used to capture moving images of the SL microcirculation in rested subjects (n=24) at 75m, 3500m (day 5) 5300m (day 16) and 5300m (day 70). Peripheral oxygen satfration (SpO_2) was measured using a pulse oximeter and haematocrit (Hct) was determined by measuring packed cell volume. From the stored images SL microcircultory flow index (MFI) [normal = 3, slow flow = 2, intermittent flow = 2, no flow = 0] was calculated for small (10-25μm) and medium (51-100μm) sized vessels according to previously described and validated guidelines. Results: Compared to 75m, median MFI was significantly reduced at all altitudes in both small ($p<0.0001$) and medium ($p=0.006$) sized vessels. The greatest reduction from baseline MFI (2.83 and 2.86) was seen at 5300m on day 70 (2.45 and 2.43 in small and medium vessels respectively). Mean SpO_2 was reduced and mean Hct increased at all altitudes ($p\leq0.001$ and $p<0.0001$ respectively). There was no direct correlation between MFI and Hct or MFI and SpO_2 at any altitude. Conclusions: Using SDF imaging, volunteers exposed to hypoxia at high altitude demonstrated significantly reduced SL blood flow in small and medium sized vessels within the microcirculation. The reduction was greatest after 70 days at altitude. This reduction may be related to increased blood viscosity secondary to a raised Hct. Alterations in blood flow within the microcirculation may contribute to the reduced oxygen consumption noted at altitude despite the normalisation of arterial oxygen content that accompanies acclimatisation. 2009.

REDUCED UTERINE ARTERY BLOOD FLOW RESTRICTS FETAL GROWTH IN GESTATIONAL HYPERTENSION AND PREECLAMPSIA AT HIGH ALTITUDE. Vaughn A Browne, Lilian Toledo-Jaldin, R. Daniela Dávila, Luis P Lopez, Henry Yamashiro, Darleen Cioffi-Ragan, Colleen G Julian, Megan J Wilson, Benjamin Honigman, Enrique Vargas, Robert Roach, Lorna G Moore. Altitude Research Center, University of Colorado Denver, Instituto Boliviano de Biologia de Altura, Wake Forest University. *Email: Vaughn.Browne@UCDenver.edu.* We recently reported that high-altitude resident Andean women who developed gestational hypertension (GH) or preeclampsia (PE) had lower uterine artery blood flow (QUA) and a higher frequency of small for gestational age (SGA) babies than normotensive controls. Here, we asked whether fetal biometry measured contemporaneously with QUA cross-sectionally at 20, 24, 28, 32, and 36 weeks of pregnancy revealed evidence of growth restriction long before delivery. We measured fetal head circumference (HC), biparietal diameter (BPD), occipitofrontal diameter (OFD), abdominal circumference (AC), femur length (FL), heart rate (FHR), and analyzed Doppler waveforms from middle cerebral (MCA) and umbilical (UmbA) arteries in normotensive controls (NORM, n=155), GH (n=11), and PE (n=21). Scatter plots and trend lines relating HC, BPD, and OFD to gestational age were similar in the three groups. However, AC and FL were smaller, and the HC/AC ratio was larger in PE and GH than in NORM at 24 weeks ($P<0.01$). FHR and UmbA peak systolic velocity (PSV) were lower in PE and GH than in NORM in each gestational age bin ($P<0.01$), while MCA PSV and the MCA/UmbA PSV ratios were higher in PE and GH, except at 36 weeks. HC was the same at birth in all groups, indicating brain sparing, but overall length was shorter in GH or PE infants. Taken together, these data suggest that fetal growth restriction was already evident in early-onset (≤34wk) PE and GH, while fetuses appeared to grow normally before symptoms developed in late-onset (>34 wk) PE and GH. Fetal cardiac output was reduced in PE and GH, and was redistributed away from the axial skeleton toward the brain. We conclude that restricted fetal growth was the consequence and not the cause of reduced QUA. 2009.

REDUCING END-TIDAL-ARTERIAL PCO2 GRADIENT IN SICK PIGLETS: A MODEL FOR NON-INVASIVE PCO2 MONITORING IN CRITICAL CARE. Joseph A Fisher[1], Jorn Fierstra[2], Jeff D Winter[3], Jelena Jovanovich[3], Andrea Kassner[3], Matthew Machina[4]. [1]Department of Anesthesiology, University Health Network and the University of Toronto, [2]Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht, the Netherlands, [3]Department of Physiology and Experimental Medicine, The Hospital for, [4]Department of Physiology, University of Toronto. *Email: joe.fisher@utoronto.ca*. Introduction: The PCO_2 in arterial blood ($PaCO_2$) is the best parameter for monitoring ventilation and acid-base changes in ventilated patients, but its measurement is invasive and expensive. Attempts have been made to use an inexpensive, easily obtained non-invasive measurement, the partial pressure of CO_2 in end-tidal gas ($PETCO_2$), as a surrogate for $PaCO_2$. Unfortunately, studies have revealed that the differences between $PETCO_2$

and $PaCO_2$ were too large and variable to be clinically useful. We hypothesized that end-inspiratory rebreathing, previously shown to equalize $PETCO_2$ and PaO_2 in healthy animals and humans, would enable $PETCO_2$ to be used as a surrogate for $PaCO_2$ in ventilated animals with lung and cardiac pathology. Methods: Eight newborn Yorkshire pigs with various combinations of acquired viral pneumonia, persistent patent ductus arteriosus, and patent foramen ovale were mechanically ventilated via a partial rebreathing circuit to implement end-inspiratory rebreathing. Arterial blood was sampled from an indwelling arterial catheter and tested for $PaCO_2$. A variety of combinations of end-tidal PCO_2 (30 to 50 mmHg) and PO_2 (35 to 500 mmHg) were tested for differences between $PETCO_2$ and $PaCO_2$ (PET-aCO_2). Results: The PET-aCO_2 of all samples was (mean ± 1.95SD) 0.4 ± 2.7 mmHg. The analysis of the paired data indicates that the chances of the observed limits of agreement occurring by chance are < 0.0001. Duplicate blood gas analysis indicated that most of the variability in Pet-aCO_2 can be accounted for by variability in arterial blood PCO_2 measurement. Conclusion: Our study is a proof of the concept that rebreathing at end-inspiration reduces PET-aCO_2 to a clinically useful range in a ventilated animal model with lung pathology and cardiac shunting. If verified clinically, this approach would reduce the frequency of arterial blood sampling required for the care of critically ill patients. 2011.

REDUCTION OF ACUTE HYPOXIC PULMONARY VASOCONSTRICTION BY ACETAZOLAMIDE AND N-METHYL ACETAZOLAMIDE IN TWO RAT MODELS OF PULMONARY HYPERTENSION. Rachel Zarndt[1], Erik Swenson[2]. [1]VA Puget Sound Health Care System, Seattle, Washington; University of California San Diego, La Jolla, California, [2]A Puget Sound Health Care System, Seattle, Washington; University of Washington, Seattle, Washington. *Email: rzarndt@ucsd.edu*. Introduction: Acetazolamide (ACZ), a carbonic anhydrase (CA) inhibitor, reduces hypoxic pulmonary vasoconstriction (HPV) by a mechanism independent of CA inhibition. We examined whether ACZ or its non-CA inhibiting analog, n-methyl acetazolamide (NMA), would alter pulmonary hemodynamics in two rat models of pulmonary hypertension (PH). Methods: Right ventricular pressure (RVP) and pulmonary vascular resistance (PVR) were measured in normoxic rats after three weeks treatment with placebo, ACZ or NMA. Rats with PH induced by either three weeks chronic hypoxia (FIO_2=0.10) or 60mg/kg monocrotaline were pretreated with placebo or ACZ. After baseline measures (FIO_2=0.21), anesthetized rats were administered an acute injection of ACZ or NMA between two 15 minute periods of hypoxia (FIO_2=0.12). Arterial and mixed venous blood and pressure measurements were taken to calculate cardiac output (Q) using the Fick equation, while PVR was estimated as RVP/Q. Results: Untreated, normoxic rats had no change in acute HPV with ACZ or NMA injection; however, chronic ACZ or NMA reduced acute HPV after an injection of ACZ (RVP: p=0.008), or NMA (RVP: p=0.0001, PVR: p=0.02). Baseline measures of ACZ- and NMA-pretreated normoxic rats revealed lower initial PVR than untreated controls (ACZ, FIO_2 =0.12, p=0.006; NMA, FIO_2 =0.21, p=0.03). While ACZ pretreatment was insufficient to significantly lower PVR in hypertensive rats, pretreat-

ment coupled with acute ACZ administration reduced HPV (RVP: p=0.02, PVR: p=0.06 - 0.09). Interestingly, the higher initial RVP observed in untreated, hypertensive rats resolved to untreated, normoxic control levels after administration of ACZ (monocrotaline: p<0.001). Conclusion: In summary, while neither ACZ nor NMA were sufficient in preventing the development of PH in chronic hypoxia or with monocrotaline, pretreatment combined with acute administration of ACZ or NMA alleviated acute HPV. Acknowledgements: Funding: VA Merit Review and TH Maren Foundation. 2011.

REGIONAL DIFFERENCES IN HUMAN CEREBRAL BLOOD VELOCITY IN RESPONSE TO HYPERTHERMIA. Patrick LL McDonald[1], Jesse G Greiner[1], Michael L Walsh[1], Matthew D White[1]. [1]Simon Fraser University. *Email: matt@sfu.ca*. Introduction: OBJECTIVE/HYPOTHESIS: To assess changes in cerebral blood velocity (CBV) in the middle cerebral artery (MCAv), posterior cerebral artery (PCAv), and basilar artery (BAv) during each of normothermic poikilocapnia (NT-PC), normothermic eucapnia (NT-EC), hyperthermic poikilocapnia (HT-PC) and hyperthermic eucapnia (HT-EC). It was hypothesized that CBV would decrease in MCA but not in the PCA or BA during hyperthermia. Methods: METHODS: Six male volunteers (Ht: 180.5 ± 4.2 cm; Wt: 77.8 ± 12.6 kg; Age: 22.2 ± 1.5 yr; mean ± SD) with NT and HT esophageal temperatures (TES) had CBV assessed with a transcranial Doppler. End-tidal CO_2 ($PETCO_2$) in EC was clamped with a breath-by-breath end-tidal forcing system. Each volunteer was passively rendered HT in a climatic chamber (~50°C, ~25% RH) while clad in a vapor impermeable suit. Analyses included a 2-way ANOVA (TES x $PETCO_2$) and means comparisons with 1-tailed, paired t-tests. The level of significance was set at α=0.05. Results: RESULTS: In both PC and EC TES increased from ~ 36.7 ± 0.3°C to ~38.0 ± 0.2°C (P<0.001). Elevating TES increased pulmonary ventilation by ~2 L/min in PC (p=0.06) and ~8 L/min in EC (P=0.03). $PETCO_2$ between NT-PC and HT-PC dropped from 41.6 ± 3.2 mmHg to 34.0 ± 4.2 mmHg (P=0.002) but remained elevated at ~42.5 mmHg (P>0.05) between NT-EC and HT-EC. There was a main effect of TES on MCAv (F=11.9, P=0.018) but no significant main effects on PCAv or BAv. The main effect of TES on MCAv was explained by significant decreases between NT-PC and HT-PC (47.5 ± 7.8 cm/s to 40.9 ± 7.1 cm/s; P=0.002) and between NT-EC and HT-EC (47.3 ± 7.9 cm/s to 42.5 ± 7.5 cm/s; P=0.04). There was no interaction between $PETCO_2$ and TES on MCAv. Conclusion: CONCLUSIONS: There were regional differences in blood velocity in the major cerebral vessels in response to hyperthermia. Cerebral autoregulation appears to maintain CBV in the PCA and BA but not in the MCA during passive hyperthermia. Acknowledgements: This study was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Canadian Foundation for Innovation. 2011.

REGULATION OF HIF BY OMEGA 3 FATTY ACIDS. Mary Taub[1], James E Springate[1], Facundo Cutuli[1]. [1]University at Buffalo, Buffalo, NY, USA. *Email: biochtau@buffalo.edu*. Introduction: The influence of omega-3 fatty acids (FAs) on growth of renal proximal tubule (RPT) cells has been studied and their influence

during hypoxia has been examined. This is particularly important because the Renal Cell Carcinoma (RCC) originates from the RPT. Moreover, the results of a 15.3 year epidemiological study indicate that a diet of fatty fish (rich in omega-3 FAs) protects against Renal Cell Carcinoma (RCC). Our hypothesis concerning the underlying molecular mechanism, is that omega-3 fatty acids elicit their effects by activating Peroxisome Proliferator-Activated Receptors (PPARs), and antagonizing Hypoxia Inducible Factor (HIF). The RCC is characterized by mutations inactivating VHL, resulting in increased HIF levels during normoxia. Methods: In order to examine this hypothesis, primary RPT cells have been examined with regards to their growth and functional properties in serum free medium. Results: The effects of PPAR gamma agonists including troglitazone, a TDZ, caused a 4-fold increase in growth, as well as Prostaglandin J2 (PGJ2; produced endogenously). In contrast, fenofibrate (a PPAR alpha agonist) had no significant affect. In order to examine our hypothesis that PPAR activation affects signaling through HIF, transient transfection studies were conducted with HRE-Luc, a HIF Regulatory Element/Luciferase construct. The level of luciferase expression via HRE-Luc increases during hypoxia. Our studies with HRE-Luc indicate that troglitazone reduces the increase in HRE-Luc gene expression under hypoxia by over 50%. Similarly, troglitazone prevented the EMT and reduction in beta catenin in hypoxia. Conclusion: Omega-3 FAs and PPAR gamma agonists stimulate growth of normal primary RPT cells, while preventing the epithelial to mesenchymal transition during hypoxia. Acknowledgements: NIH 1RO1 HL69676 to MT. 2011.

RELATION BETWEEN NITRATE AND NITRITE CONCENTRATION IN PLASMA AND OXYGEN SATURATION. Mirja Maassen[1], Marisa Nacke[2], Karina Sutmoeller[2], Henning Starke[2], Dimitrios Tsikas[3], Norbert Maassen[4]. [1]Insitute of Sport Sience, Univ Hanover, Germany, [2]Sportmedicin, Medical School Hanover, Germany, [3]Clin. Phamacology, Medical School Hanover, Germany, [4]Insitute of Sport sience, Univ Hanover, Germany. *EMAIL: Mirja.Maassen@sportwiss.uni-hannover.de* INTRODUCTION: According to Gladwin et al. hemoglobin has a nitrite reductase activity which is highest around the P50. Therefore the nitrite concentration should be dependent on the oxygen saturation. To investigate the relation between both, experiments under acute hypoxia were carried out. METHODS: 5 subjects (3 males and 2 females) served as controls under normoxia. To vary the SO_2 6 subjects (5 men and 1 woman) were connected to the "Hypoxiecator GO_2Altitude®" (BIOMEDTECH, Melbourne, Australia). The relative oxygen concentration in the inspired gas was reduced every 5 min (from 21% to 17%, 14.5%, 12%, 10.5% and 9%). In parallel blood samples were taken. Blood was sampled from a cubital vein. Acid base status was measured by an ABL 520 and plasma NO_2 and NO3 by mass-spectroscopy. In the hypoxia trial additionally [Lac] and [Glu] were determined. During both series blood flow was measured plethysmographically. No prescription related to food intake was given. To test significant differences between the time points (Oxygen concentrations) ANOVA with repeated measurements were performed. RESULTS: Normoxia: [NO_2-] and [NO3-] did not change significantly over time. Hypoxia: [NO_2-] and [NO3-] decreased significantly

in dependence on the decrease of O_2-content ($p < 0.05$; ([NO3-]: from 39.18 ± 7.59 to 38.55 ± 6.85 μM and [NO_2-] from 1.010 ± 0.142 to 0.839 ±0.068 μM). Both changes were related to the plasma pH ($p<0.05$). The change in NO3 did not correlate to SO_2 ($p= 0.56$), whereas, the difference in NO_2 correlated ($p<0.03$) significantly. Surprisingly, the change in [NO3-] correlated to the PCO_2 ($p=0.01$). No significant correlation between [NO3-] and [NO_2-] could be found CONCLUSION: Nitrate and Nitrite may serve as pool for NO production if the activity of NO-synthase is reduced by hypoxia. 2015.

RELATIONSHIP BETWEEN OXIDATIVE STRESS AND ACUTE HYPOXIC VENTILATORY RESPONSE IN HUMANS: EFFECT OF EXPOSURE TO INTERMITTENT HYPOXIA. Vincent Pialoux, Glen E Foster, Julien V Brugniaux, Cailean T Duggan, Pat J Hanly, Marc J Poulin. University of Calgary. *Email: vpialoux@ucalgary.ca.* Chronic intermittent hypoxia (CIH) is characterized by repeated episodes of hypoxia/reoxygenation, which are known to increase the acute hypoxic ventilatory response (AHVR). Animal studies suggest that oxidative stress may modulate AHVR by increasing carotid body sensitivity to hypoxia. The objective of this study was to determine i) whether four days of exposure to CIH increases AHVR and oxidative stress, and ii) the strength of the association between oxidative stress and AHVR. Ten men (29.3±1.7 years; BMI: 25.6±0.4 kg.m-2; mean±SD) were studied. Following two normoxic control days (D-4 and D0, baseline), all subjects were exposed to 4 days (D1 to D4) of CIH while awake (2 min at $PETO_2$=45 Torr and 2 min at $PETO_2$=88 Torr) 6 hours·d^{-1}. AHVR was determined using an isocapnic hypoxia protocol, consisting of seven 90-sec steps of $PETO_2$ over the range of 88 to 45 Torr. AHVR was assessed as the slope of the linear regression relating ventilation to oxygen desaturation. Oxidative stress was evaluated by measuring 8-Hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyes (MDA) and antioxidant enzymatic activities (AEA) in the plasma. All variables were analyzed by one-way repeated measures ANOVA. A Pearson's correlation test was used to analyze the relationship between oxidative stress markers and AHVR. Between baseline (mean D-4, D0) and D4, there was a significant increase in both AHVR (+83%, p=0.016) and 8-OHdG (+40%; p=0.02) whereas AEA were unchanged. Furthermore, the changes in AHVR and 8-OHdG were significantly correlated (r=0.88, p=0.002). These findings suggest that the over-generation of ROS contributes to oxidative stress in CIH. Furthermore, this human study also corroborates previous reports from animal studies that ROS over-production induced by CIH modulates increased ventilatory sensitivity to hypoxia. Supported by AHFMR and HSFC. 2009.

RELATIONSHIP OF BLOOD PRESSURE AND HYPERTENSION TO ACUTE MOUNTAIN SICKNESS. Linda Keyes[1], Sushil Pant[2], Nirajam Regmi[3], Jennifer Starling[1], Devlin Cole[4], Charles Duke[5], Luke Mather[6], Theodore McConnell[7], Matthew McElwee[4], Purshotam Paudel[8], Benoit Phelan[9], Douglas Sallade[10], Alison Sheets[11], David Twillman[1], David Young[1], Buddha Basnyat[3]. [1]Univ Colorado, [2]Khunde Hosptial, [3]Nepal Intl Clinic, [4]Case Western Reserve Univ, [5]Univ Tennessee,

[6]Univ Washington, [7]McGill Univ, [8]Tribhuwan Univ, [9]Dalhousie Univ, [10]Philadelphia College Osteopathic Medicine, [11]Longmont United Hospital. *Email: linda.keyes@aya.yale.edu.* Introduction: Hypertension has been suggested as a possible risk factor for AMS (Ledderhos 2002). We sought to determine if a relationship exists between blood pressure (BP), hypertension (HTN) and acute mountain sickness (AMS). Methods: This was an observational cohort study of subjects with and without HTN. BP, heart rate (HR), oxygen saturation (SpO2) and Lake Louise Score for AMS (LLS) were measured at 2,800m, 3,400m and 4300-4,400m in trekkers in Nepal. Hypertensive subjects (H) were defined as those with self-reported diagnosis of HTN. AMS was defined as LLS $\leq$3 with headache. MAP was estimated by the formula: MAP = [(2 x diastolic)+systolic] / 3. Results: We enrolled a total of 670 subjects; those with no HTN (NH, n=604), HTN (H, n=60), or reported borderline HTN (BH, n=6). BP values were similar in H and BH group and these were treated as one group for the analyses. A total of 528 subjects (H=48) were reevaluated at 3400m and 365 subjects (H=30) at 4400m. 107 (20%) subjects were taking acetazolamide (ACZ) at 3400m and 118 (32%) at 4400m. The overall prevalence of AMS was 16% at 3400m and 19% at 4400m. Of those with AMS at 3400m, 28% were taking ACZ and at 4400m 43% of those with AMS were taking ACZ. The prevalence of AMS was not different in those with HTN vs. those without (17% vs. 19% at 3400m, 23% vs. 14% at 4400m, $p>0.05$). There was no relationship between SBP, DBP or estimated MAP and AMS ($p>0.05$) at any altitude. SpO_2 was associated with AMS at 4400m ($P<0.05$) but not at 3400m ($p>0.05$). Conclusion: Neither absolute BP or history of HTN were associated with increased risk of AMS in this large cohort study of trekkers in Nepal. The prevalence of AMS in our study was lower than previously reported in this region, in part due to the proportion of subjects on ACZ, though interestingly a sizable fraction on ACZ still had AMS. Acknowledgements: Wilderness Medicine Society, Nepal International Clinic. 2015.

REMOTE, MOBILE TELEMEDICINE: THE SATELLITE TRANSMISSION OF MEDICAL DATA FROM MOUNT EVEREST. Jin Jong Chen[1], Terry B.J. Kuo[1], Bwo-Lwun Jang[1], Chih-Hao Huang[1], Wei Fong Kao[1]. [1]National Yang-ming University, Taipei, Taiwan. *Email: jinjong.chen518@gmail.com.* Introduction: The difficulty of a mountain and trekking trip lies in altitude and terrain. Expedition peaks are mentally and physically extremely demanding. Hiking in the high altitude, people would suffer in kinds of conditions, and hypoxia is typical one. The purpose of this investigation was to demonstrate the potential of remote, mobile telemedicine during an eight-week, high altitude mountaineering expedition to Mount Everest, world's highest summit. Methods: A 20 grams mini-wireless multichannel physiological signal recorder which can sustain for over 20 days operation without recharged had been designed for this special event. Using a mobile satellite terminal and a laptop computer both powered by a photovoltaic solar panel, ECG tracings were transmitted during the course of the ascent. One touch cell phone dialing mechanism was designed for easy signal sending operation. The data were transmitted via a mobile communications satellite to a ground station in Taiwan, a distance of over 4000 km. The data were then transferred to the public switched data

network and delivered to the National Yang-Ming University for analysis with only one second delay. Similarly, data were transmitted from the ground station to the expedition team on Mount Everest throughout the ascent. Results: Under the telemedicine monitoring, the Taiwan Everest Expedition team had finished the operation safely and successfully. Using this technique, medical diagnosis and emergency care can be facilitated in extreme and isolated locations lacking a telecommunications infrastructure. Conclusion: Such technology has applications in developing countries, disaster response efforts, remote civilian and military operations, and in space operations. 2011.

REPEATED PRE-SYNCOPE FROM INCREASED INSPIRED CO_2 IN A BACKGROUND OF SEVERE HYPOXIA: A CASE-REPORT. Jui-Lin Fan[1], Bengt Kayser[1]. [1]Univ Geneva, Geneva, Switzerland. *EMAIL: Jui-Lin.Fan@unige.ch* INTRODUCTION: Syncope is defined as a transient loss of consciousness followed by rapid recovery, and it accounts for a significant portion of emergency department visits and hospital admissions. METHODS: We describe a case of experimentally induced pre-syncope in a healthy young man when exposed to increased inspired CO_2 in a background of hypoxia. We measured partial pressure of end-tidal CO_2 ($PETCO_2$) andO_2 ($PETO_2$), arterial O_2 saturation (SPO_2), arterial blood pressure (ABP), heart rate (HR) and middle cerebral artery velocity (MCAv: index of cerebral blood flow, CBF). RESULTS: Acute severe hypoxia (FIO_2=10%) was tolerated and lowered $PETO_2$, $PETCO_2$ and SPO_2 during the two episodes by 51-58 mmHg, 3-4 mmHg and 18-19 %, respectively. At the same time, hypoxia lowered mean ABP, systolic ABP, diastolic ABP and HR by 12-20, 15-35, 11-17 mmHg and 12-9 b/min, while mean, systolic and diastolic MCAv remained relatively unchanged with hypoxia. At the end of the period of added CO_2 in hypoxia, when pre-syncope developed, mean, systolic and diastolic ABP were further lowered by 9-18, 14-30 and 3-15 mmHg, respectively, while HR remained relatively unchanged compared to hypoxia. Mean and diastolic MCAv dropped with added CO_2 by 15-20% and 51-55%, respectively, while both systolic MCAv remained relatively unchanged. Cardiac cycle time was reduced with CO_2 breathing in hypoxia, which was predominantly mediated by a reduced time in diastole. When pre-syncope symptoms were strongest (when the subject came off the mouthpiece) there was bradycardia and hypotension. We observed a distinct tri-phasic pattern in the MCAv traces, which resulted in slight retrograde blood flow at the end of systole. The mismatch of cerebral perfusion pressure and vascular tone caused a reduction in cerebral tissue oxygen saturation CONCLUSION: We speculate that this occurrence of pre-syncope was due to hypoxia-induced inhibition of brain regions responsible for compensatory sympathetic activity to relative hypercapnia. ACKNOWLEDGEMENTS: This study was supported by the Swiss National Science Foundation and the Fondation de Reuter. 2015.

REPEATED SPRINT TRAINING IN HYPOXIA IS MORE EFFICIENT THAN IN NORMOXIA. Raphael Faiss[1], Bertrand Léger[2], Olivier Dériaz[2], Grégoire P Millet[3]. [1]1:ISSUL-Dept Physiology, Faculty of Biology and Medicine, Univ

Lausanne, Switzerland 2:Institute for research in rehabilitation, SuvaCare Rehabilitation Clinic, Sion, Switzerland, [2]Institute for research in rehabilitation, SuvaCare Rehabilitation Clinic, Sion, Switzerland, [3]ISSUL-Dept Physiology, Faculty of Biology and Medicine, Univ Lausanne, Switzerland. *EMAIL: raphael.faiss@unil.ch* INTRODUCTION: While intermittent hypoxic training (IHT) was reported to evoke cellular responses via hypoxia inducible factors (HIFs) but without substantial performance benefits in endurance athletes, we hypothesized that repeated sprint training in hypoxia could enhance repeated sprint ability (RSA) in normoxia via molecular adaptations and improved blood perfusion especially in highly recruited fast twitch fibers. METHODS: 40 trained subjects completed 8 cycling repeated sprint sessions in hypoxia (RSH, 3000 m) or normoxia (RSN, 485 m). Before (Pre-) and after (Post-) training, muscular levels of selected mRNAs were analyzed from resting muscle biopsies and RSA tested until exhaustion (10-s sprint, work-to-rest ratio 1:2) with muscle perfusion assessed by near-infrared spectroscopy. RESULTS: From Pre- to Post-, the average power of all sprints during the RSA test increased ($p<0.01$) to the same extent (+6% vs. +7%, NS) in RSH and in RSN, respectively; but the number of sprints prior to exhaustion was increased in RSH (9.4 ± 4.8 vs. 13.0 ± 6.2, $p<0.01$) but not in RSN (9.3 ± 4.2 vs. 8.9 ± 3.5, NS). mRNA concentrations of HIF-1α (+55%), carbonic anhydrase III (+35%) and monocarboxylate transporter-4 (+20%) were augmented ($p<0.05$) whereas mitochondrial transcription factor A (-40%), peroxisome proliferator-activated receptor gamma coactivator 1α (-23%) and monocarboxylate transporter-1 (-36%) were decreased ($p<0.01$) in RSH only. Besides, the changes in total hemoglobin variations (Δ [tHb]) during sprints throughout RSA test increased to a greater extent ($p<0.01$) in RSH. CONCLUSION: Our investigation reports for the first time superior repeated sprint performance improvement after repeated sprint training performed in hypoxia (RSH) rather than in normoxia. RSH delayed fatigue in a repeated sprint test to exhaustion with larger muscle perfusion variations in active muscles and significant molecular adaptations. 2015.

REPRODUCIBILITY OF ACUTE MOUNTAIN SICKNESS, A CONTROLLED PROSPECTIVE STUDY IN CHILDREN AND ADULTS. Emrush Rexhaj[1], Sophie Garcin[1], Stefano Rimoldi[1], Hervé Duplain[1], Thomas Stuber[2], Yves Allemann[2], Claudio Sartori[1], Urs Scherrer[1]. [1]Department of Internal Medicine Lausanne-CHUV, Switzerland, [2]Swiss Cardiovascular Center Bern, Switzerland. *Email: Emrush.Rexhaj@chuv.ch*. Introduction: While a history of previous acute mountain sickness (AMS) is commonly used for providing advice and recommending its prophylaxis during subsequent exposure, the intra-individual reproducibility of AMS during repeated high-altitude exposure has never been examined in a prospective controlled study. Methods: In 27 non-acclimatized children and 29 adults, we assessed AMS during the first 48 hours after rapid ascent to 3450 m on two consecutive occasions 9 to 12 months apart. Results: During the first exposure 18 adults (62%) and 6 children (22%) suffered from AMS; during the second exposure 14 adults (48%) and 4 children (15%) suffered from this problem ($P<0.01$, adults vs. children). Most importantly, the intra-individual reproducibility of AMS was very different

(P<0.001) in children and adults. None of the 6 children having suffered from AMS during the first exposure suffered from AMS during the second exposure, but 4 children with no AMS during the first exposure suffered from this problem during the second exposure. In contrast, 14 of the 18 adults who suffered from AMS on the first occasion also presented this problem during the second exposure, and no new case developed in those who had not suffered from this problem on the first occasion. Conclusion: In adults, a history of previous AMS is highly predictable of the disease on subsequent exposure, whereas in children it has no predictive value. A history of a previous AMS should not incite practitioners advising against re-exposure to high altitude or prescribing drugs for its prophylaxis in children. Acknowledgements: This work was supported by the Swiss National Science Foundation, the Leenaards Foundation and the Placide Nicod Foundation. 2011.

RESPIRATORY AND LEG EFFORT SENSATION IN NORMOXIA AND HYPOBARIC HYPOXIA. GIUSEPPE MISEROCCHI, Andrea Aliverti, Antonella LoMauro, Marco Quaranta, Raffaele Dellaca, Pasquale Pompilio, Luigi Biasco, Lorenzo Cavalleri, Luca Pomidori, Manlio Milanese, Josuel Ora, Valter Fasano, Annalisa Cogo, Riccardo Pellegrino, Giuseppe Cornara, Bengt Kayser. University Milano-Bicocca. *Email: giuseppe.miserocchi@unimib.it.* Mechanisms of sensation of exertion, fatigue and exhaustion during exercise in hypoxia are not well understood. 11 non-smoking healthy male subjects (age 41[14], mean[SD] yr, height 176[3]cm, weight 75[9]kg) did incremental cycle exercise (30 watt/3 min) until exhaustion (Wmax) at sea level (SL) and after 2-3 days at 4559 meters (ALT), while leg (Le) and breathing (Br) effort sensation, heart rate (Hr), ventilation (Ve), tidal volume (Vt), respiratory rate (Rr), saturation (Sat) and work of breathing (esophageal pressure x lung gas volume loops, WOB) were monitored. Respiratory exchange ratio (RER) was higher in ALT compared to SL at any work rate (W). At altitude Wmax was 15[8]% lower while at all sub Wmax Hr, Ve and Rr were higher and Sat was lower. Up to RER values of 1, the relationship between RER and ventilation was similar in ALT and SL, while thereafter the increase of RER was larger in ALT compared to SL for similar increases of ventilation. For any level of ventilation WOB was lower at ALT because of less resistive work in spite of exaggerated expiratory gas compression. Vt vs. Ve was unaffected by ALT reflecting unchanged exercise breathing pattern. Le increased with W and Br increased with WOB, both relationships shifted upward in ALT. The shift in Le vs. W and Br vs. WOB likely reflects muscle and general hypoxemia. Br was uniquely correlated to Ve regardless of hypoxia exposure suggesting that respiratory effort sensation is exactly geared to the ventilatory requirement for gas exchange. Support: Italian Alpine Club, Polytechnic Milan, University of Geneva, University of Ferrara. 2009.

RESPIRATORY MUSCLE WEAKNESS FOLLOWING ACUTE SUSTAINED HYPOXIC STRESS IN THE MOUSE. Andrew J. O'Leary[1], Ken D. O'Halloran[1]. [1]Department of Physiology, School of Medicine, University College Cork, Cork, Ireland. *Email: andrew.j.oleary@umail.ucc.ie.* Introduction: Hypoxia is a common feature of respiratory-related diseases. There remains, however, a paucity of infor-

mation concerning the effects of sustained hypoxia (SH) on respiratory muscle performance. We assessed the effects of acute SH on ventilation and sternohyoid and diaphragm muscle contractile function. Methods: Adult male C57BL6/J mice (n=8 per group) were exposed to 1, 4 or 8 hours of SH ($F_IO_2 = 0.10$) or normoxia ($F_IO_2 = 0.21$). Whole-body plethysmography was used to record breathing during exposure. Respiratory muscles were excised post-mortem. Muscle performance was assessed ex-vivo. We examined changes in gene expression at 1, 4 and 8 hours of SH and normoxia using qRT-PCR. Results: Respiratory rate and minute ventilation were increased ($p<0.001$ and $p<0.01$ respectively, two-way ANOVA & Bonferroni post hoc test) after 10mins of SH compared with control, returning to levels equivalent to normoxia by 30mins and remaining similar to normoxia for the remainder of the 8 hour SH exposure. For sternohyoid, SH decreased tetanic force ($p=0.0683$, unpaired t-test) and half-relaxation time ($p=0.0636$), and depressed work-load ($p<0.0001$) and power-load ($p=0.0009$) relationships. For diaphragm, SH decreased tetanic force ($p=0.0334$) and power-load ($p=0.0011$) relationship. Isotonic fatigue tolerance of both muscles was improved following SH exposure. Differential changes in gene expression for PGC1α, NRF1 and NFκB1 were observed in both muscles over 1, 4 and 8 hours of SH. Conclusion: Here we show that acute SH is sufficient to cause diaphragm and sternohyoid muscle weakness with resultant decreased work and power outputs. Interestingly, both muscles demonstrate apparent increased fatigue tolerance following acute SH. Following the acute HVR at 10mins, ventilation in SH remains at normoxic levels thereafter (most likely due to a decreased metabolic O2 demand during SH exposure), suggesting that the changes observed are not due to enhanced respiratory muscle activity but relate to hypoxic stress *per se*. We aim to explore hypoxic signaling in respiratory muscle in this animal model to determine the mechanism of functional plasticity. Acknowledgements: Supported by the Department of Physiology, School of Medicine, UCC. 2015.

RESPIRATORY PLASTICITY FOLLOWING ACUTE INTERMITTENT HYPOXIA IS NOT CAUSED BY INFLAMMATION IN HEALTHY HUMANS. Andrew E. Beaudin[1], Xavier Waltz[1], Matiram Pun[1], Katherine E. Wynne-Edwards[1], Sofia B. Ahmed[1], Todd J. Anderson[1], Patrick J. Hanly[1], Marc J. Poulin[1]. [1]University of Calgary, Calgary, Alberta, Canada. *Email: abeaudin@ucalgary.ca.* Introduction: Ventilatory instability is a fundamental component of obstructive sleep apnea (OSA) pathogenesis. A key component of ventilatory stability is enhanced ventilatory chemosensitivity, with OSA patients having augmented acute hypoxic (AHVR) and hypercapnic (AHCVR) ventilatory responses. The enhanced chemosensitivity in OSA is thought to result from exposure to intermittent hypoxia (IH), but the molecular pathway is poorly understood. IH-induced oxidative stress leading to inflammation is postulated to contribute to AHVR enhancement although the role of inflammation in IH-induced respiratory changes in humans has not been examined. Employing an experimental human model of IH previously shown to increase oxidative stress and the AHVR, this study assessed the role of inflammation in IH-induced respiratory plasticity. Methods: In a double-

blind, placebo-controlled, randomized, crossover study 12 healthy males underwent 6 hours of IH. For 4 days before each IH exposure, participants ingested either 100mg lactose placebo po tid, or the non-steroidal anti-inflammatory drugs indomethacin (non-selective cyclooxygenase (COX) inhibitor; 50mg po tid) or celecoxib (selective cyclooxygenase-2 inhibitor; 200mg po bid). Pre- and post-IH AHVR and AHCVR were assessed. Results: Pre-IH, the AHVR and AHCVR were similar across all conditions (placebo, and indomethacin and celecoxib; $p \leq 0.093$). IH increased the AHVR within all drug conditions (Placebo: 2.0±0.3 vs 1.5±0.2 L/min/% desaturation, p=0.011; Indomethacin: 1.8±0.3 vs 1.4±0.2 L/min/% desaturation, p=0.026; and Celecoxib: 1.9±0.3 vs 1.4±0.2, p=0.018). The increase in AHVR was similar across all conditions (p=0.827). Post-IH, the AHCVR was increased (p=0.003) within only the celecoxib condition. Conclusion: In conclusion, inflammation does not appear to contribute to the IH-induced AHVR enhancement following an acute (6 hour) exposure. In contrast, selective COX-2 inhibition augmented the AHCVR following acute IH exposure. With respect to OSA, these findings suggest selective COX-2 inhibition could potentially exacerbate OSA severity by increasing ventilatory instability. Acknowledgements: Funded by the CIHR, HSFC, AIHS, and NSERC. 2015.

RESTING AND POST-PRANDIAL MESENTERIC BLOOD FLOW IS MAINTAINED AT 4,400M. Nicholas S Kalson , Faye Hext, Andrew J Davies , Gerry Johnson, Derrick Martin, Colin Chan, Chris Imray, Daniel Morris. Eye Care Center Vancouver General Hospital. *Email: dsm@doctors.org.uk.* Introduction Gastrointestinal symptoms are common on acute exposure to high-altitude (HA) and weight loss can be problematical in the longer term. The underlying mechanisms remain incompletely understood, but vascular shunting away from the gut to more "critical" organs might be responsible. Aim We aimed to assess superior mesenteric artery (SMA) blood flow at rest and after a standard meal on ascent to altitude. Method Thirteen healthy subjects (nine male, mean age 40 (22-72) years) with no known gastro-intestinal pathology were studied at sea-level (SL), and after 1-2 days at 4,400m. Mean velocity (systolic diastolic / 2) and SMA vessel diameter were measured using a portable Duplex ultrasound machine (Sonosite MicroMax) with the subject supine after an overnight fast (minimum 8h), and following a standard meal (1000kCal Enshake). Gastrointestinal symptoms were determined by questionnaire. Results An increase in velocity and flow were seen between rest and post-prandially at SL (velocity: 36.41±10.43 cm/s vs. 45.56±12.42cm/s, p=0.01, flow: 79.89±22.8 cm3/s vs. 138.74±52.4 cm3/s, p=0.01) and at HA (45.58±12.42 vs. 68.06±27.02, p=0.03 and 99.38±27.1 cm3/s vs. 148.10±58.8 cm3/s, p=0.01). There was no difference in post-prandial flow or velocity between SL and HA, but the increase in resting flow and velocity between SL and HA was significant (p=0.006 and 0.006 respectively). All subjects suffered reduced appetite at 4,400m. Discussion The results show that mesenteric blood flow is increased at HA due to an increase in vessel calibre and blood flow velocity. The post-prandial increase in blood flow was not damped at HA. Impaired appetite at HA is not due to reduced gut blood flow. 2009.

REVERSAL OF HYPOXIC PULMONARY HYPERTENSION BY NORMOXIA: A PILOT STUDY. Jim Milledge[1], Nick Talbot[2], Edith Kortekaas[3], Dan Martin[1]. [1]Centre for Altitude Space and Extreme Environment Medicine, Institute of Child Health (UCL),, [2]Department of Physiology, Anatomy & Genetics, University of Oxford, [3]Division of Perioperative Care and Emergency Medicine, University Medical Centre Utrecht. *Email: jim@medex.org.uk*. Introduction: Breathing a hypoxic gas mixture for 30 minutes results in pulmonary hypertension, which promptly returns to control values when air breathing is resumed. However, after some weeks or years at high altitude, breathing oxygen for a few minutes does not return the pressure to normal. We attempted to follow the time course of the development of resistance to reversal by oxygen, of pulmonary hypertension, at altitude. Methods: We measured the pulmonary artery systolic pressure (PASP) using echocardiography, in four subjects, in London and during a 9 day stay at 4,559m in the Margherita Hut on Monte Rosa. At altitude, we measured PASP breathing air and on 35% oxygen (SL PIO_2) for 30 minutes. We repeated the measurements daily. Results: Oxygen breathing reduced the PASP but not completely to SL values. The mean effect was only 67% of full correction, with no evidence of any trend in results from day 4 to day 9 at altitude. Conclusion: Results from the literature combined with this study indicate a two phase response to 30 minutes oxygen breathing after hypoxia. The change from complete to incomplete response seems to be between 30 minutes and 8 hours of hypoxia. This time indicates that remodeling cannot be the sole cause of the failure of oxygen breathing to reverse hypoxic pulmonary hypertension. Acknowledgements: This study was made possible by the organization and support of the Xtreme Alps Expedition and funded in part by Smiths Medical and Deltex Medical. 2011.

RIGHT AND LEFT VENTRICULAR FUNCTION IN LOWLANDERS WITH COPD TRAVELLING TO MODERATE ALTITUDE. Silvia Ulrich[1], Mona Lichtblau[2], Tsogyal Latshang[1], Michael Furian[1], Séverine Müller-Mottet[1], Deborah Flück[1], Silke Kuest[1], Malcolm Kohler[1], Felix Tanner[3], Ekkehard Gruenig[2], Konrad Bloch[1]. [1]Clinic of Pulmonology, University Hospital of Zurich, Switzerland, [2]Thorax Clinic at University of Heidelberg, Germany, [3]Clinic of Cardiology, University Hospital of Zurich, Switzerland. *Email: ulris@bluewin.ch.* Introduction: To investigate whether patients with Chronic Obstructive Pulmonary Disease (COPD) develop pulmonary hypertension (PH) when exposed to moderate altitude and explore predicting factors.. Methods: 37 patients, 20 men, median age 66y (quartiles 60;69) with COPD, GOLD II/III, median FEV1 57 %pred (49;71) living below 800m were recruited. Echocardiography, pulse oximetry (SpO2) and 6 min walk distance (6MWD) were assessed at 490m (Zurich) and in the first morning after patients had spent one night at 2590m (Davos Jakobshorn). Results: At 490m arterial oxygen saturation and the 6MWD were 94% (93;96) and 542m (471;585); right ventricular systolic pressure (RVSP) was 23 mmHg (18; 29), RV end-systolic and diastolic areas were 8.4cm2 (7.7;11) and 15.1cm2 (14.1;17.5); left ventricular ejection fraction (LVEF) was 67 (63;70). At 2590m, corresponding values were SpO_2 89% (87;91), 6MWD 506m (447;583), RVSP 39

mmHg (34;49), RV end-systolic and diastolic areas 10.5cm2 (8.3;12) and 15.9cm2 (13.8;19.2), LVEF 64(59;67), P<0.05, all comparisons to 2590 vs. 490m. Conclusion: In lowlanders with COPD, GOLD 2-3, hypoxemia at moderate altitude is associated with a moderate increase in RVSP, and dysfunction of both the right and left ventricle. The desaturation during 6 min. walk distance at 490 m was an independent predictor of the sPAP-increase at the higher altitude. Acknowledgements: We thank the Zurich Lung League and the Swiss National Science Foundation for financial support. 2015.

RIGHT VENTRICULAR DIASTOLIC FUNCTION IS IMPAIRED AT HIGH ALTITUDE. Bhavini Jaiswal, Edith Kortekaas, Katja Ruh, Gary P Foster, James D Anholm, Medical Expeditions. VA Loma Linda, University Medical Centre, Loma Linda University, VA Loma Linda Healthcare System. *Email: bjaiswal@hotmail.com.* Background: Ascent to high altitude (HA) reduces exercise capacity due to the fall in arterial oxygen content and possibly impaired ventricular function. Left ventricular function is normal or minimally altered by altitude; however, few data exist regarding the effects of hypoxia on right ventricular (RV) function. Hypoxic pulmonary vasoconstriction (HPV) at HA may contribute to altered RV function. If HPV contributes to RV dysfunction then inhaled iloprost, a prostacyclin analogue used in the treatment of pulmonary hypertension, may improve RV function. Objective: To determine the effects of altitude hypoxia on RV diastolic function. Methods: This double blind, placebo-controlled, randomized, cross-over trial studied 7 healthy participants (aged 24 ± 4 years) of the MedEx 2008 Expedition to Dhaulagiri, Nepal. Doppler imaging was utilized to measure tricuspid inflow (TI) E and A wave velocities, tissue Doppler (TDI) of RV free wall and septal wall of the tricuspid annulus. Measurements were performed before and after inhaled iloprost or placebo at sea level (SL) and after a 14 day trek to 5050m. Results: SpO_2 was 98% ± 1% (mean ± SD)at SL and 84% ± 5% at HA, p<0.01. TI E/A was 1.9 ± 0.2 at SL and 1.5 ± 0.2 at HA, p=0.07. TDI E/A of RV free wall was 2.0 ± 0.5 at SL and 1.6 ± 0.5 at HA, p=NS. TDI E/A of the septal wall was 2.0 ± 0.6 and 2.1 ± 0.8 at HA, p=NS. There was a trend for TI E/ TDI E (E/Eí) to increase with altitude. Iloprost had minimal effect on any of these measurements. Conclusions: HA hypoxia in healthy subjects worsens RV diastolic function based on these Doppler indices. Acknowledgements: All studies were carried out with Medical Expeditions, a charitable group that promotes high altitude research and education, http://www.medex.org.uk. 2009.

RIGHT VENTRICULAR FUNCTIONAL ENHANCEMENT DURING RAPID HIGH ALTITUDE ASCENT. N. Stuart Harris, Charles Fulco, Steven Muza, Beth Beidleman, Alan Cymerman, Kibar Yared, Peter Fagenholz, David Systrom, Malissa Wood, Arthur Weyman, Michael Picard, Aaron Baggish. Harvard Medical School, Massachusetts General Hospital, United States Army Research Institute of Environmental Medicine. *Email: nsharris@partners.org.* Background: Right ventricular (RV) response to acute afterload increase depends on the etiology of pulmonary arterial (PA) pressure rise. Hypoxia associated with high altitude ascent leads to PA vasoconstriction and increased RV afterload. The impact of rapid

ascent to high altitude on RV function has not been well characterized. Methods: Healthy males (n=10, age=20 ± 2 years) were studied at sea level (SL) and again 90 minutes after direct ascent (DA) to simulated altitude (hypobaric chamber, 4,300 meters). Echocardiography was used to determine mean PA pressure and to examine RV systolic and diastolic function. Results: DA led to increased heart rate (SL = 69 ± 5 bpm vs. DA = 77± 7 bpm, p<0.001) and reduced arterial oxygen saturation (SL = 97±2 % vs. DA = 80 ± 4 %, p<0.001). Although mean PA pressure increased significantly (SL=14 ± 3 mmHg vs. DA = 37 ± 8 mmHg, p<0.001), measurement values for RV systolic function increased significantly (table). In addition, both early and late phase RV diastolic tissue velocities increased during DA. Conclusion: Despite significant increases in afterload, there is RV functional enhancement during acute high altitude ascent. These findings suggest that the acute rise in pulmonary arterial pressure associated with rapid high altitude ascent is well tolerated by the RV. Right Ventricular Functional Parameters: SL DA (p-value) Fractional Area Change (%) 30 ± 5 41± 10 (0.003) Peak Systolic Tissue Velocity (cm/s) 9.4 ± 1.4 11.0 ± 1.1 (<0.001) Peak Strain (%) -34 ± 5 -39 ± 5 (<0.001) Stroke Volume (ml) 76 ± 13 87 ± 15 (0.02) Peak Early Diastolic Tissue Velocity (cm/s) 10.2 ± 1.5 11.0 ± 1.4 (0.02) Peak Late Diastolic Tissue Velocity (cm/s) 6.2 ± 1.6 7.1 ± 1.9 (0.02). 2009.

RISK FACTORS FOR HEADACHE WHILE HIKING ABOVE 4300 METERS. Christopher Davis, M.D.[1], Elaine Reno, M.D.[2], Brian Vestal, M.A.[3], Edward Maa, M.D.[4], and Robert Roach, Ph.D.[1.] [1]Altitude Research Center, Department of Emergency Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]Denver Health Residency in Emergency Medicine, Denver Health and Hospitals, Denver, Colorado; [3]University of Colorado Anschutz Medical Campus, School of Public Health, Biostatistics and Informatics, Research Consulting Lab, Aurora, CO [4]Division of Neurology, Denver Health and Hospitals, Denver, Colorado *EMAIL: Christopher.Davis@ucdenver.edu* INTRODUCTION: Headache is a frequent complaint after ascent to high altitude. Limitations of previous studies examining risk factors for high-altitude headache (HAH) include a focus on mountaineers and small sample size. As such, there are conflicting data regarding the role in HAH of previous headache history, gender and age. The aim of this study was to study risk factors for HAH within a population of recreational hikers above 4300 m using a cross-sectional survey design. METHODS: A convenience sample of 667 hikers participated in a written survey after descent from Mount Gray/Torreys (4349 m). Survey questions included demographics, previous headache frequency and duration, physical fitness, summit success, alcohol consumption, hydration, and hike length. Descriptive statistics, including chi-square, Fisher's exact test and T-test were used depending on the variable and the normality of its distribution. Odds ratios (OR) and 95% confidence limits (CI) were calculated to test for associations between hiker characteristics and the development of HAH. RESULTS: Sixty percent of hikers were male and 87.6% successfully summited Mount Grays/Torreys. HAH occurred in 39% of hikers, and of those, 29% reported HAH intensity as moderate or severe. Two major findings of this

study are: 1) hikers reporting HAH were on average slightly younger than those free of HAH (32.1±10.5 vs. 35.1±11.4 years, $p < 0.001$); and 2) hikers who reported headaches at home at higher than monthly frequency (104±40% vs. 84±22.6%, $p < 0.001$, OR 2.29, 95% CI 1.62-3.24) or more frequent headaches in the last 3 months (2.7±5.5 vs. 3.8±5.5 years, $p= 0.008$) were more likely to report HAH during their hike. Gender distribution, alcohol consumption, headache duration, summit success rate, and self-reported hydration status were not different between groups. While pre-hike exercise frequency was not different between the two groups, hike duration was 7% longer (357.3 ±88.6 min vs. 381.7 ±71.7 min, $p < 0.001$) in those who reported headache suggesting that headache could have slowed hiking time. CONCLUSION: Slightly younger age and a history of more frequent headaches at low altitude were associated with the development of headache while hiking above 4300 m in a group of recreational hikers. 2015.

SEPARATE EFFECTS OF ACCLIMATISATION AND CEREBRAL BLOOD FLOW ON CENTRAL SLEEP APNEA AT HIGH ALTITUDE. Keith R Burgess, Andrew Dawson, Kelly Shepherd, Marianne Swart, Kate N Thomas, Jui-Lin Fan, Rebekah A Lucas, Samuel J Lucas, James D Cotter, Karen C Peebles, Rishi Basnyat, Philip N Ainslie. University of Sydney, Peninsula Sleep Laboratory, University of Otago, Nepal International Clinic. *Email: krburgess@optusnet.com.au.* We hypothesised that partial acclimatisation and pharmacologically induced alteration of cerebral blood flow (CBF) would have separate effects on the frequency and duration of central apneas during sleep at high altitude. We studied 12 normal volunteers on four occasions over three weeks at 5050m in Nepal. Measurements included overnight polysomnography with transcranial Doppler measurement of CBF, non invasive hemodynamics and ABG analysis. They were studied at the beginning and end of their stay, and in between the control nights all subjects received oral Indomethacin (IND) 100mg and iv Acetazolamide (ACET 10mg/kg) 2 hours before sleep, in random order with placebo controls, at approximately 4 day intervals. The data from the intervention nights were compared to the mean data from the control nights. After IND, CBF fell by 22 ± 8% and the apneas lengthened from 13.9 ± 2.2 to 15.4 ± 3.3secs ($p<0.01$). Central Sleep Apnea Index (CSAI) increased from 96.4 ± 30.3 to 101 ± 28.2 apneas/hr (NS). After ACET, CBF increased by 31 ± 6% , with no effect on apnea duration but CSAI fell from 96.4 ± 30.3 to 53.7 ± 45.7 apneas/hr ($p<0.001$). During partial acclimatisation, CSAI increased from 76.9 ± 48.9 to 115.9 ± 20.2 /hr over the 12 day period ($p=0.01$), and apnea duration lengthened from 13.1 ± 2.6 to 14.6 ± 2.2 secs ($p<0.02$). Over the same period $PaCO_2$ declined from 29±3 to 26±2mmHg, and the rise (25±10%) in CBF upon initial exposure (days 1-4) returned to its sea-level values. We propose that the increase in apnea length with IND was due to reductions in CBF and cerebrovascular reactivity and increase in "loop gain". However the lengthening of the apneas due to acclimatisation must be due to mechanisms that are independent of CBF. This study was supported by the Otago Medical Research Foundation, Peninsula Health Care p/l, Air Liquide p/l, the Italian National Research Council who kindly provided use of the EV-K2-CNR research laboratory. 2009. 2009.

SERIAL PUPILLOMETRY DURING ASCENT TO 5050M. Nicholas MacLeod[1], Keita Ikeda[2], David MacLeod[2]. [1]Carolina Friends School, [2]Dept of Anesthesia, Duke Univ Medical Center, Durham NC, USA. *EMAIL: david.macleod@duke.edu*
INTRODUCTION: NeurOptics PLR-200 pupillometer records the dynamic pupillary response to an emission of white light of fixed intensity & duration. A previous study reported significant changes following a single day ascent from 300 to 3250m with return to baseline values within 2 days. Maximum pupil aperture was significantly reduced; the latency (reaction time), constriction and dilation velocities were slowed. METHODS: We conducted an observational study of pupillometry (pupil aperture [mm]: MAX & MIN; % change [(MAX-MIN)/MAX]: %CH; pupil constriction & dilatation velocity [mm/s]: CV & DV) during ascent from Kathmandu to Pyramid Lab (PL), Nepal in a cohort of 18 subjects (14 males; 4 females; ages 17 – 49) taking prophylactic acetazolamide. Measurements were performed on the right eye at baseline & 4 interval altitudes (1400, 2835, 3440, 3930, 4240m) on days 0, 1, 2, 4 & 6, respectively. All subjects ascended together except for the final ascent to 5050m - 3 separate groups over days 8 – 10. Subjects stopped acetazolamide on day of final ascent to PL. RESULTS: Results (baseline v 4240m, p-value) showed a significant increase in MAX (6.0 v 6.7, 0.0003), MIN (3.6 v 4.2, 0.0002) & %CH (59 v 63, 0.002) at all altitudes with greatest change at 4240m. CV & DV both slowed during ascent with similar significant changes. At 5050m all parameters exhibited values denoting reverse change towards baseline: MAX 6.4; MIN 3.9 & %CH 61. CONCLUSION: In conclusion CV & DV changed as predicted with ascent. MAX & MIN increased during ascent - the opposite of expected change. This suggests that acetazolamide may affect pupillary responses. This is further supported by reverse changes following drug discontinuation and further altitude ascent. ACKNOWLEDGEMENTS: This study was carried out within the framework of the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology as foreseen by the Memorandum of Understanding between Nepal and Italy, and thanks to contributions from the Italian National Research Council. 2015.

SEX DIFFERENCES IN NEUROVASCULAR RESPONSES FOLLOWING ACUTE HYPOXIA. Rachel Skow[1], Christina MacKay[1], Margie Davenport[1], Craig Steinback[1]. [1]University of Alberta. *Email: rskow@ualberta.ca.* Introduction: There is evidence suggesting differences in the sympathetic response to chemoreflex stress between sexes that may be due to differences in cyclic hormones. Previous work has shown that sympathetic nerve activity (SNA) increases during, and remains elevated following exposure to hypoxia (reduced oxygen). However, it remains unclear if and how augmented SNA affects vascular function following hypoxia and whether these changes are different between sexes. Methods: We tested the hypothesis that increased SNA following hypoxia is associated with changes vascular function (e.g. mean arterial pressure (MAP), total peripheral resistance (TPR), femoral vascular resistance (FVR)) and that the response will be different between sexes. Participants (n=16; males=9; 24±3yrs; mean ±SD) were instrumented to measure heart rate (HR), MAP, cardiac output (CO; Finometer), and femoral artery blood flow (FBF;

Doppler ultrasonography). TPR and FVR were calculated from MAP and CO or FBF, respectively. Data were collected continuously during 10-minutes of baseline, 10-minutes of hypoxia (~80% SpO_2), and up to 30-minutes of recovery. Averages (1-min) taken during baseline, the end of hypoxia and every 5-minutes during recovery were compared using two-way ANOVA for sex and time point comparisons. Results: MAP did not change during or after hypoxia compared to baseline ($p=0.494$). There were sex differences in both HR, and MAP responses ($p=0.005$ and $p=0.042$, respectively) with females showing a blunted response during hypoxia. Both FVR and TPR were decreased during hypoxia, as shown previously, however they were all not different at any time point during recovery when compared to baseline ($p>0.05$). No significant differences were observed between sexes at any time point for FVR and TPR ($p= 0.459$ and $p=0.939$, respectively). Conclusion: These data suggest that females exhibit a blunted response during hypoxia, but these differences were absent following hypoxia. Further, there are no sex differences in the vascular resistance response to or following hypoxia. Acknowledgements: Funded by the President's Grant for the Creative and Performing Arts - Human Performance Scholarship Fund (CDS) and a Queen Elizabeth II Graduate Student Scholarship (RJS). 2015.

SEX DIFFERENCES IN THE CHEMICAL AND MECHANICAL REGULATION OF BREATH-HOLDING. Craig D Steinback[1], Uday Chauhan[1], Jeff Vela[1], Christina D Bruce[2], Rachel J Skow[1], Maria Abrosimova[2], Jamie R Pfoh[2], Trevor A Day[2], Margie H Davenport[1]. [1]Faculty of Physical Education and Recreation, University of Alberta, Edmonton, [2]Department of Biology, Mount Royal University, Calgary, AB, Canada. *Email:* craig.steinback@ualberta.ca. Introduction: The mechanisms contributing to sex differences in voluntary breath-hold duration have not been fully elucidated. Methods: We studied 13 women (age 23.5±3.2 yrs) and 11 men (26.3±4.6 years) during four maximal breath-holding maneuvers designed to isolate the independent influence of lung stretch/volume and chemoreflex drive; 1) end-expiratory breath-hold(EXP), 2) end-inspiratory breath-hold (INSP) designed to isolate the influence of lung stretch/volume, 3) INSP following hyperventilation to isolate the influence of hypoxic drive (HX), and 4) INSP following hyperoxic breathing to isolate the influence of hypercapnic drive (HC). Results: Spirometry indicated men had larger forced vital (FVC; 5.8±0.9L) and total lung capacities (TLC; 7.3±1.0L) compared to women (4.2±0.5L, $p<0.001$ and 5.3±0.5L, $P<0.001$ respectively). There was no difference in EXP duration between men and women (38.9±18.4 vs 33.2±18.2s, $P=0.717$). However, INSP prolonged breath-holding compared to EXP, and men had a significantly longer INSP duration (112.4±48.3) compared to women (75.0±32.7s, $P<0.05$). Although FVC ($R2=0.189$, $P<0.05$) and TLC ($R2=0.188$, $P<0.05$) were weakly correlated to the improvement in breath-hold duration, this was only when both sexes were included in the analysis. Men had longer HX (158.1±39.4s) and HC (184.7±61.3s) breath-holds compared to women (113.8±25.6s, $P<0.01$ and 125.7±46.4s, $P<0.05$ respectively). Conclusions: After taking into account the influence of INSP, only HC duration remained significantly different between sexes. As breath-hold duration is inversely related to respi-

ratory drive, these data suggest differences in hypercapnic (but not hypoxic) drive, and to a lesser extent larger lung volumes/lung stretch, contribute to sex differences in breath-hold. Acknowledgements: Funded by NSERC and UofA HPF. 2015.

SEX DOESN'T MATTER: CAPILLARY BLOOD VOLUME RESPONSE TO EXERCISE IN MEN AND WOMEN. Melissa Bouwsema[1], Vincent Tedjasaputra[1], Michael Stickland[1]. [1]University of Alberta. *Email: mmbouwse@ualberta.ca.* Introduction: Previous work suggests that women exhibit a greater respiratory limitation to exercise due to smaller lung size compared to height-matched men. Pulmonary capillaries are recruited with incremental exercise to increase pulmonary diffusion capacity and facilitate gas exchange. Because of their lung size, women likely have fewer pulmonary capillaries to recruit, and therefore potentially a reduced pulmonary capillary blood volume (Vc) and membrane diffusion capacity (Dm) available to meet the increased oxygen demand. We hypothesized that females would have a lower Vc compared to males at rest, and exhibit a plateau in Vc response to exercise, whereas males would demonstrate a continuous increase in Vc up to maximal exercise. Methods: Height- and fitness-matched females (n=16, height: 172.6±6.9cm; 112±12% predicted relative VO_2max) and males (n=16, height: 173.3±3.4cm; 118±22% predicted relative VO_2max) performed pulmonary function tests, with total lung capacity (TLC) measured via N_2 washout at rest. At rest and during incremental exercise up to 90% of maximal intensity, hemoglobin corrected diffusion capacity (DLCO) was measured, with Vc and Dm determined via the multiple-FiO_2 DLCO technique. Results: Both groups increased DLCO, Dm and Vc with exercise intensity, but women had 20% lower DLCO ($p<0.001$), 22% lower Dm ($p<0.001$) and 18% lower Vc ($p=0.002$) compared to men across all workloads. When DLCO, Dm and Vc were corrected for alveolar volume (VA), the between-sex difference was eliminated. Conclusion: Women demonstrate a consistent reduction in DLCO, Dm and Vc during incremental exercise as compared to height- and fitness-matched men. However, our results suggest that the difference in pulmonary capillary blood volume response can be explained by lung size, and not an intrinsic sex difference in the pulmonary vasculature. Acknowledgements: Study supported by Natural Sciences and Engineering Sciences Council of Canada and Alberta Innovates Health Solutions. 2015.

SHIFTING THE OXYHEMOGLOBIN DISSOCIATION CURVE (ODC) IN VIVO. Dahlia Y Balaban, David Preiss, Alexandra Mardimae, Alex Vesely, Marat Slessarev, Richard Greene, Gustavo Zubieta, James Duffin, Joseph A Fisher. University of Toronto, Toronto, University of British Columbia, University of New Mexico, Clinica IPPA. *Email: dahlia.balaban@utoronto.ca.* The position of the ODC depends on PCO_2 because of the inverse relationship between PCO_2 and hemoglobin-oxygen affinity. The relationship between PCO_2 and the position of the ODC has been studied in vitro, but only in vivo methods can reflect metabolic changes that may occur during progressive hypoxia. Our aim was to demonstrate the ability to describe a family of in vivo ODCs by maintaining different levels of PCO_2 while inducing progressive, sustained hypoxia in breathing subjects. We used

a specialized gas blender (RespirAct™, Thornhill Research Inc., Toronto, Canada) and a sequential rebreathing circuit to prospectively target end-tidal PCO_2 ($PETCO_2$) and PO_2 ($PETO_2$), to provide conditions for measuring and shifting the in vivo ODC in 6 healthy lowlanders (2 females) within one week of arrival at 3600 m. In separate trials we maintained $PETCO_2$ at different levels, targeting that of rest, 10 mm Hg above rest and 10 mm Hg below rest. For each trial, we targeted $PETO_2$s of 100, 70, 60, 50, 40 and 35 mm Hg, in steps lasting 2 min. $PETCO_2$ and $PETO_2$ were recorded continuously and SpO_2 (Onyx II, Nonin, Plymouth MN) was recorded at the end of each PO_2 step. P50 was calculated using the Hill equation. During the three trials, $PETCO_2$ was maintained at 33 ± 0.5 (mean ± SD) (resting), 26 ± 0.4 and 42 ± 0.6 mm Hg (RMANOVA $p<0.01$). $PETO_2$ remained within ± 1.5 mm Hg (SD) for at least the last 60 s of each step in all trials. P50 values for each trial were 28.1 ± 4.4 (resting), 25.9 ± 3.4 and 31.7 ± 1.9 mm Hg $PETO_2$ (RMANOVA $p < 0.01$). We have demonstrated that prospective end-tidal gas targeting can be used to provide the conditions for describing baseline and shifted ODCs in vivo. This method may therefore be useful in studying the effect of shifts in the ODC on outcome variables, such as exercise tolerance at altitude and development of altitude-related illnesses. We thank CIHR and Thornhill Research Inc. for the generous support that made this research possible. 2009.

SLEEP DISORDERED BREATHING AND ACUTE MOUNTAIN SICKNESS IN WORKERS RAPIDLY TRANSPORTED TO THE SOUTH POLE (2835 M). Paul Anderson[1], Heather Wiste[1], Stuart Ostby[2], Andrew Miller[1], Maile Ceridon[1], Bruce Johnson[1]. [1]Mayo Clinic, [2]Mayo Medical School. *Email: Anderson.Paul1@mayo.edu.* Introduction: Sleep disordered breathing may be a risk factor for high altitude illness. Past Antarctic sleep studies suggest that rapid transport from sea level (SL) to the Amundsen Scott South Pole Station (SP, 2835m) increases risk of Acute Mountain Sickness (AMS). We analyzed sleep studies in 38 healthy polar workers to explore how sleep disordered breathing may be a risk factor for developing AMS after rapid transport to the SP. Methods: Subjects completed a baseline questionnaire, performed basic physiology tests, and were evaluated for AMS and medication use using an extended Lake Louise Questionnaire (LLQ) during their first week at SP. Participants were included in this study if they took no medications and underwent polysomnography on their first nights at SL and SP using the Vivometrics LifeShirt®. Within group changes were assessed with Wilcoxon signed rank tests and between group differences were assessed with Kruskal-Wallis rank sum tests. Results: Overall, 21/38 subjects met criteria for AMS at some time on or prior to the third morning at SP. Subjective poor sleep quality was reported by both AMS (65%) and no AMS (41%) groups. The Apnea Hypopnea Index (AHI) increased significantly in both the AMS and no AMS groups, but the difference in the increase between the two groups was not statistically significant. Increased AHI was not associated with increased AMS symptoms. Previous altitude illness ($p = 0.06$) and residence at low altitudes ($p = 0.02$) were risk factors for AMS. Conclusion: AMS was not significantly associated with sleep architecture changes or increased AHI. However, AHI sharply increased at SP (19/38 participants) primarily due to

central apneas. Those developing AMS were more likely to have experienced previous problems at altitude and reported living at lowland altitudes within the three months prior to rapid transport to SP than those without AMS. 2015.

SOS MAM, AN EXPERIMENTAL TELEMEDICINE CALL CENTER IN PROCESS; ANALYZED OF FOUR CASE REPORTS. Emmanuel CAUCHY[1], Pascal ZELLNER[1], Marie Anne MAGNAN[1], Fabrice COPPEX[2], Matthieu DE RIEDMATTEN[2], Jacques RICHON[2]. [1]IFREMMONT- Institut de Formation et de Recherche en Medecine de Montagne - Chamonix Hospital France, [2]GRIMM - Groupement d'Intervention Medicale en Montagne - Switzerland. *Email: cauchy@ifremmont.com.* Introduction: The increase of technical communication devices and the ease of data transfer by anyone from any remote area has enhanced management abilities in mountain medicine care by telemedicine. As part of a European program on the franco-swiss border (Interreg IV), two mountain medicine centers collaborated to develop a call center managed by physician experts in order to give practical advice to mountain guides in case of illness or accidental impairment during any expedition (SOS-MAM Project). Methods: Since 2012, IFREMMONT (France) and GRIMM (Suisse) have collaborated to implement a specific call center supervised by ten mountain medicine experienced physicians (6 French, 4 Swiss). Thirty mountain guides were trained for three days in advanced rescue courses then equipped with a satellite phone, and some with ambulatory transmittable ECG devices. An Internet platform adapted to ensure highest standards for privacy and medical responsibility was created and tested. For this report, four different cases of emergency calls are detailed and analyzed to demonstrate the interest and utility of such a service. Results: The first case was a French Himalayist affected by a severe high altitude pulmonary edema at 4800 meters during the ascent of Ratnachuli (Nepal). The guide already trained for the program successfully treated with an addition of three sessions of portable hyperbaric chamber, nifedipine and sildenafil protocol helping by the SOS MAM expert. The second case was a Swiss Himalayist presenting with signs of high altitude retinal hemorrhage at 7000 meter while he was attempting ascent of Cho Oyu (Nepal). Diagnosed by an ophthalmologist experimented in mountain illnesses he was advised to get down and finally saved his eye. The third was a case of chest pain at base camp of Mustagh Ata (China), ECG was performed in the field and transmitted to the expert by the SOS MAM platform attesting cardiac infarction. The fourth concerned a severe frostbite case in North Pole evaluated by satellite phone. Conclusion: The author demonstrates the platform and the successful functioning of the call center SOS-MAM Acknowledgements: Acknowledgements to Linda Keyes. 2015.

SUICIDES AT HIGH ALTITUDE. Marian Betz[1], Morgan Valley[1], Steven Lowenstein[1], Holly Hedegaard[2], Lorann Stallones[3], Benjamin Honigman[1]. [1]Altitude Research Center, University of Colorado School of Medicine, Aurora,Colorado, [2]Colorado Department of Public Health and Environment, Denver, Colorado, [3]Colorado State University, Ft Collins, Colorado. *Email: benjamin.honigman@*

ucdenver.edu. Introduction: Suicide rates are higher in high altitude areas. It has been hypothesized that chronic hypoxia plays a role, but other factors may be more important. We sought to describe the characteristics of completed suicides at high altitudes in the US. Methods: This was a cross-sectional analysis of suicides at high altitude (≥2000m) in 15 states in 2006 as recorded in the National Violent Death Reporting System. Altitude of the victim's county of residence was determined from the National Elevation Dataset, as matched by FIPS code. Results: For the 429 reported suicides, the median age was 44. Most victims were male (79%) and white (94%); more of the female victims (72%) than male victims (60%) lived in urban areas ($p<0.05$). Almost all victims (88%) had at least one stressor (recent crisis; suicide by a family member or friend; legal, physical, financial, job, or interpersonal problem) identified as a contributing factor, with no difference by gender. Most (58%) suicides were by firearm, with fewer by hanging (19%) or overdose (18%). Half the victims had a depressed mood preceding the suicide (according to family or friends), but fewer had a psychiatric diagnosis (34%) and or were receiving mental health treatment (28%). Although men and women were equally likely to have a report of a depressed mood preceding the suicide, women were more likely to have a psychiatric diagnosis (OR 3.21; 95%CI: 1.97-5.25) and to have had current (OR 3.68; 95%CI: 2.24-6.05) or prior (OR 4.75; 95%CI: 2.77-8.12) mental health care, after adjustment for age, race, Hispanic ethnicity, and rural residence. Conclusion: Suicide victims at high altitudes commonly had social stressors or depressed moods identified, but many especially men were not in mental health care when they died. A better understanding of geographic patterns of suicide, including access to care, could inform suicide prevention efforts. Acknowledgements: Supported by Grant Number 5 R49 CE001168 from the Centers for Disease Control and Prevention and by the Emergency Medicine Foundation. Its contents are solely the responsibility of the authors. 2011.

SURGICAL REVASCULARIZATION REVERSES CEREBRAL CORTICAL THINNING RELATED TO NON-ISCHEMIC CHRONIC CEREBRAL HYPOPERFUSION. Joseph A Fisher[1], Jorn Fierstra[2], David Mikulis[3], Michael Tymianski[4]. [1]Department of Anesthesiology, University of Toronto, and the University Health Network, Toronto, Canada, [2]Rudolf Magnus Institute of Neuroscience, University Medical Center, Utrecht, the Netherlands, [3]Division of Neuroradiology, Joint Department of Medical Imaging, University Health Network, Toronto, Canada, [4]Division of Neurosurgery, University Health Network, Toronto, Canada. *Email: joe.fisher@utoronto.ca*. Introduction: Chronic deficiencies in regional blood flow lead to cerebral cortical thinning without evidence of gross tissue loss, while potentially negatively impacting on neurological and cognitive performance. This is most pronounced in patients with severe occlusive cerebrovascular disease in whom affected brain areas exhibit "steal physiology", a paradoxical reduction of cerebral blood flow in response to a global vasodilatory stimulus intended to increase blood flow. We tested whether surgical brain revascularization that eliminates steal physiology can reverse cortical thinning. Methods: We identified 29 patients from our database who had undergone brain revascularization with

pre- and post-operative studies of cerebrovascular reactivity (CVR) using BOLD MRI and whose pre-operative study exhibited steal physiology without MRI-evident structural abnormalities. Cortical thickness in regions corresponding to steal physiology, and where applicable corresponding areas in the normal hemisphere, were measured using Freesurfer software. Results: At an average of 11 months after surgery, cortical thickness increased in every successfully revascularized hemisphere (n=30). Mean cortical thickness in the revascularized regions increased by 5.1% (from 2.40 mm ±0.03 to 2.53 mm ±0.03; $p<0.0001$). Conclusion: Successful regional revascularization and reversal of steal physiology is followed by restoration of cortical thickness. This is the first demonstration of regrowth of atrophied brain tissue. 2011.

SURVEYING PHYSIOLOGICAL FACTORS ASSOCIATED WITH ACUTE MOUNTAIN SICKNESS ON WHITE MOUNTAIN PEAK. Kelly Albin, Nicholas Kanaan, Michael Goodblatt, Frank Powell. University of California San Diego. *Email: kellyalbin@gmail.com.* INTRODUCTION: Acute Mountain Sickness (AMS) affects more than one quarter of people travelling above 8,200 feet elevation, but there are currently no good predictors for AMS susceptibility. Several physiological parameters have been associated with presentation of AMS in single factor studies. This study aims to comprehensively examine multiple physiological parameters in association with AMS in the field. METHODS: 223 (135m, 88f) hikers were recruited to participate in a study after ascent to White Mountain Peak (elev. 4,340m, 14,450ft). Data was collected over three years and assembled into a single data set. Subject age, sex, body mass index (BMI), days at altitude, home altitude, NSAID use was self-reported. Subject peak expiratory flow (PEF), heart rate, HbSat, blood glucose (BG), and blood pressure (BP) were measured and recorded. Subjects were evaluated for AMS using Lake Louise Scoring criteria. Factors associated with development of AMS were examined by comparing two groups: those that developed illness (AMS group) and those that did not (nonAMS group). AMS group data was further analyzed for factors associated with increased AMS severity using multivariate correlation. RESULTS: 26% of hikers were diagnosed with AMS. AMS group subjects were significantly younger ($p<0.001$), had significantly lower BP ($p<0.01$), and had significantly lower HbSat levels ($p<0.05$) than the nonAMS group (Wilcoxon Rank Sum). Subjects who took NSAIDS had a trend towards having a lower proportion of AMS illness than those who did not take NSAIDS (chi-squared, p=0.051). CONCLUSIONS: Young age, low BP, and low HbSat levels should be included in future studies as physiological predictors of AMS. BMI should not be included in prediction studies but is associated with increased AMS severity. NSAIDS may mask AMS symptoms or play a protective role in development of AMS. 2009.

SUSCEPTIBILITY TO HAPE IS ASSOCIATED WITH A MORE UNIFORM SPATIAL DISTRIBUTION OF ALVEOLAR VENTILATION. Michael D Patz[1], Rui C Sá[2], Chantal Darquenne[2], Ann R Elliott[2], Amran K Assadi[2], Rebecca J Thielmann[3], David J Dubowitz[3], Erik R Swenson[4], Kim G Prisk[5], Susan R Hopkins[5].

[1]Department of Anesthesiology, University of Washington, [2]Department of Medicine, University of California, San Diego, [3]Department of Radiology, University of California, San Diego, [4]Department of Medicine, VA Puget Sound Health Care System, University of Washington, [5]Departments of Medicine and Radiology, University of California, San Diego. *Email: mpatz@u.washington.edu.* Introduction: Uneven regional HPV is thought to incite high altitude pulmonary edema (HAPE) by increasing capillary pressure distal to less constricted vessels. We and others have shown using magnetic resonance imaging (MRI) that those susceptible to HAPE (S) but not HAPE-resistant (R) develop increased spatial heterogeneity of pulmonary perfusion in hypoxia consistent with uneven HPV as a characteristic of HAPE susceptibility. Why HPV is spatially uneven is unknown, but it may result from heterogeneously distributed alveolar PO2 stemming from heterogeneity in ventilation at baseline. Methods: We tested the hypothesis that ventilation is more heterogeneous in S than R using multi-breath inert gas washout (MBW) in normoxia and hypoxia (FIO2 = 0.125), where indices Scond and Sacin, represent heterogeneity in ventilation from conductive and respiratory airways respectively. Specific ventilation imaging (SVI), a functional proton MRI technique, was used to measure regional specific ventilation; with the RD of SVI used to quantify heterogeneity. Data were obtained in S (n=6, 1F, 5M), with a history of physician-diagnosed HAPE, and R (n =7, 1F, 6 M), frequent sojourners to > 3,500m without illness. Results: There were no significant differences between groups for age (p=0.23), height (p=0.86), FEV1 (p=0.92) or highest elevation reached (S 5,839±1703 m vs. R 5,950±543 m, p=0.87). Contrary to our hypothesis Sacin tended to be more uniform in S than R (S 0.09±0.01, R 0.11±0.03, p=0.08), and Scond and Sacin did not change significantly with hypoxia (p=0.19, 0.72, respectively). In addition, S had significantly lower ventilation heterogeneity in normoxia on SVI than R (1.30±0.60 vs2.30±0.87, p=0.04). Conclusion: Increased ventilation heterogeneity in normoxia is not a feature of susceptibility to HAPE, and does not increase with hypoxia. This suggests that the basis for uneven HPV in HAPE involves vascular phenomena. Acknowledgements: Support NIH 1R21HL118539. 2015.

SUSPENSION SYNDROME: FROM PATHOPHYSIOLOGICAL MECHANISMS TO A NEW ALGORITHM IN THE FIELD. Sandra LEAL[1], François BECKER[1], Hugo NESPOULET[1], Emmanuel CAUCHY[1]. [1]IFREMMONT- Institut de Formation et Recherche en Médecine de Montagne, Hôpital de Chamonix, France. *Email: sandra.lealmd@gmail.com.* Introduction: Suspension syndrome is defined by a set of symptoms that develop when a person is suspended motionless in a harness. This syndrome can evolve rapidly to unconsciousness and even death. To limit these risks, the rapid initial care by first responders appears to be vital, even before the arrival of emergency healthcare. Due to lack of prospective and randomized studies, few procedures are proposed in current medical literature. Most of these studies are inconclusive and sometimes contradictory, which does not allow for an appropriate assessment of outcome, nor a consensual management algorithm. Methods: The objective of the authors was to make an exhaustive analysis of previously published data's reliability concerning this topic in order to assess their value, to identify the

most likely physiological hypotheses and ultimately to propose a specific and rational algorithm for this syndrome. Results: We can distinguish two situations: when a rapid rescue can be achieved and when descent is not possible in a short delay. In the first case, if the victim is unconscious or has injuries, emergency assistance has to be called and the victim should be released from suspension as quickly as possible. If it is the latter and the subject is conscious, the person has to maintain permanent muscle activity in the lower limbs; a holding position with a saddle may be recommended. If the victim looses consciousness and nearby access to him is possible, the first responder, while waiting for emergency team, will place the victim in a fetal position, with straps or a net. The fact that there is trauma does not allow neglecting this procedure. To ensure that no more than 5% of victims present presyncopal symptoms, lowering should occur in less than 11 min. Once the victim is on the ground, the recommendation is to follow international BLS guidelines without modification. Conclusion: The existence of this syndrome is nowadays completely confirmed, but research studies have still to be undertaken in order to support our knowledge in this area. 2015.

SYSTEMIC ENDOTHELIAL DYSFUNCTION IN NORMOTENSIVE OFFSPRING OF PREECLAMPSIA. Stefano S Rimoldi, Claudio Sartori, Sophie Garcin, Mercedes Villena, Urs Scherrer, Yves Allemann. Inselspital Bern University Hospital Bern, CHUV, Instituto Bolivano de Biologia de Altura. *Email: stefano.rimoldi@insel.ch.* Background: Epidemiological studies suggest that adverse events in utero are associated with cardiovascular disease in adulthood, but the mechanisms are not known. Hypoxia may facilitate the detection of vascular dysfunction. Recently, it has been suggested that offspring of mothers having suffered from preeclampsia are predisposed to hypertension, but the underlying mechanism is not clear. We hypothesized that young normotensive offspring of preeclampsia display systemic vascular dysfunction, and speculated that high-altitude exposure may facilitate the detection of this problem. Methods: We, assessed endothelium-dependent (flow-mediated vasodilation, FMD) and -independent (glycerin trinitrate, 250 μg) vasodilation, vascular stiffness (pulse wave velocity, PWV), and central arterial blood pressure (tonometry) in 15 healthy normotensive offspring of preeclampsia (mean±SD age, 14±7 y) and 16 matched control subjects. All subjects were born and permanently living at high altitude (3600 m). Results: The major new finding was that young, normotensive offspring of preeclampsia displayed systemic endothelial dysfunction (FMD, 6.4±1.0 vs. 8.1±1.3%, P=0.0007). In contrast, arterial stiffness (PWV, 7.8±1.0 vs. 8.0±1.6 m/s) and endothelium-independent vasodilation (16.4± 2.7 vs. 15.8±3.6%) were comparable in both groups. The endothelial dysfunction was not related to a difference in the central arterial blood pressure or oxygen saturation. Conclusions: Here we provide the very first evidence that endothelial function in young normotensive offspring of preeclampsia is abnormal, whereas arterial stiffness and endothelium-independent vasodilation are still normal. We speculate that as in other populations at risk, endothelial dysfunction represents a very early step in the development of arterial hypertension and

cardiovascular disease later in life. To detect this early vascular dysfunction, the measurement of FMD is more sensitive than the assessment of PWV. 2009.

SYSTEMIC ENHANCEMENT OF BLOOD FLOW AS A POTENTIAL THERAPEUTIC STRATEGY TO COUNTERACT HIGH ALTITUDE RELATED HEALTH PROBLEMS. Daniel Radiloff[1], Yulin Zhao[1], Siqing Shan[1], David Irwin[2], Karyn Hamilton[3], Thies Schroeder[1]. [1]Duke University Medical Center, [2]University of Colorado Denver, [3]Colorado State University. *Email: drr2@duke.edu*. Introduction: Acute high altitude exposure represents a challenge to the mammalian cardiovascular system resulting in decreased exercise performance. We hypothesized a pharmaceutical strategy to counteract tissue hypoxia by increasing cardiac output, and concomitantly decreasing pulmonary vascular resistance resulting in increased blood flow, increased oxygen transport, and increased exercise performance. Methods: We evaluated hemodynamic tissue oxygenation and exercise performance parameters in female Sprague Dawley rats exposed to acute high altitude (14,000ft/4267m) and treated with a vehicle control, cardiac stimulant, vasodilator, or combinations of the two latter compounds. Results: We observed that combinational treatment with a cardiac stimulant and a vasodilator showed greater efficacy to enhance oxygenation in the leg muscles of anesthetized hypoxic rats compared to vehicle control or either drug alone. In accordance with the hypothesized mechanism, this increase in oxygenation was not accompanied by an increase in arterial hemoglobin oxygen concentration or ventilation rate, but by an increase in cardiac activity reflected by increased heart rate and pulse distension. Additionally, we observed a significant drop in pulmonary arterial pressure. Compared to all other cohorts, rats receiving combinatorial therapy exhibited a highly significant increase in exercise capacity as measured by time to fatigue in a run-to-exhaustion model. Lung wet-to-dry weight ratio measurements after treatment confirmed that the potential detrimental effect of the cardiac stimulant promoting pulmonary leak is neutralized by addition of the pulmonary vasodilator. Conclusion: Our results suggest that modulation of cardiac activity and blood pressure is a promising and viable option to facilitate rapid acclimation of unacclimatized individuals to high altitude. Since oxygen transport to tissue is restored, this strategy will likely be efficacious against all aspects of AMS that arise from hypoxemia. For this strategy to succeed, however, it is critical that any attempt to increase cardiac output is accompanied by a second drug that vasodilates the lung. Acknowledgements: This work was supported through the Defense Advanced Research Project Agency (DARPA) Prime Award Number N66001-10-C-2134. 2011.

SYSTEMIC NITRIC OXIDE METABOLISM INCREASES AMONG SHERPAS TREKKING TO LOW ALTITUDE. Cynthia Beall[1], Buddha Basnyat[2], Maniraj Neupane[3], Allison Janocha[4], Serpil Erzurum[5]. [1]Case Western Reserve University, Cleveland, OH, USA, [2]Nepal International Clinic, Kathmandu, Nepal, [3]Tribhuvan University School of Medicine, Kathmandu, Nepal, [4]Cleveland Clinic, Cleveland, OH, USA, [5]Cleveland, Clinic, Cleveland, OH, USA. *Email: cmb2@case.edu*. Introduction: The objective was to determine the extent to which nitric oxide (NO)

metabolism among Tibetans is sensitive to altitude changes. Tibetans have generally elevated levels of many measures of NO that are associated with elevated pulmonary and systemic blood flow. An earlier study found elevated exhaled NO among Tibetans residing at 4200m that further increased slightly upon breathing 100% oxygen, suggesting sensitivity to abruptly higher ambient oxygen. Methods: To conduct a more natural exposure to higher ambient oxygen the study tested eight Sherpas (five men and three women with an average age of 30 years) at their native residential altitude of 3900m and during a nine day trek to 2800m, 1600m and back to 2800m. Nitric oxide metabolism was quantified (Sievers NOA 280i) with exhaled nitric oxide to reflect pulmonary metabolism and urinary nitrite and nitrite (NOx) to reflect systematic metabolism. Results: The Sherpas at their home altitude of 3900m averaged 8.2 + 5 nmHg exhaled NO, then increased slightly to 9.5 + 0.9 (SEM) at 2800m and 13.1 + 2.2 nmHg at 1600m (repeated measures ANOVA $p > 0.05$). In contrast, urine NOx first decreased from an average baseline of 1571 + 1322 μMole/L to 459 + 206 μMole/L at 2800m and then increased markedly to 2115 + 871 μMole/L at 1600m (repeated measures ANOVA $p < 0.05$). Conclusion: In conclusion, indicators of nitric oxide metabolism differ in their response to a naturalistic exposure to higher ambient oxygen. Studies relying on a single measure may have provided an incomplete picture. Thorough understanding of nitric oxide metabolism and its role in high altitude adaptation among highlanders will require multiple measures under a variety of conditions. Acknowledgements: We thank the Sherpa trekkers for their participation. The research was supported by NSF Award BCS-0924726 to CMB. 2011.

SYSTEMIC OXYGEN EXTRACTION DURING EXERCISE AT HIGH ALTITUDE. MIchael PW Grocott[1], Mark Edsell[1], Ali Cobb[1], Kay Mitchell[1], Paula Meale[1], Daniel S Martin[1]. [1]UCL Centre for Altitude, Space and Extreme Environment Medicine (CASE Medicine), UCL Institute of Child Health, London, UK. *Email: mikegrocott@gmail.com.* Introduction: A key phase during oxygen flux process is its extraction by active tissues from arterial blood. During exercise, arterio-venous oxygen extraction $C(a\text{-}v)O_2$ is lower at altitude, than sea level {Sutton et al., 1988, J Appl Physiol, 64, 1309-21}, which would seem somewhat counter-productive in an oxygen-depleted environment. We sought to confirm this finding. Methods: $C(a\text{-}v)O_2$ was calculated using arterial and central venous blood during exercise in 5 subjects at 75m and then at 4559m (after 6 days at altitude). Subjects performed an incremental ramp test to exhaustion whilst sequential, simultaneous measurements of arterial and central venous oxygen partial pressure (PaO_2), oxygen saturation (CaO_2) and haemoglobin concentration ([Hb]) were made. Results: Mean PaO_2 and SaO_2 were reduced from 13.2 (±1.3) kPa and 97.6 (±0.7) % at 75m to 6.6 (±0.5) kPa and 87.0 (±3.4) % at 4559m, respectively at rest. Mean [Hb] remained unchanged; 14.2 (±0.8) g/dl at 75m and 14.5 (±1.5) g/dl at 4559m. Thus mean resting arterial oxygen content (CaO_2) declined from 195.6 (±11.8) ml/l at 75m to 176.9 (±14.3) ml/l at 4559m. Mean sea level VO_2max was 3450.0 (±905.7) ml/min, which was reduced by 26.8 % to 2526.4 (±653.8) ml/min at altitude. At sea level the mean resting $C(a\text{-}v)O_2$ was 73.8 (±11.1) ml/l and increased to 112.9 (±28.4) ml/l at maxi-

mum exercise. At 4559m mean resting $C(a\text{-}v)O_2$ 62.6 (±4.5) ml/l and at maximum exercise was 95.1 (±23.8) ml/l. Conclusion: Despite the significant reduction in PaO_2, SaO_2 and CaO_2 at rest after ascent to 4559m there was no increase in the $C(a\text{-}v)O_2$ at rest or at maximum exercise; in fact, there is a tendency for absolute oxygen extraction to decline at altitude. Acknowledgements: The Xtreme Alps Research Group; Smiths Medical; Deltex Medical. Some of this work was undertaken at University College London Hospital–University College London Comprehensive Biomedical Research Centre, which received a proportion of funding from the United Kingdom Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme. 2011.

SYSTEMIC VASCULAR DYSFUNCTION IN CHRONIC MOUNTAIN SICKNESS. Stefano S Rimoldi, Sophie Garcin, Mercedes Villena, Urs Scherrer, Yves Allemann, Claudio Sartori. Inselspital Bern University Hospital Bern, CHUV, Instituto Bolivano de Biologia de Altura. *Email: stefano.rimoldi@insel.ch.* Background: Chronic mountain sickness (CMS) is characterized by chronic hypoxemia, erythrocytosis and pulmonary hypertension. While there is evidence that CMS is associated with pulmonary endothelial dysfunction, there is no information on the systemic circulation. We speculated that patients suffering from CMS are at risk to develop systemic endothelial dysfunction and augmented stiffness of the great arteries, two early markers of vascular disease and important independent predictors of cardiovascular risk. Methods: We assessed endothelium-dependent (flow-mediated vasodilation, FMD) and -independent (glycerin trinitrate, 250 μg) vasodilation, vascular stiffness (pulse wave velocity, PWV), and central arterial blood pressure (tonometry) in 18 normotensive male subjects with CMS (mean±SD age, 53±11 y, Hb>20g/L) and 16 matched control subjects. All participants were born and permanently living at high altitude (3600 m). Results: The major new finding was that subjects with CMS displayed marked vascular dysfunction in the systemic circulation. FMD was 25 percent smaller (5.2±1.0 vs. 6.8±1.7%, P=0.0028) and PWV was significantly faster (10.2±2.1 vs. 8.6±1.3 m/s, P=0.007) in subjects with CMS than in controls. Endothelium-independent vasodilation (12.4±2.4 and 13.7±2.2%, P=0.62) and central arterial pressure (systolic 108±12 vs. 110±10, P=0.55, diastolic pressure 83±7 vs. 83±9 mmHg, P=0.8) were similar in the two groups. As expected, arterial oxygen saturation was lower in the patients than in controls (86.5±2.3 vs. 90.2±2.1%, P<0.001). There was a significant direct relationship between oxygen saturation and FMD (r=0.33, P=0.049). Conclusions: These findings provide the first evidence that normotensive patients suffering from CMS display systemic vascular dysfunction. Chronic hypoxemia may, at least in part, contribute to this dysfunction. We speculate that CMS represents a novel risk factor for systemic cardiovascular disease. 2009.

THE COGNITIVE EFFECTS OF HYPOXIA IN ADOLESCENTS: EXPERIENCE FROM TWO HIMALAYAN EXPEDITIONS. Mary Slingo[1], Fionna Lowe[2], Andrew Pieri[3], Jan Stygall[4], Chris Imray[5]. [1]Department of Physiology, Anatomy and Genetics, University of Oxford, England, [2]Derbyshire, Leicestershire and

Rutland Air Ambulance, England, [3]British Schools Exploring Society, London, England, [4]Unit of Behavioural Medicine, University College London, England, [5]Coventry and Warwickshire County Vascular Unit, University Hospitals Coventry and Warwickshire NHS Trust, England. *Email: mary.slingo@dpag.ox.ac.uk*. Introduction: Neuro-cognitive changes have been reported at high altitude, but it is unclear whether these are predictive of acute mountain sickness (AMS). We developed a battery of 'field' tests, attempting to determine whether changes in neuro-cognitive function were related to the development of AMS in adolescents. Methods: The study was conducted during 35-day expeditions to Ladakh in 2009 and 2010. Comparable ascent profiles were followed, reaching a maximum altitude of 6000m. Three paper-based cognitive tests, comprising trail making A and B (TMTA/B), controlled oral word association (COWA), and auditory verbal learning (AVL) were used. In 2009, the wobble board and cognitive tests were performed at 5 altitudes from 0-5200m. The cognitive tests were repeated (using 4 altitudes) in 2010. Results are means ± standard errors. Results: In 2009, 16 individuals (mean age 16.9 years) completed the cognitive tests and 13 (17.8 years) undertook the wobble board. 11 individuals (10 for the AVL; 17.7 years) completed the cognitive tests in 2010. Performance in cognitive tests improved, between the lowest and highest altitudes, in both expeditions (2010 data shown) – TMTA 27±2 seconds (low) vs. 20±2 (high; $p < 0.05$); TMTB 55±7 seconds vs. 43±4 ($p < 0.01$); COWA 36±3 words vs. 43±3 ($p < 0.01$); AVL (first 5 trials total) 53±2 words vs. 61±2 ($p < 0.01$). There was no change in wobble board performance – 24±3 contacts vs. 22±4 ($p = 0.4$). No difference in performance was found in individuals with AMS. Conclusion: The Lake Louise system for the detection of AMS is widely used. Our tests were unable to predict individuals with AMS. The learning effects appeared greater than that of altitude, making the tests impractical for use on expedition where access to control data is not readily available. Acknowledgements: British Schools Exploring Society. 2011.

THE CONTRIBUTION OF ARTERIAL BLOOD GASES IN CEREBRAL BLOOD FLOW REGULATION AND FUEL UTILIZATION IN MAN AT HIGH ALTITUDE. Christopher Willie[1], David MacLeod[2], Kurt Smith[1], Nia Lewis[1], Glen Foster[1], Keita Ikeda[3], Ryan Hoiland[1], Philip Ainslie[1]. [1]School of Health and Exercise Sciences, University of British Columbia, British Columbia, Canada, [2]Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA, [3]Department of Anesthesiology, University of Virginia School of Medicine, Charlottesville, VA, USA. *Email: ckwillie@gmail.com.* Introduction: The effects of partial acclimatization to high altitude (HA; 5050m) on cerebral metabolism and cerebrovascular function have not been characterized. We hypothesized 1) increased cerebrovascular reactivity (CVR) at HA; and, 2) that CO_2 would affect cerebral metabolism more than hypoxia. Methods: The partial pressures of arterial O_2 (PaO_2) and CO_2 ($PaCO_2$) were manipulated at sea level (SL) to acutely simulate HA exposure; at HA, arterial blood gases at SL were simulated, and CVR and ventilatory sensitivity via steady-state euoxic $PaCO_2$ alterations were assessed. Arterial-jugular venous differences were measured to calculate cerebral metabolic rates and determine cerebral blood

flow (CBF) by the Fick principle. Transcranial Doppler ultrasound (TCD) was also used to estimate CBF. Results: We observed: (1) At SL, the isocapnic reduction in PaO2 to 40 ± 1 mmHg (SaO2: 79 ± 1 %; mean ± SD) elicited a 45 ± 16% increase in CBF, whereas hypocapnic hypoxia caused a 21 ± 16 % reduction in CBF. At 5050m (PaO2 = 41 ± 2 mmHg) normalization of PaO2 (99.0 ± 3.5 mmHg) decreased CBF by 17 ± 6.7%. (2) Independent of altitude, acute manipulation of oxygen did not affect cerebral metabolism, whereas there was a trend for reduced cerebral metabolic rate of glucose with hypercapnia. (3) Partial acclimatization to HA yielded a steeper CO_2-H+ relation in both arterial and jugular venous blood; yet, (3) cerebrovascular reactivity did not change, despite (5) MAP-CO2 reactivity being doubled at HA (SL 1.2 ± 0.74 %/mmHg versus HA 2.8 ± 1.0 %/mmHg), thus indicating (6) effectively maintained cerebral autoregulation. (7) TCD underestimated CVR in hypercapnia at both altitudes implying dilation of the middle cerebral artery. Conclusion: Despite an increased PCO2-H+ slope and elevated MAP-reactivity at HA, the brain adapts effectively to chronic hypoxic hypocapnia; it retains similar fuel utilization and sensitivity to changes in $PaCO_2$, and displays effective autoregulation. Acknowledgements: Supported by the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology, and the Italian National Research Council. C.K.W. was a Vanier Canada graduate scholar. P.N.A. is supported by a Canada Research Chair and NSERC Discovery Grant. 2015.

THE DIFFERENCES BETWEEN SHERPA AND LOWLANDER MICROCIRCULATION ON ASCENT TO HIGH ALTITUDE. Edward Gilbert-Kawai[1], Jonny Coppel[1], Jo Court[1], Michael Grocott[1], Daniel Martin[1](presenting author). [1]UCL Centre for Altitude, Space and Extreme Environment Medicine, London, UK. *Email: e.gilbert@ucl.ac.uk.* Introduction: Anecdotal evidence suggests Sherpas demonstrate extraordinary tolerance to hypoxia at high altitude, the physiological basis for which is unknown. We explored the theory that improved microcirculatory flow may provide a means to aid successful adaptation. Methods: The study was undertaken as part of the Xtreme Everest 2 expedition [1]. Images of the sublingual microcirculation were obtained from 64 Sherpas (SH) and 69 Lowlanders (LL) using the CytoCam video-microscope [2]. Repeated measures were taken at 'Sea Level' (LL: 35m, SH 1300m)), immediately after an 11 day ascent to Everest Base Camp (5300m), and on descent to Kathmandu (1300m). Values were obtained describing capillary density (Total Vessel Density (TVD)), and the microcirculatory flow within said vessels (Microvascular Flow Index (MFI)). Results: Our data demonstrated that there was no difference in TVD between cohorts at Sea Level (LL: median 9.34 mm/m2 (inter quartile range 7.45-11.3), SH: 8.45 mm/m2 (7.70-9.36), p = 0.345), and Kathmandu (LL: 8.69 mm/m2 ((8.31-11.37), SH: 9.34 mm/m2 ((8.5-10.1), p = 0.626), but Sherpa capillary density was significantly greater at Everest Base Camp (LL: 10.52 mm/m2 ((8.90-11.34), SH: 13.83 mm/m2 ((11.4-14.5), p = 0.047). The MFI was also significantly greater in Sherpas at Everest Base Camp (LL: 2.66 (2.45-2.97), SH: 3.00 (2.88 -3.00), p = 0.00), but not at Sea Level (LL: 2.96 (2.62-3.00), SH: 2.81 (2.60–2.98), p = 0.130) and Kathmandu (LL: 2.84 (2.52-3.00), SH: 2.97 (2.75-3.00), p = 0.180). Conclusion:

This is the first study of Sherpa microcirculation. We demonstrate that in hypoxia, Sherpas exhibit both greater capillary density and microcirculatory blood flow. As teleological reasoning would suggest that this allows a greater oxygen flux both per unit time, and per unit volume of tissue, it may in part explain Sherpas' apparent hypoxia tolerance. 2015.

THE EFFECT OF "LIVE HIGH – TRAIN LOW" ON VO2MAX AND POTENTIAL UNDERLYING MECHANISMS – A DOUBLE BLINDED PLACEBO CONTROLLED STUDY. Paul Robach[1], Christoph Siebenmann[2], Robert Jacobs[3], Peter Rasmussen[2], Nikolai Nordsborg[4], Dominik Pesta[5], Erich Gnaiger[5], Victor Diaz[2], Andreas Christ[6], Julia Fiedler[7], Nadine Crivelli[7], Niels Secher[8], Aurélien Pichon[9], Marco Maggiorini[6], Carsten Lundby[2]. [1]Ecole Nationale de Ski et d'Alpinisme, Chamonix, France, [2]Center for Integrative Human Physiology, Zurich, Switzerland, [3]Institute of Veterinary Physiology, Zurich, Switzerland, [4]Department of Exercise and Sport Sciences, Copenhagen, Denmark, [5]Department of Transplant Surgery, Innsbruck, Austria, [6]Intensive Care Unit DIM, Zurich, Switzerland, [7]Hôpital La Vallée, Le Sentier, Switzerland, [8]The Copenhagen Muscle Research Centre, Copenhagen, Denmark, [9]Laboratoire Réponses Cellulaires et Fonctionnelles à l'Hypoxie, Bobigny, France. *Email: paul.robach@jeunesse-sports.gouv.fr*. Introduction: To test the hypothesis that the improvement in maximal oxygen uptake (VO_2max) following live high-train low (LHTL) intervention is related exclusively to hypoxia-induced increases in total hemoglobin mass (Hbmass, measured by carbon-monoxide rebreathing), we measured VO_2max before intervention and again after intervention, before and after the altitude-induced increases in Hbmass had been abolished by means of isovolumic hemodilution (phlebotomy + plasma expander reinfusion). Methods: Starting with 2 weeks of lead-in training, 16 endurance-trained athletes were then assigned in a double blinded placebo controlled manner to ≥16 hours of daily exposure, during four weeks, to either normoxia (placebo, n=6) or normobaric hypoxia equivalent to 3000m altitude (LHTL, n=10). Training was supervised during the whole study. Cycling efficiency was measured and furthermore, we obtained skeletal muscle biopsies to quantify mitochondrial oxidative capacity and efficiency. Results: Four-week intervention induced a small non-significant increase in VO_2max, irrespective of treatment (LHTL: 1.5%; placebo: 2.0%). Hbmass was found to be slightly increased in five (out of ten) LHTL subjects, but this was not accompanied by a concurrent increase in VO_2max. In those subjects, isovolemic hemodilution provoked a 5.6% decrease in VO_2max. Cycling efficiency at 150 or 200W was altered neither by intervention nor LHTL. Neither maximal capacity of oxidative phosphorylation nor mitochondrial efficiency was modified by intervention or LHTL. Conclusion: Our results suggest that 1) LHTL has no positive effect on VO_2max, 2) the changes in VO_2max after LHTL are not mediated by Hbmass, and 3) LHTL does not improve muscle efficiency. Acknowledgements: This study was funded through grants obtained from Bundes Amt für Sport (BASPO, Switzerland), TeamDanmark (Denmark) and Institut National du Sport, de l'Expertise et de la Performance (INSEP, France). 2011.

THE EFFECT OF CHANGES MEAN BLOOD PRESSURE ON MCAV RESPONSE TO INCREASES IN END-TIDAL PCO_2. Rosemary Regan, Marat Slessarev, Jay Han, Alexandra Mardimae, Dahlia Balaban, Stephanie Dorner, Cathie Kessler, James Duffin, Greg Wells, Joseph Fisher. University of Toronto. *Email: Rosemary.regan@utoronto.ca.* Middle cerebral artery blood velocity ('MCAV', a surrogate for cerebral blood flow, 'CBF'), and mean blood pressure (BP), change in tandem with end-tidal PCO_2 ($PETCO_2$). It is not known to what extent the physiologic changes reflect separate responses to the same stimulus and to what extent increases in mean BP augments CBF. Our aim was to surmise these effects from MCAV and BP responses to 2 separate grades of $PETCO_2$ changes. A prospective end-tidal targeting system (RespirAct™, TRI, Toronto, Canada) was used to provide a standardized, repeatable, and stable isoxic ($PETO_2$ resting) square wave step changes in $PETCO_2$ in 12 seated healthy subjects (2 F). Each subject performed 2 protocols (P1, P2). $PETCO_2$ was first fixed at either resting $PETCO_2$ (P1) or 5 mmHg below resting (P2) for 5 min (control). $PETCO_2$ was then raised by 10 mmHg (P1, P2) for 10 min before returning to control values. We monitored MCAV (ST3, Spencer Technologies) and BP (Nexfin, BMEYE). MCAV response (percent change) was larger in protocol P1 than P2 (137% ±10% vs. 128% ± 10%, P = 0,02). Similarly, increases in mean BP were greater in P1 than P2 (6.51 ± 2.1 vs. 3.45 ± 2.5 mmHg respectively, p=0.008). We conclude that both changes in CBF and BP are related more strongly to absolute values of $PETCO_2$ than to the magnitude of changes in $PETCO_2$. Additionally, there may be a threshold in $PETCO_2$ for increases in BP. 2009.

THE EFFECT OF CHRONIC HIGH ALTITUDE EXPOSURE ON PULMONARY ARTERY PRESSURE. Matthias P Hilty[1], Andrea Mueller[1], Katja Auinger[1], Christoph Siebenmann[2], Peter Rasmussen[2], Mike Hug[2], Daniela Flueck[2], Mario Widmer[2], Stefanie Keiser[2], Carsten Lundby[2], Marco Maggiorini[1]. [1]Medical Intensive Care Unit, Univ Hospital of Zurich, [2]Center for Integrative Human Physiology (ZIHP), Institute Physiology, Univ Zurich. *EMAIL: matthias@hilty.info* INTRODUCTION: Acute exposure to high altitude is known to increase pulmonary artery pressure (PAP) as a result of hypoxic pulmonary vasoconstriction. However, the evolution of PAP after several weeks of acclimatization is not conclusively known. Our hypothesis is that chronic high altitude exposure leads to a persistent and in its severity constant elevation of PAP. METHODS: 7 healthy male subjects ascended to 3450m above mean sea level within one day and remained at high altitude for 4 weeks without interruption. Noninvasive hemodynamic assessment was performed using a standardized echocardiography (ECHO) protocol at low altitude at 488m (LAB), at the end of high altitude week 1 through 4 (HA1-HA4), and one week after descent to baseline altitude (LAD). Parameters measured included tricuspid valve regurgitation jet maximum flow velocity and pulmonary vascular resistance (PVR). The results obtained by ECHO were validated by measurements using a pulmonary artery catheter (PAC) at LAB and HA3. Peripheral oxygen saturation was measured during ECHO measurements. RESULTS: During chronic HA exposure, invasively measured sPAP increased from 20±2 to 27±4mmHg (p=0.02).

There was no significant difference in sPAP measured by ECHO from HA1 to HA4 (26±4, 25±4, 25±6, 24±5mmHg, p=0.93) and between LAB and LAD (17±4, 19±2mmHg, p=0.3). There was a significant increase in PVR measured invasively (p=0.02), with no significant difference between HA1 and HA4 measured by ECHO (p=0.55). Peripheral oxygen saturation increased significantly from HA1 to HA4 (p=0.04). The correlation coefficient between sPAP measured by ECHO and by PAC was r=0.65 (p=0.01). Bland Altman analyses showed a bias of -2mmHg, the 2SD confidence intervals being -12/+8mmHg. CONCLUSION: Chronic exposure to 3450m for 4 weeks leads to a significant and persistent increase in PAP compared to LA, which is fully reversible one week after return. We speculate that persistently elevated PAP during chronic exposure may be due either to a constant downregulation of endogenous vasodilator mechanisms or a remodeling of the pulmonary resistant vessels, which is reversible within one week after return to low altitude. 2015.

THE EFFECT OF CHRONIC HYPOXIA ON TAIL VASODILATION OF RATS. Viviana Cadena, Glenn J Tattersall. Brock University. *Email: viviana.cadena@brocku.ca.* Animals respond to hypoxia by reducing body temperature (Tb). This is the result of metabolic depression and a transient increase in heat loss through peripheral vasodilation. After a day of continuous hypoxia, Tb returns to pre-hypoxic values but metabolic rate remains slightly depressed. The mechanisms by which Tb is elevated after exposure to chronic hypoxia are poorly understood. We studied the role of heat loss through tail vasodilation in the rat, in the maintenance of normothermic Tb during chronic hypoxia. A group of rats was acclimated to hypobaric hypoxia (362 mmHg atm, equivalent to 10 % O_2) for two weeks while a control group remained in normoxia. At the end of the acclimation each animal was exposed to 45 min step increments of 1 °C, from 24 to 33 °C after which the animal was returned to its acclimatory condition. This was done during two consecutive days; one in normoxia and one in hypoxia (10 % O_2). Tb was continuously recorded and surface temperatures gathered using infrared thermal imaging, from which tail temperature (Ttail) was later determined. Sudden increments in Ttail were taken as an indication of tail vasodilation. Hypoxic acclimated animals exhibited ambient temperature thresholds for tail vasodilation ~2 °C higher than controls ($P < 0.05$). Differences in this threshold were not observed between acute exposures to hypoxia and normoxia in either hypoxic acclimated or control animals. The results from this study indicate a decrease in heat loss through changes in peripheral vasomotion in hypoxic acclimated animals, and highlight the importance of heat conservation mechanisms in the maintenance of Tb in conditions of chronic hypoxia, such as those encountered in high altitude environments. 2009.

THE EFFECT OF HIGH ALTITUDE AND NORMOBARIC HYPOXIA ON FUEL METABOLISM IN ENDURANCE-TRAINED MEN. Liga Plakane, Juris Aivars. University of Latvia Faculty of Biology. *Email: auce@lanet.lv.* Hypoxia and physical stress under high altitude, lasting a number of weeks, as well as acute normobaric hypoxia cause changes in energy metabolism. The purpose of this study was to quantitatively evaluate plasma glucose concentration and the level of high

altitude hypobaric hypoxia in endurance-trained climbers. An additional goal was to evaluate the type and rate of fuel oxidation within the body during a three-step (50 W, 100 W, 150 W) cycle ergometer exercise test under short-term normobaric hypoxic (15% O_2) conditions.The measurement of morning-fasted plasma glucose (MFPG), fatty acids, insulin, cortisol and complete blood chemistry screen before high altitude climbing and 3-5 days after return to sea level was performed in a clinical laboratory. MFPG was also measured during three high altitude expeditions upon reaching an altitude milestone. Before and after the expeditions an evaluation of gas exchange (O_2 uptake, expired CO_2), metabolic rate, and physical working capacity was performed. The results show that MFPG levels were significantly ($p<0.005$) lower during the entire high altitude climbing period than MFPG at sea level before and after the expeditions. Moreover, the daily decreased MFPG level was strongly and significantly correlated ($r=-0.67$, $p<0.001$) with the altitude at which the athlete spent the previous night. The respiratory exchange ratio (RER) was lower both at rest and during exercise under acute normobaric hypoxia than under normoxic conditions. Analysis of the results allows us to conclude that both normobaric and hypobaric hypoxia cause immediate adaptive changes in energy metabolism, resulting in a decrease in carbohydrate utilization and increased lipid utilization. Acknowledgements: This study was supported by the European Social Foundation. 2009.

THE EFFECT OF HYPOBARIC HYPOXIA ON LEFT VENTRICULAR MECHANICS. Mike Stembridge[1], Philip Ainslie[2], Michael Hughes[1], Eric Stöhr[1], James Cotter[3], Sam Lucas[3], Nia Lewis[2], Keth Burgess[4], Rob Shave[1]. [1]Cardiff Metropolitan Univ, Cardiff, UK, [2]Univ British Columbia Oakanagan, Kelowna, Canada, [3]Univ Otago, Dunedin, New Zealand, [4]Univ Sydney, Sydney, Australia. *EMAIL: mstembridge@cardiffmet.ac.uk* INTRODUCTION: The purpose of this study was to investigate left ventricular (LV) rotation, twist and strain ('LV mechanics') to better understand systolic and diastolic LV function after partial-acclimatization to 5050m. We hypothesized that exposure to high altitude (HA) would result in increased LV twist, rotation and strain. METHODS: Eleven healthy lowlanders (2 female; aged 32±7 years) were studied at sea-level and after 10±3 days at 5050m. LV volumes, transmitral early (E) and late (A) diastolic flow velocities, systolic myocardial velocity (S'), pulmonary artery systolic pressure (PASP) and LV mechanics were measured using standard and speckle-tracking echocardiography. Plasma volume (PV) changes were approximated from hemoglobin and hematocrit. RESULTS: As reported in previous studies, exposure to HA significantly increased resting heart rate (17±22%), PASP (51±24%) and mean arterial pressure (22±14%), and decreased PV (-21±7%, n=7), E/A (-20±12%), end -diastolic (-18±15%), -systolic (-24±14%) and stroke volume (-11±20%) (all $p<0.05$) whilst S' was unaltered ($p=0.90$). A decrease in basal rotation at 5050m (6.2±1.7 vs. 3.7±2.0°, $p<0.05$) coincided with an increase in both systolic and diastolic LV eccentricity index (septal flattening) ($p<0.05$). In contrast, HA led to a doubling of peak apical systolic rotation (6.9±2.7 vs. 15.5±4.8°, $p<0.01$) and peak systolic and diastolic rotation rate (52±24 vs. 99±37 and -62±16 vs. -131±32° s-1 respectively,

p<0.01) whilst time to peak rotation fell ~10% (p<0.01). Furthermore, apical circumferential strain (-25±5 vs. -29±6%, p<0.01) and strain rate (-1.5±0.3 vs. -2.2±0.7 s^{-1}, p<0.01) increased. Changes in overall LV twist mirrored those at the apex. CONCLUSION: In conclusion, ~10-d exposure to HA resulted in increased LV afterload, decreased LV preload and concomitant increases in apical rotation and deformation. Enhanced apical function may help to maximize stroke volume when faced with a unique hemodynamic challenge at HA. ACKNOWLEDGEMENTS: Nepal Health Research Council and EV-K2-CNR. 2015.

THE EFFECT OF LOW AND MODERATE DOSE DOPAMINE ON INDUCIBLE ANATOMICAL INTRAPULMONARY SHUNTS. Tracey Bryan[1], Sean Van Diepen[1], Mohit Bhutani[1], Miriam Shanks[1], Rob Welsh[1], Michael Stickland[1]. [1]Department of Medicine, University of Alberta. *Email: michael.stickland@ualberta.ca*. Introduction: Recent work has shown that anatomical intrapulmonary shunts can be recruited during exercise. Dopamine (DA) is a pulmonary vascular vasodilator and has been shown to cause hypoxemia and increase shunt fraction (Qs/Qt) when used in the critical care setting. Increased circulating DA has been observed with exercise, and it is possible that DA contributes to intrapulmonary shunt recruitment, either by increasing cardiac output, or by acting directly on the pulmonary vasculature via dopaminergic receptors.The purpose of this study was to examine the effect of low- to moderate-dose DA on anatomical intrapulmonary shunt, gas exchange, and shunt fraction in healthy individuals. Methods: Nine healthy subjects with no baseline intracardiac shunt received DA infusions in incremental doses (2-10ug/kg/min) while in the supine position. At each dose an echocardiogram was performed to calculate cardiac output and agitated-saline contrast was administered to evaluate anatomical intrapulmonary shunt. Echocardiographic shunting was scored on a four-point scale by a blinded reviewer. An arterial blood gas was also obtained at each step to assess pulmonary gas exchange and shunt fraction. Results: Incremental DA resulted in a progressive increase in cardiac output with no significant change in pulmonary artery systolic pressure. No subject had significant anatomical intrapulmonary shunt at baseline; however, 5 of 9 subjects developed significant anatomical intrapulmonary shunt with DA. Shunt fraction (Qs/Qt) increased with DA, while the alveolar to arterial PO_2 difference (AaDO2) was unchanged. There was no increase in oxygen consumption (VO_2) with DA; thus mixed venous O_2 content increased during DA infusion. Conclusion: DA appears to recruit anatomical intrapulmonary shunts resulting in an increase in shunt fraction. DA increased cardiac output and correspondingly mixed venous O_2 content; therefore, $AaDO_2$ was unaffected despite an increase in shunt fraction. Acknowledgements: Funding: Canadian Institutes of Health Research. 2011.

THE EFFECT OF RIOCIGUAT ON GAS EXCHANGE, EXERCISE PERFORMANCE, AND PULMONARY ARTERY PRESSURE DURING ACUTE ALTITUDE EXPOSURE. Jon Andrews[1], Stefanie Martina[1], Michael Natoli[1], Aaron Walker[1], John Freiberger[1], Richard Moon[1]. [1]Department of Anesthesiology, Center for Hyperbaric Medicine and Environmental Physiology, Duke University Medical

Center, Durham, NC. *Email: jon.andrews@dm.duke.edu.* Introduction: Impairment of exercise performance during altitude exposure is caused primarily by lower arterial oxygen content leading to reduced oxygen delivery to exercising muscles. Ultimately, hypoxemia results in hypoxic pulmonary vasoconstriction (HPV), causing pulmonary hypertension with further ventilation/perfusion (VA/Q) mismatch and PO2 decline. Therefore, if the HPV could be attenuated by pharmacologic blockade, then VA/Q matching, arterial PO2, and exercise performance should improve. The nitric oxide (NO) pathway has been targeted in this context with sildenafil, but NO is limited at high altitude, which may explain the variable effectiveness of this approach. Riociguat is a soluble guanylate cyclase stimulator that does not require NO in its mechanism. We hypothesize that riociguat will decrease pulmonary artery pressure and improve gas exchange and exercise performance at altitude. Methods: Subjects instrumented with radial and pulmonary artery (PA) catheters performed incremental exercise tests on a bicycle ergometer at a simulated altitude of 15,000 feet before and after administration of riociguat. The following parameters were measured during rest and exercise: radial artery and PA pressures, heart rate, ventilation, cardiac output, arterial and mixed venous blood gases and O2 saturation, alveolar-arterial PO2 difference, and arterial oxygen delivery before and after administration of the drug. Results: Preliminary results demonstrate decreased pulmonary artery pressure and pulmonary vascular resistance at all stages of exercise, while saturation of arterial hemoglobin was slightly, though not significantly increased. Conclusion: On the basis of preliminary results, we conclude that at mild levels of exercise intensity at 15,000 feet simulated altitude, a one-time dose of 1.0 mg riociguat appears to decrease pulmonary artery pressure but does not significantly improve arterial oxygenation or peripheral oxygen delivery. Acknowledgements: This research was made possible thanks to a $10,000 Hackett-Auerbach grant from the Wilderness Medical Society. 2015.

THE EFFECT OF SILDENAFIL AND ACETAZOLAMIDE ON HYPOXIC SLEEP ARCHITECTURE. Robert P Frantz, S Lalande, Eric M Snyder, Thomas P Olson, Minelle L Hulsebus, Marek Orban, Virend K Somers, Bruce D Johnson. College of Medicine Mayo Clinic. *Email: frantz.robert@mayo.edu.* The objective of this study was to determine the effects of sildenafil and acetazolamide with hypoxic exposure on SaO_2, periodic breathing and apnea/hypopnea during sleep. Methods: Polysomnograms were obtained on 8 volunteers receiving sildenafil 40 mg (S), acetazolamide 125 mg (A) or matching placebo (P) tid x four doses in double-blind fashion starting 8 hrs prior to hypoxic exposure over 3 separate overnight stays in an altitude tent with an O_2 concentration of 12.5%. A repeated measures 1-way analysis of variance was performed to test for drug effect. Results: Acetazolamide but not sildenafil improved mean sleep SaO_2 (A: 86.1 ± 0.9, S: 83.5 ± 1.5, P: 81.5 ± 1.8%, $p<0.04$) with a smaller percentage of total sleep time spent with SaO_2 of 70-79% (A: 1 ± 1, S: 20 ± 9, P: 43 ± 13%, $p<0.02$; $p=0.17$ for S vs P). Sleep efficiency was improved with acetazolamide when compared to sildenafil. Total sleep time and total arousals were not different between conditions but there was a 17% reduction in arousals secondary to periodic breathing with acetazol-

amide. The hourly episodes of central or obstructive apnea were not different between conditions. Acetazolamide increased the duration of hypopnea during sleep when compared to sildenafil and placebo (A: 21.8 ± 0.7, S: 16.6 ± 0.9, P: 17.5 ± 0.9 sec, $p<0.01$). Conclusions: Acetazolamide improves hypoxic sleep SaO_2 but is associated with longer duration of hypopnea. Acetazolamide improved sleep efficiency in comparison to sildenafil. Sildenafil did not have any significant effects on SaO_2 or periodic breathing during single overnight exposure. This study was funded by Mayo Foundation and NIH Grant No. HL71478. 2009.

THE EFFECTS OF GRADED HYPEROXIA ON CEREBRAL BLOOD FLOW IN HEALTHY HUMANS: DATA AND META-ANALYSIS. Danielle Sorenson[1], Megan Crosby[1], Vanessa Mattson[1], Nathan Hart[1], Trevor Day[1]. [1]Mount Royal University. *Email: tday@mtroyal.ca.* Introduction: Stroke is the third leading cause of death in Canada, and effective early treatments are of critical importance to limit damage. During a stroke, the blood supply to brain tissue is reduced or blocked (ischemia), which can cause irreversible damage to brain tissue if not treated and reversed quickly. Emergency personnel routinely provide 100% oxygen to patients suspected of having a stroke. However, increases in the fraction of inspired oxygen (hyperoxia) can cause cerebral vasoconstriction, potentially exacerbating ischemia. Methods: We investigated the effect of graded hyperoxia on cerebral blood velocity in the right (R) and left (L) middle cerebral artery (MCAv) using transcranial Doppler ultrasound. In random order, 12 participants (23.7±3.6yrs, 24±3.9kg/m2, four males) received five different concentrations of inspired oxygen by mouthpiece: 15% (hypoxic control), 21% (room air), 40%, 70% and 100% for five minutes each, all with coached isocapnia (±1 Torr CO_2). Results: Mean R-MCAv was only significantly lower in extreme hyperoxia (100%) compared to 15% O2 (48.5±4.3 vs. 50.8±4.3 cm/s, P=0.02, n=12). Mean L-MCAv was not different across all treatments (P=0.09, n=10). We also compared and plotted the responses of previous studies of brain blood flow and hyperoxia (both isocapnia and poikilocapnia), with similar results, depending in part on the CO_2 status. Conclusion: Our data and meta-analysis suggest that hyperoxia has minimal effects on brain blood flow in healthy individuals. However, further studies are needed to fully characterize the confounding effects of CO_2 (i.e., poikilocapnia and isocapnia), within-subjects. In addition, further research is required in populations experiencing ischemic stroke, particularly when tissues are initially hypoxemic, and relative hyperoxia may cause vasoconstriction within the dynamic range. Results in healthy individuals should be compared with elderly or clinical populations with caution, and further investigation is required. 2015.

THE EFFECTS OF LOWER BODY POSITIVE AND NEGATIVE PRESSURE ON HYPOXIC VENTILATORY DECLINE. Michael S Koehle, Luisa V Giles, Michael Walsh, Matthew D White. University of British Columbia, Simon Fraser University. *Email: koehle@interchange.ubc.ca.* PURPOSE: Lower body negative pressure (LBNP) has been shown to augment the hypoxic ventilatory response (HVR) in humans. The present study investigated the effects of LBNP and lower

body positive pressure (LBPP) on hypoxic ventilatory decline (HVD) in humans. METHODS: Seven individuals (3 females and 4 males) were tested in a supine position with the lower body supported inside a hypo/hyperbaric chamber. During each test the participant was exposed to LBNP at -37.5 mmHg, LBPP at +37.5 mmHg and to ambient pressure (LBAP) at 0 mmHg in a random order. Blood pressure, expired gases and O_2 saturation were continuously recorded. Hypoxia was administered in a single step to a $PETO_2$ of 50 mmHg for 20 min. For all tests $PETCO_2$ was maintained at the pre-hypoxic resting level. The HVR was defined as the ventilatory change from hypoxia onset to peak VE divided by a theoretical SaO_2 using $PETO_2$ (ScO_2). The HVD was calculated as the percent decrease in ventilation from peak to the twentieth minute of hypoxia. RESULTS: The HVR was significantly greater during LBNP (1.94±0.54 $L{\cdot}min^{-1}{\cdot}\%ScO_2^{-1}$) than during ambient pressure (1.34±0.37 $L{\cdot}min^{-1}{\cdot}\%ScO_2^{-1}$; p=0.039) and LBPP (1.37±0.23 $L{\cdot}min^{-1}{\cdot}\%ScO_2^{-1}$; p=0.025). Hypoxic ventilatory decline was not significantly different across the three conditions (p=0.144). Both mean arterial pressure and pulse pressure were not affected by 37.5 mmHg of either positive or negative lower body pressure (p=0.941, and p=0.275, respectively). CONCLUSION: These data suggest that LBNP increases HVR, while LBPP has no effect on HVR. The increased HVR in LBNP is likely mediated through altered central integration of baro- and chemoreceptor afferents. Since LBNP increases HVR without affecting HVD, these findings indicate that HVD occurs at the level of the peripheral chemoreceptors and not centrally. 2009.

THE EFFECTS OF NORMOBARIC HYPOXIC TRAINING ON HEMOGLOBIN AND HEART RATE IN MOUNT EVEREST CLIMBERS. Wei-Fong Kao. National Yang-Ming University, Taipei, Taiwan. *Email: wfkao@vghtpe.gov.tw*. Introduction: To investigate the effects of hypoxic training (HT) on hemoglobin and heart rate (HR) in Mount Everest Climbers. Methods: Four Taiwanese who enrolled in the 2009 Mount Everest summit program participated in this study. All of them had successfully completed six summits of the Seven Summits between 2006 and 2008. Two of the four climbers received Normobaric HT in Taiwan before leaving for Mount Everest. Climbers took 100 mg Ferrum hausmann twice a day since 12 days prior to the HT. The training climbers slept in the hypoxic room for 10 days with about 8 hours each night. The oxygen concentration of training ranged from 14% to 10%. Blood samples were collected 12 days prior to (baseline) and 5 days after hypoxic training (AHT), and 11 days and 1 month after the day of summiting Mount Everest. Wearable ECG devices were used to record and transmit the ECG of climbers during the course of Mount Everest climbing. The daily maximal and minimal HR during last attempt to summit the Mount Everest were traced from the ECG device. Results: Four Taiwanese, one female and three male with a mean age of 39±6 years (range from 34 to 48 years old), who planned to summit the Mount Everest in 2009 participated in this study. Three of the Four Climbers, including two climbers receiving HT, summited Mount Everest successfully. The average hemoglobin level of the 2 male climbers who received HT was 13.6±0.2 g/dl before training and 16.9±0.4 g/dl after training. The hemoglobin level was significant elevated

after training ($p<0.001$). The hemoglobin level of the male climber who did not receive hypoxic training and did not summit was 14.1 g/dl at baseline and 14.8 g/dl before leaving for Nepal. Clinically, the two climbers who received HT had less discomfort than the other two climbers who did not receive. The maximal HR that we recorded from our climbers on the summit-day was 150 /min at the peak of Mount Everest. Conclusion: Normobaric HT made a significant elevation of the hemoglobin level. Climbers who received normobaric HT summited Mount Everest successfully. The maximal HR that we recorded from our climbers on the summit-day was 150 /min at the peak of Mount Everest. 2011.

THE EFFECTS OF SILDENAFIL AND ACETAZOLAMIDE ON BREATHING EFFICIENCY DURING HYPOXIC EXERCISE. Robert P Frantz, Sophie Lalande, Eric M Snyder, Thomas P Olson, Marek Orban, Minelle L Hulsebus, Virend K Somers, Bruce D Johnson. College of Medicine Mayo Clinic. *Email: frantz.robert@mayo.edu.* The ability to work efficiently at altitude is impaired by the reduced arterial oxygen tension. Acetazolamide may improve systemic saturation (SaO_2) by stimulating ventilation but increases the work of breathing. Sildenafil may also improve SaO_2 at altitude. The purpose of this study was to determine the effects of acetazolamide and sildenafil on ventilatory control and breathing efficiency during submaximal steady state hypoxic exercise. Fifteen volunteers received sildenafil 40 mg (S), acetazolamide 125 mg (A) or matching placebo (P) tid x four doses in double-blind fashion starting 8 hours prior to hypoxic exposure over 3 separate overnight stays in an altitude tent with an O_2 concentration of 12.5%, and then performed hypoxic exercise on a stationary bicycle for 10 min at a workload of 40% of VO_2peak. There was no difference in exercising VO_2 between conditions. SaO_2 was greater with acetazolamide compared to placebo and sildenafil. As expected, acetazolamide increased ventilation and reduced $PETCO_2$ compared to placebo and sildenafil. Breathing efficiency as measured by VE/VCO_2 decreased with acetazolamide in comparison to placebo and sildenafil (P: 41.5 ± 1.0, S: 40.4 ± 1.3, A: 45.4 ± 1.0, $p<0.01$). Sildenafil did not change breathing efficiency while acetazolamide reduced breathing efficiency during hypoxic exercise. This study was funded by Mayo Foundation and NIH Grant No. HL71478. 2009.

THE END-TIDAL-TO-ARTERIAL CO_2 GRADIENT AND CEREBROVASCULAR REACTIVITY. Michael Tymko[1], Tom Kuca[2], Ryan Hoiland[1], Lindsey Boulet[1], Josh Tremblay[1], Bryenna Pinske[1], Alex Williams[1], Anthony Bain[1], Glen Foster[1]. [1]University of British Columbia - Okanagan, [2]University of Dalhousie. *Email: mike.tymko@alumni.ubc.ca.* Introduction: The end-tidal partial pressure of CO_2 ($PETCO_2$) is often considered a surrogate for the arterial partial pressure of CO_2 ($PaCO_2$), however, during hypercapnia a $PETCO_2$-to-$PaCO_2$ gradient (PET-$PaCO_2$) exists such that $PETCO_2$ overestimates $PaCO_2$. We sought to determine if the PET-$PaCO_2$ affects the cerebrovascular reactivity (CVR) profile and whether it could be corrected for in subjects with (n=8) and without (n=7) a patent foramen ovale (PFO). We hypothesized that (1) the PET-$PaCO_2$ would be less in those with a PFO during hypercapnia due to venous admixture, (2) hypercapnic CVR would be lower

when plotted against $PETCO_2$ compared to $PaCO_2$, and (3) a $PaCO_2$ prediction algorithm will appropriately correct for the PET-$PaCO_2$. Methods: $PETCO_2$ was controlled by end-tidal forcing in steady-state steps of -8, -4, 0, +4, and +8mmHg from baseline in normoxia (NX1; $PETO_2$=94.3±1.3mmHg) and hypoxia (HX1; $PETO_2$=50.8±0.1mmHg). Tests were repeated following correction for the PET-$PaCO_2$ (NX2 and HX2). During each test internal carotid artery blood flow (QICA), and temperature corrected $PETCO_2$, and $PaCO_2$ were measured at the end of each $PETCO_2$ step. CVR was calculated using linear regression analysis in the hypocapnic and hypercapnic ranges by indexing the percent change in QICA against $PETCO_2$ and $PaCO_2$. Results: In both conditions, a PET-$PaCO_2$ was present in hypercapnia ($P<0.05$) but not hypocapnia, and was unaltered by PFO. CVR assessed using $PETCO_2$ was lower compared to CVR determined using $PaCO_2$ during normoxia (6.0±0.7 vs. 7.5±0.9%/mmHg; $P<0.001$) and hypoxia (7.1±0.5 vs. 8.0±0.5%/mmHg; $P=0.01$). The prediction equation derived from previous work minimized the difference between $PaCO_2$ and the target $PaCO_2$ ($tPaCO_2$) during +4mmHg (NX2: $tPaCO_2$-$PaCO_2$=0.0±0.2mmHg, $P=0.894$; HX2: $tPaCO_2$-$PaCO_2$=-0.2±0.2mmHg, $P=0.403$) and +8mmHg for HX2 ($tPaCO_2$-$PaCO_2$=0.0±0.3mmHg, $P=0.860$) but not NX2. Conclusion: In conclusion, care must be taken when comparing PETCO2 CVR to PaCO2 CVR. Acknowledgements: NSERC and CFI. 2015.

THE HUMAN CAROTID BODY TRANSCRIPTOME WITH FOCUS ON OXYGEN SENSING AND INFLAMMATION. Jessica Kåhlin[1], Souren Mkrtchian[1], Constancio Gonzalez[2], Machico Shirahata[3], Malin Jonsson Fagerlund[1], Lars I Eriksson[1]. [1]Karolinska Institutet, Stockholm, Sweden, [2]Univ Valladolid-CSIC, Valladolid, Spain, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. *EMAIL: jessica.kahlin@karolinska.se* INTRODUCTION: The CBs regulate the hypoxic ventilatory response (HVR) through oxygen sensing and signaling. Our aim is to characterize the CB transcriptome with focus on oxygen sensing genes in unique human CB tissue. Animal CBs are extensively studied in contrast to human, and we hypothesize that there are differences in the expression of oxygen sensing genes in human and mouse CBs. METHODS: CBs from patients undergoing radical neck dissection were studied with microarray and PCR. The resulting gene lists were compared with transcriptomes of mice CBs from two published studies (Balbir et al, Am J Physiol Lung Cell Mol Physiol, 2007 and Ganfornina et al, J Physiol, 2005) and also with other tissues in public databases using on-line tools. RESULTS: The human CB expressed ~ 13,500 genes and has a unique tissue profile. It overexpresses genes in inflammation in comparison with brain and adrenal gland. We demonstrated molecules in oxygen sensing such as cystathionine λ-lyase, heme-oxygenase 2, AMP-kinase, superoxide dismutase and NADPH-oxidase 2 and 4. In the human CB two essential oxygen sensing K+ channels were expressed (TASK-1 and Maxi-K) and also components of the systemic inflammatory response (toll-like receptors 1 and 4 and cytokines of both the early and late response). We found similarities but also clear differences in gene expression between human and mouse CBs. CONCLUSION: The CB is the peripheral regulator of breathing during hypoxia. We show key oxygen sensing components in

the human CB, as well as inflammatory response elements. Distinct differences between human and animal CB gene expression are shown, however animal CB function data can not easily be translated onto humans. This calls for further studies on human CB oxygen sensing and signaling function. ACKNOWLEDGEMENTS: This work was supported by grants from the Swedish Research Council (No. 521-2011-152 (L.I.E.)), the Stockholm County Council (ALF project 20090321 (L.I.E.)), Post-Doc project 108042 (M.J.F.), Ministerio de Ciencia e Innovacio Spain, grant no. BFU2007-61848 (CG), Instituto Carlos III, grant no. CIBER CB06/06/0050 (CG), NHLBI HL81345 of the USA (MS). 2015.

THE HYPOXIA ASSOCIATED FACTOR (HAF) MODULATES THE SWITCH FROM HIF-1α TO HIF-2α DURING CHRONIC HYPOXIA AND PROMOTES TUMOR SELF-RENEWAL AND INVASION. Mei Yee Koh[1], Robert Lemos Jr[1], Garth Powis[1]. [1]U.T.M.D. Anderson Cancer Center, Houston TX USA. *Email: mykoh@mdanderson.org*. Introduction: Critical mediators of the hypoxic response are the hypoxia inducible factors, HIF -1α and HIF-2α, which play distinct roles in cancer progression (1). Generally, HIF-1α regulates acute metabolic changes required for hypoxic survival, whereas HIF-2α is important for adaptation to chronic hypoxia, including the maintenance of cancer stem cells. However, the mechanism determining HIF-1α versus HIF-2α activation is unclear. The hypoxia associated factor (HAF), which is overexpressed in cancer, is an E3 ubiquitin ligase that ubiquitinates HIF-1α independently of oxygen and pVHL, thus targeting HIF-1α for proteasomal degradation, without affecting HIF-2α levels (2). Our objective was to delineate the role of HAF in modulating HIF activity and its effects on hypoxic tumor progression. Methods: HAF and HIF-1/2α binding domains were mapped using in vitro binding assays. HIF-1α or HIF-2α specific target genes were identified using microarrays, whereas the effects of HAF modulation on HIF-1/2α specific transcription were confirmed using qRT-PCR and luciferase reporter assays. Relevant downstream outcomes were assessed using FACs, invasion transwells, 3D assays and an intracranial glioblastoma mouse model. Results: HAF binds to HIF-2α at a different site than HIF-1α, and increases HIF-2α transactivation without causing HIF-2α degradation. HAF levels decrease during acute hypoxia, but increase following chronic hypoxia, resulting in the inhibition of HIF-1α specific target genes (eg. CA-IX, DDIT4), and the activation of HIF-2α specific target genes (eg. MMP9, PAI-1, OCT3/4). In glioblastoma cells, the HAF-induced switch to HIF-2α dependent transcription promotes invasion and enriches the proportion of CD133+ tumor stem cells. This increases the rate of tumor take, increasing tumor morbidity in vivo. Conclusion: HAF, by degrading HIF-1α and promoting HIF-2α transactivation, provides a mechanism by which cancer cells can switch between HIF-1α to HIF-2α dependent transcription in response to changes in hypoxic duration and intensities. Acknowledgements: NIH Grants CA095060, CA17094, CA098920 and CA127001. 1. Gordan JD, and Simon MC. Curr Opin Genet Dev 17: 71-77, 2007; 2. Koh MY, Darnay BG, and Powis G. Mol Cell Biol 28: 7081-7095, 2008. 2011.

THE HYPOXIA INDUCIBLE FACTOR (HIF) THREATENS HYPOXIC SURVIVAL. Goran E Nilsson, Miriam Oijordsbakken, Stian Ellefsen, Jonathan Stecyk, Kare-Olav Stenslokken. University of Oslo. *Email: g.e.nilsson@imbv.uio.no.* The crucian carp (Carassius carassius) is an extremely anoxia tolerant animals, being able to survive without oxygen up to half a year. HIF is a transcription factor known to stimulate the transcription of various hypoxia related genes, including erythropoietin and vascular endothelial growth factor (VEGF), thereby promoting the formation of red blood cells and angiogenesis. To assess the role of HIF in the anoxic survival of the crucian carp brain, crucian carp were exposed to severe hypoxia ($pO_2 \approx 5$ mmHg) for 10 days. Both the protein and mRNA levels of HIF1-α were reduced during hypoxia, while the level of Factor Inhibiting HIF (FIH-1) increased 4-fold. Although there were significant increases in both VEGF-A and VEGF-C (but not VEGF-D), there was a reduced capillary density in the brain after 10 days of hypoxia. Thus, while HIF is likely to be important for local responses to tissue hypoxia in many organisms, a well-adapted facultative anaerobe like the crucian carp probably has to suppress HIF levels and HIF responses during global hypoxia (i.e. hypoxia that is affecting all tissues simultaneously). To allow HIF to stimulate angiogenesis in all tissues would probably be maladaptive, since the fish would have to pay high costs for cell proliferation and for pumping increased blood volumes. In fact, our results show that not even the brain, which has particularly high energy needs, is allowed an increased capillary density in hypoxia. 2009.

THE IMPACT OF ACUTE NORMOBARIC HYPOXIA ON THE RESTING HEART RATE AND CORRECTED QT INTERVAL (QTC) OF HEALTHY VOLUNTEERS. Rahul K Mukherjee, Jayna Gadawala, George W Rodway, Jeremy S Windsor. UCL, The University of Utah School of Medicine. *Email: mukherjee79@hotmail.com.* Objective: To measure the resting heart rate and corrected QT interval (QTc) of a group of healthy volunteers. Methods: 10 healthy male and female volunteers were placed in a supine position and exposed at random to four different fractions of inspired oxygen (FIO_2)-0.12, 0.15, 0.18 and 0.21. Each period of exposure lasted 15 minutes and was interrupted by a period of 30 minutes of normoxic exposure (FIO_2=0.21). A 12-lead digital electrocardiogram (Welch Allyn,UK) was obtained every five minutes from which the heart rate and corrected QT interval was obtained. The QTc was calculated using Bazett's Formula (QTc=QT/Å„RR). Repeated measures analysis of variance (RMANOVA) was used to test the effect of different levels of FIO_2 on QTc. Results: With decreasing FIO_2 levels (0.21, 0.18, 0.15, and 0.12) the mean heart rate increased from 67.4 to 76.2, 77.5 and 80.5 beats per minute. Meanwhile, the mean QTc increased from 388.3 to 397.4, 398.7 and 407.2 milliseconds. RMANOVA demonstrated that a significant difference was observed in the HR between different levels of FIO_2 $F(3, 29) = 31.03$ ($p < 0.001$). Tukey post-hoc testing showed that the HR at $FIO_2 = 0.21$ was lowest and differed significantly from the HR at FIO_2=0.12, 0.15 and 0.18. However no significant difference was seen between the three hypoxic mixtures. RMANOVA demonstrated that a significant difference was observed in QTc between different

levels of FIO_2 $F(3, 29) = 76.45$ ($p < 0.001$). Tukey post-hoc testing showed that the FIO_2= 0.12 period had the highest QTc and differed significantly from the QTc at FIO_2=0.15, 0.18 and 0.21. An additional RMANOVA was conducted to determine if QTc changed over the three measurements within the 15 minute hypoxic exposure (5, 10, and 15 minute measures). Post-hoc testing reported no difference in the effect of the three time intervals studied (p=0.996, p=0.886, p=0.958). Conclusion: Exposure to acute normobaric hypoxia resulted in an increase in heart rate and QTc interval. 2009.

THE IMPACT OF CHRONIC HYPOBARIC HYPOXIA ON THE RESTING HEART RATE, CORRECTED QT INTERVAL AND ARTERIAL OXYGEN SATURATION OF TREKKERS ASCENDING TO MT EVEREST BASE CAMP. Rahul K Mukherjee, Jayna Gadawala, JW Blaz, R Wong, George W Rodway, Jeremy S Windsor. UCL, University of Utah College of Nursing. *Email: mukherjee79@hotmail.com.* Study Objective: To measure the resting heart rate, corrected QT interval (QTc) and arterial oxygen saturation (SaO_2) of a group of healthy trekkers during an ascent to Mt Everest Base Camp (5600m). Methods: 17 men and 20 women aged between 19 and 28 (mean: 23 years) undertook a trek from Lukla (2850m) to Mt Everest Base Camp (5300m) over 13 days. A resting arterial oxygen saturation (SaO_2) (Nonin Onyx, Nonin, Minnesota, USA) and 12-lead digital electrocardiogram (Welch Allyn PCR-100i, Welch Allyn, Aston Abbotts, UK) were obtained from each participant in London (75m), and within two hours of arrival at Namche Bazaar (3500m), Pheriche (4250m) and Gorak Shep (5150m). The corrected QT interval (QTc) was calculated using Bazett's Formula (QTc=QT/ÅRR). The relationship between QTc, gender, and SaO_2 was assessed using Generalized Estimating Equations (GEE) modelling. Results: Mean heart rate increased from 62.4 (London), to 73.8 (Namche Bazaar), 76.4 (Pheriche) and 80 beats per minute (Gorak Shep). Mean QTc increased from 396.2 (London), to 430.8 (Namche Bazaar), 437.1 (Pheriche) and 446.5 ms (Gorak Shep). Mean arterial oxygen saturation fell from 98.6 (London), to 83.6 (Namche Bazaar), 81.0 (Pheriche) and 75.3 % (Gorak Shep). All values were significantly different to sea level ($p<0.05$). An increase in QTc was found to be associated with a reduction in SaO_2 ($p<0.001$) and being female (p=0.023). A 1% fall in SaO_2 was associated with a 2.86 ms increase in QTc. Conclusion: In a group of 37 healthy trekkers ascending to Mt Everest Base Camp, resting heart rate and QTc were found to increase whilst SaO_2 fell. The fall in SaO_2 was strongly associated with an increase in QTc calculated using Bazett's Formula. 2009.

THE INCIDENCE AND SEVERITY OF ACUTE MOUNTAIN SICKNESS ARE NOT REPEATABLE ACROSS TWO 12-HOUR NORMOBARIC HYPOXIA EXPOSURES. Martin MacInnis[1], Sarah Koch[1], Kristin MacLeod[1], Eric Carter[1], Radha Jain[1], Michael Koehle[1], Jim Rupert[1]. [1]School of Kinesiology, Univ British Columbia. *EMAIL: martin@alumni.ubc.ca* INTRODUCTION: A previous history of acute mountain sickness (AMS) is often reported to be a strong risk factor for the recurrence of AMS; however, the repeatability of AMS has not been measured in a

sham-controlled experiment. METHODS: Subjects (n = 25) slept three times in a normobaric hypoxia chamber [twice at an FIO_2 of 0.126 (hypoxia) and once at an FIO_2 of 0.186 (sham)], with a minimum of 2 weeks between exposures. AMS severity (Lake Louise Score; LLS), heart rate (HR), oxygen saturation (SPO_2), mean arterial pressure (MAP), and the fraction of exhaled nitric oxide (FENO) were measured before subjects entered the chamber (hour 0) and before subjects exited the chamber (hour 12). Intraclass correlations (ICC), Cronbach's alpha (α), and Kendall's W were used to measure repeatability. RESULTS: Relative to the sham condition, hypoxia decreased SPO_2, increased HR and MAP ($p < 0.05$), but did not affect the FENO ($p > 0.05$). The incidence of AMS (84% vs. 56%; $p = 0.065$) was greater on the first hypoxic night compared to the second hypoxic night, and the agreement between LLS on the two hypoxic nights was low ($W = 0.23$). The HR (ICC = 0.84; $\alpha = 0.92$), MAP (ICC = 0.68; $\alpha = 0.81$), and FENO (ICC = 0.78; $\alpha = 0.88$) were highly repeatable across the two hypoxic nights, but the repeatability of SPO_2 was moderate (ICC = 0.39; $\alpha = 0.54$). CONCLUSION: Physiological responses to hypoxia were moderately or strongly repeatable, but subjective ratings of AMS symptoms were much less repeatable. Acclimation to the chamber, and not acclimation to hypoxia, is likely the reason for discordant subjective responses across hypoxic exposures. ACKNOWLEDGEMENTS: Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC). MM was supported by an NSERC Canada Graduate Scholarship. 2015.

THE INDEPENDENT EFFECTS OF PAO_2 AND $PACO_2$ ON BRAIN O_2 DELIVERY (DO_2) AT ALTITUDE. David Preiss, Dahlia Balaban, Alexandra Mardimae, Marat Slessarev, Dick Greene, Alex Vesely, Joseph Fisher. Boston University, Thornhill Research, Thornhill Research, Thornhill Research, New Mexico Highlands University, University of British Columbia, Thornhill Research. *Email: davidapreiss@gmail.com.* Introduction: At altitude, the hypoxic ventilatory response (HVR) increases O_2 saturation of hemoglobin by increasing alveolar PO_2, and shifting the oxyhemoglobin dissociation curve (ODC) leftward through reduced arterial PCO_2 ($PaCO_2$). $PaCO_2$ is an important determinant of cerebral blood flow (CBF) and thereby DO_2. We studied how the independent control of $PaCO_2$ during hyperpnea influences DO_2 at altitude. Methods: Five (3M) healthy lowlanders at 3800m for one week breathed via sequential rebreathing circuit and a custom gas blender designed to dissociate ventilation from end-tidal PO_2 ($PETO_2$) and $PETCO_2$ (Respiract™, TRI Toronto, Canada). $PETCO_2$ was clamped at resting level and $PETO_2$ was targeted at 100mmHg to 70, 60, 50, 40, and 35, 100 mmHg at 2 min intervals. This was repeated with $PETCO_2$ targeted at 10 mmHg below resting value. We measured tidal gas concentrations, pulse oximetry (SpO_2, Nonin) middle cerebral artery velocity (MCAV) and calculated relative DO_2 (rDO_2=SpO_2 x MCAV) for each step $PETO_2$. Each ODC was calculated as a line of best fit through each set of SpO_2 and $PETO_2$. Results: The subjects' resting $PETO_2$, $PETCO_2$, SpO_2 and rDO_2 values at altitude were 59.7±2.9mmHg, 35.4±2.9mmHg, 90.5±1.6% and 1006±231, respectively. From resting levels, an iso-oxic left shift of the ODC ($PETCO_2$ = 27.3±2.9 mmHg), increased SpO_2 to 93.5±1.2% but reduced rDO_2 to 813±200.

However, an isocapnic increase in $PETO_2$ raised SpO_2 (93.4±1.2%) and left rDO_2 unchanged (998±234). Indeed, all increases in SpO_2 on the left-shifted ODC had lower rDO_2 than its counterpart-point on the control ODC. Conclusions: This first in vivo ODC study at altitude indicates that increasing SpO_2 through hyperventilation results in a lower rDO_2 compared to that if the resting ODC position were maintained. Brisk HVR and reduced CBF may be protective for HACE despite lower rDO_2. 2009.

THE INFLUENCE OF ACUTE HYPOXIA ON THE DISTRIBUTION OF NOX SPECIES IN BLOOD. Mirja Maassen[1], Marisa Nacke[2], Dimitrios Tsikas[3], Norbert Maassen[1]. [1]Institute for Sports Science, Leibniz University Hannover; Germany, [2]Institute for Sports Medicine, Medical School Hannover; Germany, [3]Institute for Clinical Pharmacology, Medical School Hannover; Germany. *Email: Mirja.Maassen@sportwiss.uni-hannover.de.* Introduction: In the present investigation we investigated the effect of decreasing SO_2/PO_2 on [NO3] and [NO_2] in plasma and red cells by applying systemic hypoxia in vivo at rest. Methods: To vary the SO_2 12 subjects were connected to the „Hypoxiecator GO2Altitude®“. The relative oxygen concentration in the inspired gas was stepwise reduced. In parallel blood samples were taken from a cubital vein. Acid base status was measured. In plasma and haemolysate [NO_2] and [NO3] were determined by GC-mass-spectroscopy. To test significant differences between means a paired t-test was used. Additionally regressions were calculated. The level of significance was set to $p<0.05$. Results: The mean cubital venous PO_2 at the end of the hypoxia trial was 24.6 ± 4.5 mmHg and SO_2 was 45.8 ± 12.7%. In plasma the decrease in [NO_2-] during the experiment was not significant ($p>0.14$). However, the decrease was significantly correlated to a decrease SO^2 ($p<0.008$). [NO3-] decreased significantly during the experiment ($p<0.05$). The change in [NO3] correlated to SO_2 ($p<0.04$). [NO3-] in red cells did not change during the experiments. Intracellular [NO3-] was significantly lower than in plasma ($p<0.03$). [NO_2] in plasma was significant lower than in red cells. The concentration in red cells was by far higher than expected from the Donnan-Equilibrium. With decreasing SO_2 [NO_2] in red cells increased significantly ($p<0.05$). Conclusion: In the range of the P50 there seems to be a net shift of NO_2 from the plasma into the red cells. By that the nitrite reductase activity of HB might be supported. Since the intracellular [NO3] remains constant, the decrease in [NO3] in plasma seems to be caused by extracellular effects, probably catalysed by Xanthine oxidase. With this pathway an NOS-independent way to produce NO might function under hypoxic condition. Acknowledgements: Supported by Leibniz University Hannover (WIF II). 2015.

THE INFLUENCE OF ALTITUDE TRAINING ON BLOOD GLUCOSE AND LACTATE FLUCTUATION DURING AN INCREMENTAL EXERCISE TEST IN JUNIOR ELITE SPEED SKATERS. Taketeru Maegawa[1], Natsumi Suzuki[2], Hidenobu Kobai[3], Toshiyuki Homma[4], Daisuke Kumagawa[1], Toshiharu Yokozawa[1]. [1]Department of Sports Sciences, Japan Institute of Sports Sciences, Tokyo, Japan, [2]Sports Research & Development Core, University of Tsukuba, Ibaraki, Japan,

[3]Aizawa Hospital, Nagano, Japan, [4]Faculty of Sport and Health Science, Ritsumeikan University, Shiga, Japan. *Email: taketeru.maegawa@jpnsport.go.jp.* Introduction: Glucose utilization and blood lactate kinetics are important physiological determinants of endurance exercise performance. In addition, altitude training improves glucose control. The aims of this study were 1) to examine the effects of altitude training on glycolysis during exercise and 2) to assess sex differences on lactate and glucose curves and the adaptive changes caused by altitude training. Methods: Elite speed skaters (9 boys and 10 girls) participated in an incremental pedaling exercise test performed before and after altitude training. Altitude training is the "living high-training low" model. The participants stayed at Park City, Utah (2,200 m) and trained at Salt Lake City, Utah (1,300 m) for 16 days. The incremental exercise test was performed to observe the lactate and glucose curve and to determine the lactate threshold (LT) and glucose threshold. The test began at 117.6 W and 78.4 W for boys and girls, respectively, and the intensity was increased by 23.5 W (or 15.7 W) every 3 min until the participants could no longer maintain their pedal cadence (80 rpm). Fingertip blood lactate and glucose concentration were measured every 3 min and after exhaustion. Results: The major finding was sex differences in the training effect on the blood glucose transition. A significant downward shift in the glucose curve was noted in girls only. In both sexes, altitude training caused the lactate curve to significantly shift to the right (i.e., a lower lactate concentration at a given workload). Additionally, the peak power output and power at LT improved in both sexes. Conclusion: These findings suggest that altitude training improves aerobic glycolysis during exercise. For girls in particular, altitude training improves blood glucose uptake without blood lactate accumulation. 2015.

THE INFLUENCE OF ONE SESSION OF INTERMITTENT HYPOXIA ON THE RETICULOCYTE AGE. Norbert Maassen[1], Armin Finkel[1], Kerstin Püllmann[2]. [1]Inst. of Sport Science, Univ Hannover, Germany, [2]Inst. Clin. Chemistry, Medical School, Hannover, Germany. *EMAIL: norbert.maassen@sportwiss.uni-hannover.de* INTRODUCTION: Some years ago, we found that after 10 sessions of intermittent hypoxia (IH; 9% oxygen) the reticulocyte count and the soluble transferrin receptor were enhanced, however, the total hemoglobin mass was unchanged. We believed that neocytolysis might be the cause for the constancy of the red cell volume. To investigate that, we tested whether IH causes a decrease in the number of young red cells. METHODS: 10 male subjects took part in the crossover study. The IH session lasted 90 min (5 min at 21 % and 5 min at 9 % oxygen in the inspired gas; Hypoxicator, Biomedtech, Australia). 9 cycles were performed. As a control, the subjects performed a trial under continuous normoxia. Blood was sampled from a cubital vein before, at the end and after 1, 3, 6 and 24 h to determine acid base parameters (ABL 520, Radiometer) and erythrocyte parameters (Sysmex XE-5000, Horgen). Heart rate and capillary oxygen saturation was measured by oximetry (Hypoxicator). Statistics: Two-way ANOVA for repeated measurements was performed. RESULTS: The mean PO_2 in arterialized blood was 95 torr under control conditions and 35 torr at the end of the hypoxic periods. Mean oxygen saturation was 95 and 70%, respectively. Haemoglobin and haematocrit were not different

between the two conditions. However, 24 hours after the start of the IH trial the reticulocyte count was significantly lower than after the control trial ($p<0.005$). The immature fraction of the reticulocyte was significantly lower after IH ($p<0.05$). The decrease appeared between one and three hours after terminating the IH session ($p<0.001$). The difference remained until the next day. CONCLUSION: IH caused a decrease of the fraction of the young reticulocytes. This might be a sign of neocytolysis. 2015.

THE POTENTIAL ROLE OF PERINATAL HYPOXIA IN THE ETIOLOGY OF CHRONIC MOUNTAIN SICKNESS. CG Julian, E Vargas, RD Dávila, S Niermeyer, A Rodrigues, C Salinas, LG Moore. Altitude Research Center and the Dept of Pediatrics, University of Colorado Denver, Instituto Boliviano de Biología de Altura, Bolivia and Wake Forest University. *Email: Colleen.Julian@ucdenver.edu.* Chronic mountain sickness (CMS) is a progressive disease of unknown etiology that develops after years of high altitude residence. Excessive erythrocytosis (EE), considered to be a preclinical phase of CMS, provides a useful tool identifying causal factors in CMS. OBJECTIVE: To determine the association between perinatal hypoxia, impaired pulmonary function and/or respiratory control and EE. METHODS: Respiratory function during sleep and wakefulness, cardiopulmonary characteristics and the incidence of perinatal hypoxia were compared in young males (18-25yrs) with EE ($Hb \geq 18.3$g/dl; n=10) and healthy controls (n=10) in Bolivia (3600m-4100m) matched by age- and altitude of residence. Exclusion criteria were confounding disease, birth at low altitude (<2500m), or extensive time spent <2500m (i.e. residence within the past 15yrs, total of >1yr over the past 10yrs or >1mo over the past year). Respiratory function was assessed using standard spirometry, cardiopulmonary characteristics by echocardiography and EKG, respiratory patterns during sleep by standard polysomnography, and pregnancy/perinatal complications by medical record review and maternal interviews. Group comparisons were made using independent t-tests or $\chi 2$, as appropriate. RESULTS: Hemoglobin and hematocrit were greater in EE versus controls. All subjects had normal FVCs, FEV1 and FEV1/FVC. EE subjects had lower FEF25-75 than controls (all, $p<0.05$), suggesting alterations in the peripheral airways. Cardiopulmonary characteristics were similar between groups, except for more frequent RVH in EE ($p<0.05$). EE subjects had more central apneas ($p<0.05$), and tended to have lower SaO_2 during sleep. Perinatal hypoxia [i.e., premature delivery, supplemental oxygen at delivery or birth to a preeclamptic mother] was more common in EE than controls. CONCLUSIONS: These data support the hypothesis that perinatal hypoxia may impair the development of pulmonary structure and/or ventilatory function during sleep thereby increasing susceptibility to CMS in later life. 2009.

THE PULMONARY ARTERY PRESSURE RESPONSE TO HYPOXIA DOES NOT CHANGE WITH PROLONGED HIGH ALTITUDE EXPOSURE. Andrew M Luks, Denny Z Levett, Erik R Swenson, Michael P Grocott, Caudwell Xtreme Everest Research Group. University of Washington, University College London. *Email: andrew_luks@yahoo.com.* Objective: Hypoxic pulmonary vasoconstriction

(HPV) triggers a rise in pulmonary artery (PA) pressure with acute hypoxia but it is unclear if the magnitude of response changes with longer exposures. We determined whether PA pressure responses to hypoxia change during prolonged high altitude sojourns. Methods: Right ventricular systolic pressure (RVSP) was measured by echocardiography in healthy volunteers breathing room air at sea-level and following 30-minute hypoxic exposures simulating ambient oxygen tensions in Namche Bazaar (NB), elevation 3500 m (FIO_2 0.13) and Everest Base Camp (EBC), elevation 5300 m, (FIO_2 0.10). RVSP measurements were repeated at two stages of a trek to EBC while breathing ambient air and hypoxic and hyperoxic gas mixtures equivalent to oxygen tensions achieved during sea-level testing sessions (NB, Day 3-4 of trek, FIO_2 0.32, 0.21, 0.16; EBC, Days 11-12: FIO_2 0.42, 0.27, 0.21 and 0.18). For each location, RVSP vs. SpO_2 was plotted and the slope of the relationship was determined. $\Delta RVSP/\Delta SpO_2$ was compared between the testing locations. Results: 11 subjects completed testing at all three locations. Comparable degrees of hypoxemia were achieved under the different testing conditions at London, NB and EBC. There were no significant changes in $\Delta RVSP/\Delta SpO_2$ between the three locations. Administration of supplemental oxygen sufficient to raise SpO_2> 96% decreased RVSP to 23.8 ± 8.5 at NB (P = NS compared to ambient air RVSP in London, mean RVSP 21. 5 ± 7.3) and to 25.06 ± 7.6 at EBC (P = .03 compared to London, P= NS compared to NB). Conclusions: RVSP responses to hypoxia and thus HPV do not change during 11-12 days of hypobaric hypoxic exposure. The lack of full correction to sea-level RVSP values after 11-12 days at altitude suggests the onset of early pulmonary vascular remodeling that may progress if the high altitude sojourn is continued. 2009.

THE RESPONSE OF MUSCLE SYMPATHETIC NERVE ACTIVITY TO HYPOXIA CAN BE INFERRED FROM HEART RATE VARIABILITY ONLY WHEN BREATHING IS CONTROLLED. Lindsay D DeBeck, Stewart R Petersen, Kelvin E Jones, Michael K Stickland. University of Alberta. *Email: debeck@ualberta.ca.* Is it possible to non-invasively detect the adaptive response of an individual's autonomic nervous system to generalized hypoxia? The purpose of this study was to determine if breathing parameters influenced the validity of inferring a direct measure of the sympathetic nervous system: muscle sympathetic nerve activity (MSNA), from a non-invasive indirect measure: heart rate variability (HRV). Eight healthy males were exposed to hypoxia (FIO_2=0.11) for seven minutes during spontaneous and controlled (isocapnic, 20 breaths·min^{-1}, constant tidal volume; VT) breathing conditions, which were presented in random order and separated by 10 minutes of breathing room air. Continuous recordings of MSNA (peroneal nerve), heart rate, beat-by-beat blood pressure, pulse oximetry and respiration were obtained. To determine the effect of hypoxia on physiological variables during spontaneous and controlled breathing a repeated measures analysis of variance was used ($\alpha \leq 0.05$). Hypoxic exposure decreased end tidal O_2 to 42 and 49 mmHg respectively during spontaneous and controlled breathing. VT and minute ventilation increased during spontaneous breathing, while by design, VT and minute ventilation were held constant during controlled breathing. Hypoxic exposure increased

MSNA (bursts·min^{-1}) and heart rate in both breathing conditions. However, increases in blood pressure and low frequency HRV in normalized units (LFnu) were only evident with controlled breathing. The increased sympathetic modulation of heart rate with hypoxic exposure is evident when breathing is controlled. Parallel responses in the sympathetic markers of HRV (LFnu) and MSNA were observed with hypoxia when breathing is controlled. During spontaneous breathing, the attenuation of the HRV response to hypoxia underscores the importance of respiratory modulation of the autonomic response to hypoxia. Supported by the Department of National Defence. 2009.

THE ROLE OF ADRENALINE AND NITRIC OXIDE IN THE REFLEX HINDLIMB VASODILATATION ELICITED BY STIMULATION OF THE SUPERIOR LARYNGEAL NERVE IN THE URETHANE ANAESTHETISED RAT. Edward T. O'Connor[1], Ken D. O'Halloran[1], James F.X. Jones[1]. [1]University College Dublin. *Email: edward.oconnor@ucd.ie*. Introduction: The cardiorespiratory effects of carotid body (CB) and aortic body (AB) stimulation are well described. Superior laryngeal nerve (SLN) paraganglia are structurally similar to the glomus cells of the CB and are excited by both sodium cyanide (NaCN) and hypoxia in vitro. SLN stimulation with either NaCN or hypoxia results in a dilatation of the hindlimb vasculature in anaesthetised rats. The aim of this study was to examine the role of adrenaline and nitric oxide in this response. Methods: Wistar rats were anaesthetised with 20% urethane (1.5 g/kg i.p.). Both SLNs were cut close to the larynx preserving the region of bifurcation, an area that reliably contains glomus tissue, with the free ends placed in 20µl tissue baths containing Tyrode's solution. The SLNs were stimulated by adding NaCN (0.1 mg/ml) directly to the baths. Next, the beta adrenergic receptor antagonist propranolol was administered (1 mg/kg i.v.), and SLN stimulation was repeated. The nitric oxide synthase (NOS) inhibitor L-NNA was then administered (10 mg/kg i.v.), and a final SLN stimulation trial was performed. Results: SLN stimulation with NaCN caused hindlimb vasodilatation as a result of changes in mean arterial pressure (MAP) and femoral blood flow. SLN stimulation with NaCN caused a significant decrease in MAP (-12.6 ± 9.1%) (t-test) which was not significantly affected by beta blockade (-9.0 ± 9.9%), but was abolished by NOS inhibition (1.4 ± 2.3%) (one-way repeated measures ANOVA). Hindlimb conductance increased with SLN stimulation (5.9 ± 4.7%), and this was diminished after both beta blockade (1.8 ± 3.3%) and NOS inhibition (1 ± 2.3%). Conclusion: These results suggest that both adrenaline and NO have a role in the reflex hindlimb vasodilatation elicited by SLN stimulation, which may be part of a hypothalamic defence response. All values reported are mean ± S.D. Acknowledgements: We wish to acknowledge support of The Wellcome Trust (UK) and the School of Medicine and Medical Sciences UCD, Dublin. 2011.

THE ROLE OF THE AUTONOMOUS NERVOUS SYSTEM IN THE REGULATION OF THE PULMONARY AND SYSTEMIC CIRCULATION DURING HIGH ALTITUDE EXPOSURE. Matthias P Hilty[1], Andrea Mueller[1], Katja Auinger[1], Christoph Siebenmann[2], Peter Rasmussen[2], Mike Hug[2], Daniela

Flueck[2], Mario Widmer[2], Stefanie Keiser[2], Carsten Lundby[2], Marco Maggiorini[1]. [1]Medical Intensive Care Unit, Univ Hospital of Zurich, [2]Center for Integrative Human Physiology (ZIHP), Institute Physiology, Univ Zurich. *EMAIL: matthias@hilty.info* INTRODUCTION: Animal studies indicate that sympathetic blockade enhances hypoxic pulmonary vasoconstriction. The effect of an autonomous nervous system blockade on pulmonary hemodynamics at high altitude in humans has not been studied yet. METHODS: 7 healthy male subjects were investigated at low altitude (LA, 488m) and 3 weeks after rapid ascent to 3450m. Hemodynamic measurements were performed using a radial artery and a pulmonary artery catheter. Cardiac output (CO) was measured using the transpulmonary lithium dye dilution method. Calibrated pulse contour analysis was used to measure CO continuously (PCCO). Blockade of the autonomous nervous system was performed in two steps, first using the non-selective beta-receptor blocker propranolol (BB) and second the m-acetylcholinergic receptor blocker glycopyrronium bromide (DB). To assess the autoregulation properties of the pulmonary circulation a bilateral thigh cuff release maneuver (TC) was performed at baseline, after BB and after DB. RESULTS: HA exposure significantly increased heart rate (HR), mean pulmonary artery pressure (mPAP) and pulmonary vascular resistance (PVR), whereas all other variables did not change. At LA and HA, BB decreased HR and CO and increased mPAP, PVR and systemic vascular resistance (SVR) significantly ($p<0.05$) while mean systemic arterial pressure (mSAP) remained unchanged. No changes in right and left ventricular filling pressures were observed. At LA and HA, DB reversed completely the effect of BB on the systemic and pulmonary circulation. At LA and HA, TC maneuver caused within seconds a significant drop in SVR leading to a 20-25% decrease in mean systemic arterial pressure ($p<0.05$) and an increase in PCCO by 2.5 l/min ($p<0.05$) at baseline, with BB and DB. TC maneuver decreased PVR significantly ($p<0.05$) at LA, HA and this irrespectively of the condition tested. CONCLUSION: The results of the present study indicate that propranolol increases PVR at low and high altitude by approximately 60% irrespective of hypoxic vasoconstriction. The capability of the pulmonary circulation to vasodilate after TC maneuver, irrespectively of the condition tested, suggests a hypoxia and autonomous nervous system independent mechanism, likely endothelium dependent. 2015.

THE ROLE OF THE TRKB RECEPTOR IN THE HYPOXIC-INDUCED RESPONSE OF ADRENAL CHROMAFFIN CELLS. Angela Scott[1], Colin Nurse[1]. [1]McMaster Univ. *EMAIL: scottan@mcmaster.ca* INTRODUCTION: Adrenomedullary chromaffin cells (AMCs) are neurosecretory cells that are activated in response to hypoxic stress and are responsible for many of the resulting physiological responses. Once activated via a direct sensing mechanism at birth or via cholinergic stimulation postnatally, the AMCs release catecholamines into the circulation that act on target organs. Although this response has been well characterized, the signaling cascades and cellular mechanisms that regulate hypoxia-induced catecholamine release by AMCs remain largely undefined. The aim of this study was to further elucidate the molecular machinery that govern the synaptic plasticity

of AMCs and regulate the hypoxic stress response. RESULTS: Brain-derived neurotrophic factor, a signalling molecule responsible for synaptic adaptations within the CNS, and its receptor the tyrosine-related kinase receptor B (TrkB) are expressed in the adrenal chromaffin cells throughout development. Given this, we hypothesized that TrkB is involved in hypoxia-induced synaptic release of catecholamines by AMCs. To test this, we used amperometric methods to measure catecholamine secretion by AMCs isolated from juvenile (P14) rat pups and exposed to 48 hours of normoxia or hypoxia (2% oxygen). We demonstrated that a specific agonist for TrkB induces catecholamine release from cultured primary AMCs, which is significantly enhanced when cells were exposed to hypoxia. The increase in catecholamine secretion in hypoxic versus normoxic cells was due to an increase in the number of responsive cells and an increase in the secretion frequency of individual cells. Preliminary findings show that this response is calcium-mediated and prevented by exposure to tyrosine kinase antagonists. Microarray studies on immortalized chromaffin cells suggest that TrkB expression is regulated by hypoxia-inducible transcription factor 2 and significantly enhanced during hypoxic stress. Thus, TrkB receptor activation appears to play a significant role in regulating hypoxia-induced catecholamine release by AMCs. 2015.

THE SPIRONOLACTONE VS. ACETAZOLAMIDE (SPACE) TRIAL FOR PREVENTION OF ALTITUDE SICKNESS. Ken Zafren, Buddha Basnyat, Jeffrey H Gertsch. Stanford University, Himalayan Rescue Association, University of California, San Diego - School of Medicine. *Email: zafren@alaska.com.* Context/objective: Previous small trials have reported that spironolactone, a mild diuretic, may effectively prevent acute mountain sickness (AMS). Design: Prospective, double-blind, randomized, placebo-controlled trial conducted from Oct-Nov 2007, ISRCTN#77054547. Participants were recruited at 4280m/4358m on the Mount Everest base camp approach. Interventions: Participants received (with 3 doses before ascent) spironolactone 50 mg, acetazolamide 250 mg, or placebo twice daily. 311 healthy Western trekkers were enrolled; 251 completed the trial. Outcome measures: The Lake Louise AMS Score (LLQ) was used to evaluate AMS as the main outcome measure (score of >3). Secondary outcome measures included severe AMS (LLQ of >5), headache, and severe headache (LLQ headache score of >2). Results: For the main outcome of AMS incidence placebo was 13/64 (20.3%), acetazolamide 10/95 (10.5%, vs. placebo p=0.09), and spironolactone 27/92 (29.3%, vs. placebo p=0.21). For incidence of severe AMS only acetazolamide was more effective than placebo. For incidence of headache and severe headache, no significant difference was found between either drug and placebo. Conclusions: Spironolactone was not effective in preventing AMS or headache at altitude, and against expectations trended towards worsening the clinical scenario. The data serve to direct clinical focus away from simple diuretic agents, and towards agents such as acetazolamide with a rational mechanism for antagonizing pathophysiological processes underlying the spectrum of acute altitude-associated neurological disease. 2009.

THE SYSTEMIC INFLAMMATION OF ALVEOLAR HYPOXIA IS INITIATED BY A MEDIATOR RELEASED FROM ALVEOLAR MACROPHAGES. Norberto C Gonzalez, Jie Chao, Zachary Viets, John G Wood. University of Kansas Medical Center. *Email: ngonzale@kumc.edu.* Alveolar hypoxia (10 % O_2 breathing) produces widespread systemic inflammation in rats. Recent evidence suggests that the inflammation is not triggered by the low tissue PO_2 but by activation of mast cells by a mediator released from alveolar macrophages. If this is correct, the following should apply: 1. neither mast cells nor resident tissue macrophages should be directly activated by hypoxia, and 2.isolated mast cells would be activated when in contact with hypoxic alveolar macrophages, but not with hypoxic tissue macrophages. Objective: To determine if hypoxia activates isolated alveolar macrophages, tissue macrophages, and tissue mast cells, and to study the microvascular response to supernatants of these cultures. Methods: Rat mesenteric microcirculation intravital microscopy was combined with primary cultures of alveolar and peritoneal macrophages and peritoneal mast cells. Results: a. Isolated peritoneal mast cells did not degranulate with hypoxia. b. Hypoxia induced a respiratory burst in alveolar, but not in peritoneal macrophage cultures. The respiratory burst was blocked by dexamethasone. c. Mast cells degranulated when immersed in supernatant of hypoxic alveolar macrophages, but not in supernatant of hypoxic peritoneal macrophages d. Supernatant of hypoxic alveolar macrophages, but not of hypoxic peritoneal macrophages produced mast cell degranulation and inflammation when applied to mesentery of normoxic rats. e. Hypoxia induced release of a mast cell secretagogue, monocyte chemoattractant protein-1, from alveolar, but not peritoneal macrophages or mast cells. Conclusions: A mediator released by hypoxic alveolar macrophages activates mast cells and triggers systemic inflammation. Reduced systemic tissue PO_2 and activation of systemic tissue macrophages do not play a role in this phenomenon. The inflammatory response could contribute to the systemic effects of conditions associated with alveolar hypoxia. Supported by NIH HL39443, AHA 0815652G. 2009.

THE TIGHT FIT BRAIN HYPOTHESIS: NO CORRELATION BETWEEN HEADACHE AND ANTHROPOMORPHICALLY MEASURED INTRACRANIAL VOLUME. Daniel Martin, Nicky Kolfschoten, Jules Harvey, Mike Grocott, Mark Wilson, for the Caudwell Xtreme Everest Research Group . University College London. *Email: dan.s.martin@gmail.com.* Introduction: The pathophysiology of Acute mountain sickness (AMS) remains unclear. Ross proposed the "Tight Fit" hypothesis in 1985 suggesting that those with greater cranial spinal compliance (greater CSF volumes to accommodate neuronal swelling) would be less susceptible to a rise in intracranial pressure and the headache of AMS. Hypothesis: In a large number of subjects, those with greater skull volumes will have fewer and less severe headaches with hypobaric hypoxia. Methods: 198 volunteers (125 male, 73 female) were recruited as part of the Caudwell Xtreme Everest Medical Research Expedition. Each subject had extracranial measurements taken at sea level (skull width, length and height) to enable estimation of Intracranial

Volume (ICV) using the Lee-Pearson formula. Subjects ascended from 1300m to 2800m by plane, then to 5300m by foot over 11 days. Headache score (from 0 to 3 - as part of the Lake Louise Score) was recorded daily and the cumulative score from each morning after an increase in altitude was summed for each individual. An independent sample T test was performed to compare male and female ICV. Non-parametric data was analysed using Spearman's rho. Results: Complete data was available for 173 subjects (113 male, 60 female. Mean age 44.2±13.6 years). The mean ICV in males (1483.42mls) and females (1284.77mls) differed significantly ($p< 0.001$); subsequent data was analysed in male/female subgroups. The median cumulative headache score in both groups was 3. In those that completed the trek, no correlation between estimated ICV and headache score was demonstrated. There was a trend towards a negative correlation between headache and age ($p=0.08$). Conclusion: There is no correlation between anthropomorphic ICV and altitude headache. This may reflect concurrent larger brain volumes in those with larger skull volumes, but it may also imply oversimplification of the "Tight Fit" hypothesis. MRI studies will help assess this further. 2009.

THE USE OF LIVE LOW - TRAIN HIGH PROTOCOL FOR THE ENHANCEMENT OF ENDURANCE PERFORMANCE AND AEROBIC CAPACITY. Tadej Debevec, Mojca Amon, Michail E Keramidas, Stelios N Kounalakis, Igor B Mekjavic. Jožef Stefan Institute. *Email: tadej.debevec@ijs.si.* Altitude training is used by athletes, not only to enhance their altitude, but also at sea level performance. The "live low-train high" protocol involves endurance training in a simulated altitude environment, thus theoretically providing acclimatization and training benefits concomitantly. However, the previous studies showed equivocal results. Therefore we investigated the effect of a four week LL-TH protocol on aerobic capacity and endurance performance in hypoxia and normoxia. 18 fit male subjects were assigned to either a control "live low-train low" (LL-TL), or LL-TH group. Each subject performed submaximal endurance training on a cycle ergometer 1 $h \cdot d^{-1}$ for 5 $d \cdot wk^{-1}$. The exercise intensity was such that the heart rate was maintained at a level corresponding to 50% of peak power output determined prior to the onset of the training. Subjects in the LL-TL group trained at ambient (normoxic) conditions, whereas subjects in the LL-TH group performed their training in a climatic chamber with oxygen content at 12%. By design, the exercise training protocol was identical in duration and relative intensity between the groups. Prior to, during, and upon completion and 10 days after the training regimen, subjects' aerobic capacity (VO_2peak) and endurance performance were determined under both, normoxic and hypoxic conditions. There were no significant changes in any of the group over the course of the training in measured hematological parameters or pulmonary function. Mean (SD) normoxic VO_2peak increased significantly only in the LL-TL group ($p<0.005$) from 45.7 (6.1) to 53.9 (3.9) $ml \cdot kg^{-1} \cdot min^{-1}$. There were no changes in the hypoxic VO_2peak in both groups. The LL-TL group exhibited significant improvements in normoxic, but not hypoxic, endurance performance ($p<0.001$). The LL-TH group also improved normoxic, but not hypoxic, endurance perfor-

mance ($p<0.05$). We conclude that LL-TH does not provide benefits over LL-TL for either altitude or sea level performance. 2009.

THREE DIMENSIONAL ANALYSIS OF OXYGEN DISSOCIATION CURVE DURING LONG TERM POIKILICAPNIC HYPOXIA. Shigeru Masuyama[1], Nao Kurita[1], Shinji Fukushima[1], Atsuo Hamada[1]. [1]Travellers Medical Center, Tokyo Medical University Hospital, Tokyo Japan. *Email: s_masu@za2.so-net.ne.jp.* Introduction: Sigmoid feature of Oxygen Dissociation Curve (ODC) is qualitatively given by the Hill's equation ($SaO_2/100=k*PO_2\text{^}n/(1+k*PO_2\text{^}n)$, where n=Hill's constant and k=(1/P50)^n. It is important that pHa, $PaCO_2$ and then P50 stay constant. During progressive hypoxia with unsustained $PaCO_2$ level, Hill's equation would not be kept accurate on two-dimensional plane. It would be projection mapping on SaO_2/PaO_2 plane from SaO_2/PaO_2/pHa space. Our purpose is to demonstrate three dimensional ODC during long term poikilocapnic hypoxia. Methods: Ten healthy non-smoking volunteer males (from 21 to 43 years old) were monitored in a hypoxic room for five hours. Their arterial blood were sampled at FiO2=0.21, 0.15, 0.13, 0.12, and 0.10 and the blood gases were analyzed by Rapid lab 1265, Bayer Health Care at Laboratory Center of Tokyo Medical University Hospital, Tokyo Japan. Results: Relationship among PO_2, PCO_2, pH and SO_2 of arterial blood were investigated. 1. The SaO_2/PaO_2 plotting shifted to the left as well as upward compared to Hill's DOC with pH=7.4 constant. 2. pHa distributed from 7.4 to 7.5. 3. $PaCO_2$ was significantly and negatively correlated with pHa. 4. PaO_2 had weak negative correlation with pHa. 5. SaO_2 was not correlated with pHa nor $PaCO_2$, while significant correlation was found between SaO_2 and $PaCO_2$ among only high ventilatory responders to hypoxia. Conclusion: It is suggested that Hill's equation should be expanded to three-dimensional SaO_2/PaO_2/pHa(or $PaCO_2$) space. Wide deviation of SaO_2 and large bias between SaO_2 and SpO_2, which was observed frequently at severe poikilocapnic hypoxia, might be explained well in this space. Acknowledgements: Funding for this research was provided by the Travellers Medical Center, Tokyo Medical University Hospital, Tokyo Japan. 2015.

THROMBELASTOGRAPHY DURING ASCENT TO ALTITUDE. Jim Pate, Patrick Doyle, Andre Vercueil, Daniel Martin, Michael Grocott. UCL - CASE, Charing Cross Hospital, King's College Hospital, University College London. *Email: runawayjimuk@gmail.com.* Objective: Studies examining the effect of hypoxia on coagulation have produced conflicting results. As part of the Caudwell Xtreme Everest (CXE) human physiology research expedition, this study sought to further explore the link between hypobaric hypoxia at altitude and coagulation. We hypothesize that coagulation will be increased on ascent to altitude. Methods: Subjects were 17 healthy volunteers who trekked to Mt Everest Base Camp (EBC, 5300m) over 13 days. Measurements were made three times; at sea level (75m) in Pheriche (4250m) and at EBC. Venesection was performed at each altitude and coagulation was assessed using thrombelastography (TEG). A HaemoscopeÆ TEGÆ Model 5000 was used to perform TEG analysis on kaolin activated blood.

The TEG variables R-time (R), K-time (K), α angle (α) and maximum amplitude (MA) were recorded. These variables represent time to clot initiation, clot dynamics, velocity of clot formation and maximum clot strength respectively. Peripheral oxygen saturation (SpO_2) was recorded at each altitude using pulse oximetry. A repeated measures ANOVA with Bonferroni correction compared TEG values at different altitudes. Results: Mean values for R and K increased significantly from sea level (R = 9.1±1.3min, K = 2.4±0.7min) to EBC (R = 11.5±2.9min, K = 4.8±1.4min). Mean K and MA decreased significantly from sea level (α = 58.5±6.4°, MA = 66.4±4.0mm) to EBC (α = 41.1±9.3°, MA = 62.2±5.2mm). Mean SpO_2 decreased significantly from sea level (SpO_2 = 97.8±1.2%) to EBC (82.8±4.5%). ANOVA p values for R-time, K-time, α angle, MA and SpO_2 were $p=0.011$, $p<0.001$, $p<0.001$, $p=0.02$ and $p<0.001$ respectively. SpO_2 did not significantly correlate with any TEG value. Conclusion: The changes in the TEG values indicate a decrease in blood coagulation properties from sea level to high altitude. Thrombo-embolic conditions are believed to occur more frequently at altitude; however, our findings do not support a role for increased coagulation in their pathogenesis. 2009.

TRANSFORMATION OF OBSTRUCTIVE SLEEP APNOEA AT SEA LEVEL TO CENTRAL SLEEP APNOEA AT HIGH ALTITUDE; INFLUENCE OF CEREBRAL BLOOD FLOW. Keith R Burgess, Andrew Dawson, Kelly Shepherd, Marianne Swart, Kate N Thomas, Jui-Lin Fan, Rebekah A Lucas, Samuel J Lucas, James D Cotter, Karen C Peebles, Rishi Basnyat, Philip N Ainslie. University of Sydney, Peninsula Sleep Laboratory, Nepal International Clinic, University of Otago. *Email: krburgess@optusnet.com.au.* Under special circumstances Obstructive Sleep Apnoea (OSA) and Central Sleep Apnoea (CSA) can occur in the same patient at different times; the transformation of OSA at sea level to CSA at high altitude and simulated high altitude have been reported (1). Those reports lacked measures of ventilatory response or cerebral blood flow that might help explain the underlying physiological mechanisms. Here, we report data from one otherwise healthy subject (54 years) who participated in experiments investigating the effects of pharmacological-induced alterations in cerebral blood flow velocity (CBFv) during sleep monitored with full polysomnography. At sea-level he had mild OSA (AHI = 14/h), which was completely resolved at high altitude (5,050m) and replaced with severe CSA (AHI = 108/h). During wakefulness, whilst his resting CBFv was unaltered at high altitude from that at sea-level, his cerebrovascular response to CO_2 was reduced by 38% and the ventilatory response to hypercapnia was elevated (0.1 to 1.0 l/min/mmHg); $PaCO_2$ fell from 40 to 25 mmHg following ascent to altitude. Since reductions in CBF-CO_2 sensitivity are important determinates of eupnoeic ventilation, hypercapnic ventilatory sensitivity and breathing stability, these factors may partly explain the exacerbation of CSA. Although the mechanisms by which OSA is replaced with CSA at altitude are unclear, hypoxic-induced alterations in chemoreflex stability and upper airway muscle activity are likely to be critical factors. 1. Burgess et al Respirology 2004. 2009.

TRANSPULMONARY PASSAGE OF SALINE CONTRAST BUBBLES CONTRIBUTES TO WORSENED PULMONARY GAS EXCHANGE EFFICIENCY WHILE BREATHING 60% O_2. Steven Laurie[1], Kara Beasley[1], Jonathan Elliott[1], Jerold Hawn[2], Andrew Lovering[1]. [1]Department of Human Physiology, University of Oregon, Eugene, OR, USA, [2]Oregon Heart & Vascular Institute, Springfield, OR, USA. *Email: slaurie@uoregon.edu*. Introduction: The reduction in pulmonary gas exchange efficiency that occurs with increased exercise intensity, measured by the alveolar-to-arterial oxygen difference ($AaDO_2$), can be caused by diffusion limitation, ventilation-perfusion (V/Q) heterogeneity, and/or all sources of shunt. In subjects breathing 60% O_2, diffusion limitation and V/Q heterogeneity are eliminated as potential contributors to the $AaDO_2$ during submaximal exercise, leaving only shunt. Thus, an $AaDO_2$ greater than that predicted by a 0.5% post-pulmonary shunt would be due to intrapulmonary shunt. Methods: Nine subjects exercised at 75% VO_2max breathing an FIO_2=0.60. Saline contrast echocardiography was performed to detect transpulmonary passage of bubbles and arterial blood gas samples were collected in glass syringes, analyzed immediately, and were tonometry and body temperature corrected. Results: Using the average measured alveolar PO_2 (372Torr), VO_2 (2.95L), arterial saturation (98.6%), total hemoglobin (14g/dL), and an estimated cardiac output (25L/min), a post-pulmonary shunt of 0.5% resulted in a predicted $AaDO_2$ of 20.4Torr. Echocardiography revealed significant bubble contrast in the left ventricle in 5/9 subjects with a mean $AaDO_2$ of 33.9Torr. The 4 subjects not demonstrating significant bubble contrast in the left ventricle developed a mean $AaDO_2$ of 17.9Torr. Conclusion: During exercise at 75% of VO_2max, breathing 60% O_2 prevents intrapulmonary shunts from opening in some, but not all subjects. Those subjects demonstrating transpulmonary passage of saline contrast bubbles have an $AaDO_2$ wider than predicted from post-pulmonary shunt alone. The wider $AaDO_2$ can be accounted for by a 1% total shunt. Thus, a 0.5% intrapulmonary shunt explains the additional gas exchange impairment during exercise at 75% of VO_2max in subjects breathing 60% O_2. Acknowledgements: APS Giles F. Filley Memorial Award; Oregon Health & Science University MRF Grant #0820; Dept of Defense #DM102758 TATRC. 2011.

TREATMENT OF HIGH ALTITUDE PULMONARY EDEMA IN THE HIMALAYA: PHERICHE, SPRING 2010. Barbara Jones[1], Buddha Basnyat[2], Colin Grissom[3]. [1]University of Utah Division of Pulmonary and Critical Care Medicine, [2]Himalayan Rescue Association, [3]Intermountain Medical Center, Division of Pulmonary and Critical Care Medicine. *Email: barbara.jones@hsc.utah.edu*. Introduction: Recent consensus guidelines for the management of high altitude pulmonary edema (HAPE) emphasize descent and oxygen as the cornerstones of management. When descent and oxygen are unavailable, nifedepine has been shown to decrease pulmonary hypertension and reduce oxygen requirements in patients with HAPE. Phosphodiesterase inhibitors, dexamethasone, and acetazolamide have also been proposed as potentially beneficial medications for treatment of HAPE. However,

no medication has been shown to provide additional benefit when oxygen and descent are available. AIM: To review the characteristics and management of 25 HAPE cases evaluated at the Pheriche Himalayan Rescue Association Medical Aid Post for the Spring 2010 season. Methods: Retrospective case series. Results: Twenty-five patients presented to the Pheriche Aid Post with a primary diagnosis of HAPE. Eight were Nepalese, and 17 were foreigners. The average altitude at onset of symptms was 4952m; 88% of patients had descended to the aid-post after developing symptoms at a higher altitude. The average presenting SpO_2 was 64%. 20% of patients also presented with physical signs of ataxia and were managed for suspected concurrent high altitude cerebral edema (HACE). 56% of all patients were hospitalized overnight. 80% of all patients were treated oxygen. Of the 5 patients not given oxygen, 3 had resolving HAPE, and 2 descended on foot with Nifedepine. 68% of all patients were given Nifedepine, 24% were given dexamethasone, 24% were given sildenafil, and 28% were given acetazolamide. There was one death, thought to be due to HAPE in the setting of an additional underlying cardiac disease. All but one patient (who ascended against medical advice) descended: 5 by helicopter, 14 by foot, and 4 by horse. Of the patients who descended on foot, none had a supply of oxygen. Conclusion: A significant number of patients presenting to the Pheriche medical aid post with HAPE were given nifedepine, dexamethasone, sildenafil, or acetazolamide in addition to oxygen and descent. Over half of the patients were hospitalized, then continued their descent after treatment with oxygen overnight. If rapid descent is not available, adjunct medications may have a role for patients with HAPE in addition to oxygen; however, the safety and efficacy of this practice has not yet been established and should be further studied. Acknowledgements: Thanks to the Himalayan Rescue Association, Bhuwan Acharya for data assistance with collection, and the University of Utah for financial support. 2011.

ULTRAMARATHON ACONCAGUA: CLINICAL AND BIOCHEMISTRY IMPACT. Alvaro Emilio Ortiz Naretto[1], Sebastián Donato[2], Rodrigo Duplessis[2], Ana Laura Bertone[3], Susana der Parsehian[3], Mariana Vilariño[4], Miriam Patricia Pereiro[4]. [1]Muñiz Hospital, Buenos Aires City, Argentina, [2]Asociación Argentina de Medicina para la Altura (AAMpA), [3]HMI Ramón Sardá, Buenos Aires City, Argentina, [4]Fiorito Hospital, Avellaneda, Buenos Aires, Argentina. *Email: miriam.pereiro@gmail.com.* Introduction: A high altitude running competition of 25 and 50 km was held in the Aconcagua Provincial Park on November 29th 2014 with a cumulative elevation gain of 1590 meters. 25 K competitors had to acclimatise during 24 h above 2000 masl before the start while 50 K ones, 48 h before the start. Exercise under Hypoxia leads to excretion of catecholamines, inflammatory response and muscular damage. Marathon training gives protection but High Altitude competitions produce an additional stress factor. Methods: In order to determine it, before the start, questionnaires about previous mountaineering experience, allergic response (aqua test), fatigue/anxiety/sleep quality visual scales were done to the competitors. Blood and salivary samples were taken before and after the competence. Lake Louise Score and fatigue and pain visual scales were done also

once they finished. Results are represented as mean values and standard deviation. Significance p<0.05. Results: 451 runners participated on the competition. Clinical status and biochemistry profile were evaluated on 31 of them (17 males) before and 15 before and after the competition. 77.4 % had previous mountaineering experience. All aqua tests were less than 5 points. Fatigue questionnaire, above 15 in 80 % of the participants (9-48); anxiety: 41 (0-80); sleep quality: 32 (0-93). After the competition: LLS: 1.3 (0-3); effort visual scale: 64 % (30-85); pain visual scale: 67% (45-90); dyspnoea visual scale: 33% (10-50). There was elevation of neutrophils (p<0.001), Nt-proBNP levels (p<0.05), Troponin T high sensitivity (p<0.01), CPK (p<0.01), myoglobin (p<0.01) after the competition. No significant difference between men and women. Salivary samples showed no inflammatory or immunologic changes. Initial Secretory Ig A levels: 5.6 mg% (0.12-14.82), CRP 0.135 (<0.1-0.442). Conclusions: We observed an elevation of the White cells (neutrophils), muscular damage (CPK, troponin and myoglobin changes). Fatigue visual scale was 15 or more points in 80 % of the competitors. There was neither laboratory nor clinical evidence of allergic response. 2015.

ULTRAMARATHON ACONCAGUA: HEMATOLOGICAL PROFILE OF ATHLETES. Alvaro Emilio Ortiz Naretto[1], Sebastián Donato[2], Rodrigo Duplessis[2], Ana Laura Bertone[3], Susana der Parsehian[3], Mariana Vilariño[4], Miriam Patricia Pereiro[4]. [1]Muñiz Hospital, Buenos Aires City, Argentina, [2]Asociación Argentina de Medicina para la Altura (AAMpA), [3]HMI Ramón Sardá, Buenos Aires City, Argentina, [4]Fiorito Hospital, Avellaneda, Buenos Aires, Argentina. *Email:* miriam.pereiro@gmail.com Introduction: A high altitude running competition of 25 and 50 km was held in the Aconcagua Provincial Park on November 29th 2014, with a cumulative elevation gain of 1590 meters. 25 Km competitors had to acclimatise during 24 hs before the start while 50 Km ones, 48 hs before the start, above 2500 masl. Methods: The initial sample was performed the evening before the competition and the subsequent two hours after the marathon. Values are expressed as mean and range. p<0.05 was considered significant. 451 runners participated on the competition. Clinical status and hematological profile were evaluated on 31 of them (17 males) before and 15 (11 males) before and after the competition. Pre -competitive levels were different between males (M) and females (F) for the following parameters : Hematocrit: M:48.7 (43.6-53.1) %, F:44.4 (38.9-49.8) %, p < 0.01; Hemoglobin M:15.6 (13.6-16.8) g%, F:14.1 (11.8-15.4) g%, p < 0.01; Ferritin M:41.3 (10.7-121.5) ng/ml, F: 266 (45.9-673) ng/ml p < 0.01. There were no differences in the levels of iron, folic acid, B12 vitamin, transferrin or transferrin saturation. The latter inversely correlated with the perception of anxiety and positively with the trainability of the previous days. Results: In studies post marathon was an increase in white blood cells count (6.3 (2.9-10.6) vs 12.6 (8.5-16.1)/ mm3) at the expense of the absolute value of neutrophils (3.3 (1.7-8.1) vs. 9.7 (7.2-14.0)/mm3) and monocytes (0.278 (0.060 to 0.655)/mm3) vs 0.717 (0.300-1.300)/mm3) without changes in lymphocytes or platelet count. Conclusions: The blood profile of athletes who participated in the study did not modify their performance, since there were no differences in responses to Lake Louise test, the intensity

of effort, the characteristics of pain after exercise or their difficulty breathing perceived. Acknowledgements: We thank the participants who contributed selflessly to the study. 2015.

UNDERSTANDING PREECLAMPSIA AT HIGH ALTITUDE: ARE IMMUNOLOGICAL FACTORS INVOLVED? R Daniela D Dávila, Vaughn A Browne, Lilian Toledo, Henry Yamashiro, Colleen G Julian, Megan J Wilson, Enrique Vargas, Robert Roach, Lorna G Moore. Altitude Research Center, University of Colorado Denver, Instituto Boliviano de Biologia de Altura, Wake Forest University. *Email: Daniela.Davila@ucdenver.edu.* Preeclampsia (PE), a major pregnancy complication marked by an exaggerated systemic inflammatory response, is more common at high than low altitude. Early- (<34wks) compared to late-onset PE (>34wks) is marked by lower uterine artery blood flow (UABF) and higher rates of infant mortality. It is not known whether chronic hypoxia increases the maternal systemic inflammatory response or whether variations in maternal cytokine levels are associated with UABF differences characteristic to early vs. late onset PE. Hypothesis We hypothesized that chronic hypoxia exaggerates the maternal systemic inflammatory response during pregnancy and that this, in turn, contributes to the increased incidence of PE and reduced UABF at altitude. Materials and methods We performed cross-sectional studies in 19 PE (early=9, late=10) and 19 normal pregnant women living at high altitude (3600m)and 19 normal pregnant women at low altitude (400m) all matched by gestational age. UABF was measured by Doppler ultrasound. Maternal plasma pro- and anti-inflammatory cytokine levels were quantified using a multiplex bead-based assay. Results IL-1B, IL-6, IL-8, TNFa and IL-1ra levels decreased at high altitude during pregnancy. Pro-inflammatory IL-6 and IL-8 were elevated in early and late onset PE ($p<0.05$, both), however neither pro- nor anti-inflammatory cytokine levels differed between early and late-onset PE. Women with early-onset PE had lower UABF compared to those with late-onset PE or normal pregnancy. Higher levels of pro-inflammatory IL-1B and IL-8 decreased UABF in late-onset PE ($p<0.05$). Conclusions An augmented inflammatory response during pregnancy does not appear to contribute to the greater rate of PE observed at high than low altitude. Moreover, since pro-inflammatory cytokine levels were related to lower UABF only in late onset PE it is unlikely that an exaggerated inflammatory response during pregnancy contributes to the development of early- vs.late-onset PE. HL079647,HL07171. 2009.

USE OF OXYMIND® IN THE ARTERIAL OXYGENATION AND NEUROPSYCHOLOGICAL PERFORMANCE AT 5050 M IN A POPULATION OF WORKERS FROM THE ALMA PROJECT. Fernando A Moraga[1], Ivan Lopez[2], Alicia Morales[3], Victor Varela[3]. [1]Universidad Católica del Norte, Coquimbo, Chile, [2]ALMA, Calama, Chile, [3]INDURA, Santiago, Chile. *Email: fmoraga@ucn.cl.* Introduction: It is estimated that labor activities at high altitude in Chile will increase from 60,000 to 120,000 workers by the year 2020. It is known that oxygenation of bedrooms and the work place improve the quality of life for workers at high geographic altitudes <5000 m. The objective of the present study was to determine the

effect on arterial oxygenation and neuropsychological performance variables at 5050 m in a population of workers from the Project ALMA (Atacama Large millimeter/submillimeter array) in the Chajnantor Valley, Chile. Methods: We evaluated oximetry and continuous heart rate (Nonin 3100), blood pressure (SBP/DBP), and neuropsychological tests (Minimental Test, Rey Complex Figure Test, and color discrimination test) under oxygenated environment conditions and subsequently in an oxygenation module (Oxymind® FiO2 increase equivalent to 2800 m). Results: The use of Oxymind® at altitude of 5050 m, simulating an altitude of 2800 m, increased blood oxygenation from 84+/-0.8 to 90+/-0.81% ($p<0.00001$), decreased heart rate frequency from 90+/-8 to 77+/-12 lpm ($p<0.01$) and reduced diastolic blood pressure from 96+/-3 to 87+/-5 mmHg ($p<0.01$). Neuropsychological tests demonstrated that the mental cognitive state of the workers increased from 19 to 31 points ($p<0.02$). Furthermore, the Rey complex figure test (memory) revealed a significant increment from 35 to 70 ($p<0.0001$). Conclusion: The results demonstrate that the use of an oxygenated module system (Oxymind®) at 5050m, simulating an altitude equivalent to 2800 m, significantly improves arterial oxygenation and heart rate and reduces diastolic blood pressure, and also enhances neuropsychological performance in workers exposed to an altitude of 5050 m. Acknowledgements: We are grateful to all volunteers of ALMA that participated in the study. 2015.

VARDENAFIL SUPRESSES THE LUNGS' REACTION TO HYPOXIA. Kleinsasser Axel[1], Hlastala Michael P.[2], Truebsbach Susan[1]. [1]Dept Anesthesiology & CCM, MUI Innsbruck, [2]Dept Physiology, Univ Washington, Seattle. *EMAIL: axel.kleinsasser@i-med.ac.at* INTRODUCTION: Hypoxic pulmonary vasoconstriction (HPV) is a physiologic response, known to be unevenly distributed through the lungs. HPV is attenuated by phosphodiesterase-5 (PDE-5) inhibitors (ie; vardenafil). We tested the hypothesis that vardenafil reduced the redistribution of pulmonary blood flow in hypoxia. METHODS: We studied four anesthetized pigs using fluorescent microspheres. Lungs were ventilated with an FIO_2 of 0.21 for 20 minutes, followed by 20 minute periods of FIO_2 of 0.10, 0.125, and 0.15 (random order). Regional blood flow and ventilation were measured using IV infusion of 15 um and inhalation of 1 um fluorescent microspheres. Vardenafil (1 mg/kg IV) was then given. Lung pieces were clustered according to their response to hypoxia prior to vardenafil into three primary clusters: unchanged, increased or decreased relative blood flow. RESULTS: Before vardenafil, clusters were spatially similarly distributed among the animals. After vardenafil, the response of blood flow to hypoxia was significantly dampened in all three clusters such that they had the same low resistance to blood flow at the maximum level of FIO_2 of 0.10. Pulmonary arterial pressure was significantly lower after vardenafil (21±2 vs 44±5 cmH2O at FIO_2 of 0.10) while arterial oxygenation was not affected. CONCLUSION: Vardenafil markedly reduced pulmonary blood flow redistribution during global hypoxia without affecting gas exchange. This might be useful in the therapy of pulmonary arterial hypertension, high altitude pulmonary edema or right heart failure. Future research might be adressed to pulmonary function and pulmonary arterial blood pressure of COPD patients - particularly during exercise. 2015.

VASCULAR DYSFUNCTION IN CHILDREN CONCEIVED BY ASSISTED REPRODUCTIVE TECHNOLOGIES. Sophie Garcin, Stefano F Rimoldi, Thomas Stuber, Hervè Duplain, Marc Germond, Pascal Nicod, Yves Allemann, Claudio Sartori, Urs Scherrer. University Hospital, Inselspital, CPMA. *Email: garcins0@etu.unige.ch.* Environmental influences acting early in life predispose to cardiovascular disease in adulthood. Assisted reproductive technologies (ART) have allowed millions of infertile couples to have children. ART involves the manipulation of embryos at a time when they may be particularly vulnerable to external disturbances. The safety of ART for long-term health is, therefore, of utmost importance, but there is little information. Hypoxia induces exaggerated pulmonary hypertension and systemic vascular dysfunction in persons displaying endothelial dysfunction. We hypothesized that high-altitude exposure may facilitate the detection of vascular dysfunction in children born after ART. We, therefore, measured flow-mediated vasodilation (FMD) and pulse wave velocity (PWV) on the brachial artery, and systolic pulmonary-artery pressure (PAP) in 65 children born from ART (mean age 11±2y) and 57 controls at high-altitude (3450m). The major new finding was that children born after ART displayed marked vascular dysfunction both in the systemic and the pulmonary circulation. FMD was roughly 25 percent smaller (6.6±1.8 vs. 8.8±1.7%, P<0.0001) and PWV significantly faster (10.2±2.0 vs. 8.3±1.7 m/sec, P<0.0001) in children born after ART than in controls. Similarly, the PAP was roughly 30 percent higher in offspring of ART than in controls (39±11 vs. 30±9 mm Hg, P<0.0001). This vascular dysfunction was not related to dyslipidemia or altered glucose homeostasis, since lipid, glucose and insulin plasma concentration, as well as glucose tolerance were comparable in the two groups. In contrast, oxidative stress, as assessed by isoprostane plasma concentration, was significantly higher in children born after ART than in controls (83±41 vs. 55±28 pg/ml, P<0.001). These findings demonstrate for the first time that children born after ART display marked vascular dysfunction that may be related to increased oxidative stress and predispose them to premature cardiovascular disease later in life. 2009.

VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS SOLUBLE RECEPTOR IN ACUTE MOUNTAIN SICKNESS. Kai Schommer[1], Neele Wiesegart[1], Christoph Dehnert[1], Peter Bärtsch[1]. [1]Department of Sports Medicine, Medical Clinic, University Hospital Heidelberg, Germany. *Email: kai.schommer@gmx.de*. Introduction: There is a controversy about vascular endothelial growth factor (VEGF) in acute mountain sickness (AMS). One small study (n=20) suggests that the ratio between free plasma VEGF and its soluble receptor (sFlt-1) is essential and that lower sFlt-1 is associated with AMS. The purpose was to examine the association between plasma VEGF and sFlt-1 and AMS in a larger group. Methods: The parameters were measured in a study on 42 subjects which primarily aimed at investigating the effects of hypoxic training for prevention of AMS. Plasma was collected at lowland (100m) before (LA1) and after (LA2) a 4 week period of bicycle training (3 times per week over 60min in hypoxia or normoxia) and after the night spent at 4559m. Subjects ascended to 4559m within 20 hours ascending on foot above 3000m. The primary endpoint AMS was defined as a Lake Louise Score ≥5

and AMS-C score ≥0.70 after the night spent at 4559m. Results: 11 subjects were excluded from analysis (2 violation of study protocol, 1 HAPE, 8 early terminations because of severe AMS). Training modalities (in hypoxia or normoxia) did not influence VEGF and sFlt-1. Therefore, data of both training groups are analyzed together. VEGF decreased significantly after training ($p<0.01$), whereas sFlt-1 remained unchanged ($p=0.36$). At the study endpoint, 18 subjects had AMS (AMS+), 13 were well (AMS-). In AMS+ (AMS-) plasma VEGF was 110.0 ±74.9 pg/ml (104.2 ±81.5; $p=0.74$) at LA1, 63.4 ±40.4 (73.3 ±49.7; $p=0.54$) at LA2 and 87.9 ±61.6 (103.9 ±80.7; $p=0.54$) at 4559m. Corresponding values for sFlt-1 in AMS+ (AMS-) were 80.8 pg/ml ±13.1 (82.2 ±16.7; $p=0.97$), 79.1 ±10.5 (79.6 ±15.9; $p=0.92$) and 138.9 ±28.4 (134.7 ±30.7; $p=0.70$), respectively. Absolute values or changes of plasma VEGF and sFlt-1 at low and high altitude did not correlate with AMS scores. Conclusion: Our data do not provide evidence for a role of VEGF and sFlt-1 in the pathophysiology of AMS. Measurements in plasma do, however, not exclude paracrine effects of VEGF at the tissue level. 2011.

VENTILATORY AND CEREBRAL HEMODYNAMIC RESPONSES TO HYPOXIA, HYPERCAPNIA AND HYPOCAPNIA DURING 5 DAYS AT 4350 M. Samuel Verges[1], Thomas Rupp[1], François Esteve[2], Carsten Lundby[3], Pierre Bouzat[2], Stéphane Perrey[4], Paul Robach[5], Patrick Levy[1]. [1]HP2 laboratory, INSERM U1042, Joseph Fourier Univ, Grenoble, France, [2]Grenoble Institute Neurosciences, INSERM U836, Grenoble, France, [3]Institute Physiology, Univ Zurich, Zurich, Switzerland, [4]Movement To Health (M2H), Montpellier-1 Univ, Euromov, Montpellier, France, [5]Ecole Nationale de Ski et d'Alpinisme, Chamonix, France; HP2 laboratory, INSERM U1042, Joseph Fourier Univ, Grenoble, France. *EMAIL: sverges@chu-grenoble.fr* INTRODUCTION: This study investigated changes in ventilatory and cerebral hemodynamic responses induced by several days at high altitude. METHODS: Eleven healthy subjects were rapidly exposed to 4350 m, for 5 days. At sea level, at day 1 and 5 at altitude, subjects were exposed during 10-min periods to i) normoxia (end-tidal oxygen partial pressure ($PetO_2$) = 100 mmHg), ii) isocapnic hypoxia ($PetO_2$=55 mmHg, $PetCO_2$ similar to normoxia), iii) hypercapnic hypoxia ($PetCO_2$ +5 and +12 mmHg, $PetO_2$=55 mmHg) and iv) normoxic hypocapnia ($PetCO_2$ -15 mmHg). The following parameters were measured during each period: ventilation, blood velocity in the middle cerebral artery (MCAv) with transcranial Doppler and pre-frontal oxygenation index (TOI), oxy- (HbO_2), deoxy- (HHb) and total hemoglobin (HbTot) changes with near-infrared spectroscopy. RESULTS: The isocapnic hypoxic ventilatory response was enhanced compared to sea level after 5 days at altitude only. MCAv increased in response to isocapnic hypoxia at altitude only, as soon as day 1. Pre-frontal deoxygenation (TOI and HbO_2) induced by isocapnic hypoxia was significantly reduced after 5 days at altitude while the increase in HHb tended to be reduced and HbTot was similar. The hypoxic hypercapnic ventilatory response was enhanced after 5 days at altitude, while a tendency for a smaller response was observed after 1 day at altitude compared to sea level. During hypercapnia at altitude (day 1 and 5), the MCAv increase tended to be lower, pre-frontal oxygenation increased less and HHb was less reduced

compared to sea level, with no effect on HbTot response. Hemodynamic responses to normoxic hypocapnia did not change at altitude. CONCLUSION: These data show distinct patterns of changes in hypoxic, hypercapnic and hypocapnic ventilatory and cerebral hemodynamic responses during a 5-day stay at high altitude. Cerebral oxygenation was better preserved in response to hypoxia but less enhanced in response to hypercapnia at altitude compared to sea level. ACKNOWLEDGEMENTS: We thank the SMTEC company for providing the gas mixing device, Atys Medical for providing the TCD device, the Rhône-Alpes Region for financial support, the CNRS and the ENSA for supporting the VALLOT 2011 project. 2015.

VENTILATORY AND CEREBROVASCULAR RESPONSES TO HYPOXIA FOLLOWING INTERMITTENT HYPOXIA: IMPACT OF PROSTANOID INHIBITION. Andrew E. Beaudin[1,7], Matiram Pun[1,7], Christina Yang[10], Craig D. Steinback[1,7], Lea Bond[5,9], Andrea De Souza[5,9], Katherine E. Wynne-Edwards[5,9], Todd J. Anderson[4,6,7], Sofia B. Ahmed[2,6,7], Patrick J. Hanly[2,5,7], and Marc J. Poulin[1,3,5,6,7,8] Department of Physiology & Pharmacology[1], Medicine[2], Clinical Neurosciences[3], and Cardiac Sciences[4], Hotchkiss Brain Institute[5], Libin Cardiovascular Institute of Alberta[6], Faculties of Medicine[7], Kinesiology[8], Veterinary Medicine[9], and Science[10] University of Calgary, AB, Canada. *EMAIL: abeaudin@ucalgary.ca* INTRODUCTION: Obstructive sleep apnea (OSA) is characterized by exposure to intermittent hypoxia (IH) during sleep. IH is purportedly the primary pathway producing the augmented acute ventilatory response (AHVR) and blunted cerebral blood flow (CBF) response to hypoxia in OSA, although the mechanisms are unclear. A potential mechanism may involve prostanoids and IH-induced inflammation. METHODS: In a double-blind, placebo-controlled, randomized, crossover study we examined the role of prostanoid inhibition on IH-induced changes in resting ventilation (Ve) and CBF velocity (CBFv), and the AHVR and CBF response to hypoxia. On two occasions separated by >4 days, 12 males underwent 6 hours of IH preceded, and followed by, an acute isocapnic hypoxia test. Four days before each IH exposure, subjects ingested (po tid) either 50mg of indomethacin (INDO; non-selective cyclooxygenase inhibitor) or 100mg lactose placebo (PLBO). RESULTS: Compared to PLBO, INDO resulted in higher pre-IH resting Ve (10.3±1.2 vs 9.7±1.4 L/min; $p<0.01$), lower end-tidal partial pressures of CO_2 ($Petco_2$; 35.4±2.2 vs 36.8±2.0 mmHg), higher end-tidal partial pressure of O_2 (Peto2; 89.2±4.0 vs 86.8±3.3 mmHg) and a lower CBFv (52.7±7.9 vs 57.6±10.6 cm/s; $p=0.01$). Post-IH Ve, $Petco_2$, $Peto_2$, and CBFv were similar between INDO and PLBO ($p\geq0.15$). The AHVR was similar between INDO and PLBO before (1.4±0.6 vs 1.5±0.7 L/min/%-desaturation; $p=0.65$) and after IH (1.7±0.9 vs 2.0±1.0 L/min/%-desaturation; $p=0.36$). Irrespective of INDO or PLBO, IH increased the AHVR (1.9±0.9× vs 1.4±0.6 L/min/%-desaturation; $p<0.01$). Furthermore, irrespective of IH, CBFv response to hypoxia was greater with INDO (1.1±0.4 vs 0.9±0.4 %/%-desaturation; $p=0.01$). CONCLUSION: In conclusion, prostanoid inhibition increased resting VE, decreased CBFv, but did not alter the AHVR. Irrespective

of IH, CBF response to isocapnic hypoxia was greater with INDO compared to PLBO ACKNOWLEDGEMENTS: Funded by the NSERC, AIHS, CIHR, and the HSFC. 2015.

VENTILATORY CHEMOSENSITIVE ADAPTATION TO INTERMITTENT HYPOXIC EXPOSURE. Keisho Katayama, Koji Ishida, Ken-ichi Iwasaki, Miharu Miyamura. Nagoya University, Nihon University School of Medicine, Tokai Gakuen University. *Email: katayama@htc.nagoya-u.ac.jp.* The purpose of this study was to clarify the change in hypoxic ventilatory response (HVR) at different levels of hypoxia, durations, and periods of intermittent hypoxia. The HVR was measured by using a progressive isocapnic hypoxic test. HVR was estimated as the $\Delta VI/\Delta SaO_2$ and was presented a positive numbers. Hypoxic tent system was utilized for intermittent hypoxia. In experiment 1, the O_2 levels were maintained at 12.3±0.2% for the first group and at 15.5±0.1% for the second group. Each group spent 1 hour/day for 1 week in the tent. In experiment 2, the first group spent 1 hour/day, while the second group spent 3 hours/day in the tent. The O_2 level was maintained at 12.3±0.2%, and the period was 1 week for each group. In experiment 3, the period was 1 week for the first group and 2 weeks for the second group. The O_2 level was maintained at 12.3±0.2%, and the duration/day was 3 hours for each group. In the experiment 1, intermittent exposure to 12.3% O_2 induced an increase in HVR, whereas exposure to 15.5% O_2 did not. In the experiment 2, HVR enhanced ($P<0.05$) after 1 and 3 hours/day of hypoxia. The magnitude of increased HVR after 3 hours/day did not differ from that after 1 hour/day of intermittent hypoxia. In the experiment 3, an increase ($P<0.05$) in HVR was found after 1 and 2 weeks of intermittent hypoxia. The magnitude of the increase in the HVR after 2 week of exposure was not different from that following 1 week. From these results, it is suggested that level of hypoxia during intermittent hypoxia could be a main factor for enhancing ventilatory chemosensitivity to hypoxia, when the duration/day and period of intermittent hypoxia are short. 2009.

VENTILATORY PATTERN AND OXYGEN SATURATION(SAO_2) IN LOWLANDERS (LL) AND HIGHLANDERS (HL) DURING EXERCISE AT HIGH ALTITUDE (HA). Annaluisa Cogo, Luca Pomidori, Elisa Paolucci, Luciano Bernardi. Ev-K2-CNR Committee. *Email: evk2cnr@evk2cnr.org.* Ventilatory response is a hallmark of HA acclimatization. We aimed to compare ventilation in LL and HL at rest and during a standardized exercise. Methods: 2 groups of healthy subjects were studied at Shigatse (4200m, Tibet) : 6 HL, M (age 17-19yrs); 6 acclimatized LL (3M,3F) (age 26-35 yrs). All were monitored by means of portable respiratory inductive plethysmography (LifeShirt, VivoMetrics, CA, USA) both at rest and during strenuous walking on a slope with a 50m difference in height. Results: Ventilatory parameters at rest, at 50% and 80% of the predicted maximal heart rate(HRmax) are reported. In HL the breathing rate(RR) was 22.4(1.3), 36.0 (2.6)*∞, 47.2(3.2)* br/min; the tidal volume (TV) was 381(56), 703(104)*∞, 1264(122) * mL; the ventilation (VE) was 10.1(1.2), 24.2(2.4)*∞ , 58.4(4.2) * L/min; SaO_2% was 85.3(1.1) , 85.3(1.5) ∞, 81.0(1.4) *. In LL , RR was 18.4 (1,7),

24.3(2.4)*∞, 35.1(1.9) * br/min; TV was 445(67), 778(129)*∞, 1316(127)* mL; VE was 7.6(0.6), 17.3(2.9)*∞, 44.5(3.0)* L/min; SaO_2% was 83.3(0.8) ,79.9(1.1)*∞, 74.6(1.5)*. HL vs LL show a significantly higher RR at each point [22.4(1.3) vs 18.4 (1,7); 36.0 (2.6) vs 24.3(2.4); 47.2(3.2) vs 35.1(1.9) p<0.01], a significantly higher VE at 80%HRmax [58.4(4.2) vs 44.5(3.0) p=0.02] and a significantly higher SaO_2% during exercise (at 50% and 80% HRmax): [85.3(1.5) vs 79.9(1.1); 81.0(1.4) vs 74.6(1.5) p<0.05]. The % increase in VE, RR and TV was not different in the 2 groups. We conclude that acclimatized LL and HL have the same increase in ventilation during exercise at HA but highlanders' ventilation is characterized by higher RR in all condition and a higher VE during strenuous exercise. This different ventilatory pattern in the HL is accompanied by lower oxygen desaturation during exercise. This fact suggests the adaptation of body's ability to cope with strenuous exercise and hypoxic stress. *p<0.05 versus rest ∞p<0.05 80%HR max Funded By: IMONT, Ev-K2-CNR. 2009.

VENTILATORY RESPONSE TESTING IN THE RAT: INFLUENCE OF THE HYPOMETABOLIC EFFECT. Barbara Morgan[1], Russell Adrian[1], Jerome Dempsey[1]. [1]Univ Wisconsin-Madison. *EMAIL: morgan@ortho.wisc.edu* INTRODUCTION: The purpose of this study was to evaluate the suitability of several methods for quantifying the ventilatory responses to hypoxia in conscious rats. METHODS: On two separate days, we measured tidal volume and respiratory frequency (barometric plethysmography), body temperature (telemetry), arterial oxygen saturation (SpO_2; MouseOx), and VO_2 and VCO_2 (gas analysis) during exposure to graded hypoxia in adult Sprague-Dawley rats. In a subset of rats, blood pressure and heart rate were measured by telemetry. Data were collected during the final 5 minutes of 15-minute periods in which inspired oxygen fraction (FIO_2) was maintained at 0.21, 0.15, 0.12, and 0.09. Ventilation and ventilatory equivalents for O_2 and CO_2 (VE/VO_2 and VE/VCO_2) were expressed relative to FIO_2 and also relative to SpO_2. Stimulus:response relationships were determined either by linear regression analysis or curve fitting logistic regression analysis, as appropriate. RESULTS: Hypoxic exposure produced substantial increases in the ventilatory equivalents for O_2 and CO_2 that were caused not only by increases in breathing frequency and tidal volume but also by progressive reductions in VO_2 and VCO_2. The highest day-to-day reproducibility for the measurement of the hypoxic ventilatory response was obtained when VE/VCO_2 was expressed relative to SpO_2. CONCLUSION: Based on our findings, we propose that the ideal way to measure and express the ventilatory response to hypoxia in the rat is to plot VE/VCO_2 vs. SpO_2. This approach takes into account two important factors in the rodent's response to hypoxia that are commonly not considered: the reduction in metabolic rate that accompanies hypoxic exposure and the interindividual variation in circulating stimulus level (SpO_2) for a given FIO_2. ACKNOWLEDGEMENTS: Funded by: NHLBI (HL 105365). 2015.

VISUAL ANALOGUE SELF-ASSESSMENT OF ACUTE MOUNTAIN SICKNESS IN ADOLESCENTS: EXPERIENCE FROM TWO HIMALAYAN EXPEDITIONS. Mary Slingo[1], Fionna Lowe[2], Andrew Pieri[3], Chris Imray[4].

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, England, [2]Derbyshire, Leicestershire and Rutland Air Ambulance, England, [3]British Schools Exploring Society, London, England, [4]Coventry and Warwickshire County Vascular Unit, University Hospitals Coventry and Warwickshire NHS Trust, England. *Email: mary.slingo@dpag.ox.ac.uk*. Introduction: Recent studies have explored the value of visual analogue scales (VAS) as an alternative to the Lake Louise system (LLS) for the self-assessment of acute mountain sickness (AMS). An advantage of VAS is the removal of the individual's need to categorise their symptom severity. We developed a novel VAS system to assess its use for adolescents. Methods: The study was conducted during 35-day expeditions to Ladakh in 2009 and 2010. Comparable ascent profiles were followed, reaching a maximum altitude of 6000m. LLS and VAS AMS scores were recorded each morning. LLS comprised the standard questionnaire (LLS(total)). VAS comprised 100mm lines for each LLS symptom (5 in total); VAS scores for all symptoms were summed to give a total for each day (VAS(total)). In 2010 an additional, separate, line was used to score an individual's overall 'altitude sickness' (VAS(subjective)). Results: 38 individuals (mean age 17.5 years) participated in 2009; 27 participated in 2010 (17.5 years). 760 LLS scores, with corresponding VAS, were recorded in 2009; 529 in 2010. There was a significant correlation between LLS(total) and VAS(total) on both expeditions (rho 0.8, $p < 0.01$ in 2009; rho 0.7, $p < 0.01$ in 2010), which held true for all symptom category scores. Excluding scores of zero for both LLS and VAS, the correlation remained (rho 0.8, $p < 0.01$; rho 0.6, $p < 0.01$). In 2010, there were significant correlations between VAS(subjective) and either VAS(total) (rho 0.8, $p < 0.01$) or LLS(total) (rho 0.5, $p < 0.01$). Conclusion: At the expedition's end the participants were asked which self-assessment method they preferred. 77% (26/34) chose VAS. We demonstrate that VAS is a reliable and user-friendly alternative to LLS for the detection of AMS in adolescents. Acknowledgements: The British Schools Exploring Society 2011.

WEEKEND WARRIORS IN THE KHUMBU: INCREASING AGE AND POLYPHARMA IN HIMALAYAN TREKKERS. Luke Mather[1], Benoit Phelan[2], Matthew McElwee[3], Devlin Cole[3], Charles Duke[4], Theodore McConnell[5], Sushil Pant[6], Purshotam Paudel[7], Nirajan Regmi[8], Douglas Sallade[9], Alison Sheets[10], Jennifer Starling[11], David Twillman[11], David Young[11], Buddha Basnyat[8], Linda Keyes[11]. [1]University of Washington, [2]Dalhausie University, [3]Case Western University, [4]University of Tennessee, [5]McGill University, [6]Kunde Hospital and Mountain Medicine Society of Nepal, [7]Tribhuwan University, [8]Nepal International Clinic, [9]Philadelphia College of Osteopathic Medicine, [10]Longmont United Hospital, [11]University of Colorado. *Email: lfmather@uw.edu.* Introduction: The number of trekkers in the Khumbu valley has dramatically increased over the past 10 years (Nepal Tourism Board). Our goal was to characterize the current demographics of individuals trekking in the Khumbu Valley, Nepal. Methods: Individuals were enrolled from October 7th- November 2nd, 2014 in Lukla, Nepal at 2,860m. Subjects were enrolled on a volunteer basis, after arrival by plane from Kathmandu, Nepal, elevation 1304m. We collected demographic data as a part of a larger study

on the effects of altitude on blood pressure and risk of AMS. Results: We enrolled 670 individuals, roughly 7% of the trekkers who entered the park during our enrollment period. Those enrolled were 59% male, ranging in age from 18-76 yrs. The mean age was 47.5 and the median age was 50, 15% >60 yrs. Individuals originated from 38 countries, 33% from United States and Australia. 95% of these individuals lived <1000m, median of 67m. The mean self-reported BMI was 23.8 (95%CI 23.6-24.1), with 31% classified as overweight and 4% obese. Self-reported activity levels were, sedentary 1%, average 41%, athletic 45%, and very athletic 9%. Participants reported a number of preexisting medical problems including hypertension 9%, high cholesterol 8%, migraines 6%, thyroid dysfunction 6%, asthma 5%, heart disease 2% and diabetes mellitus 2%. Of the 276 individuals (41%) taking medications the most common medications were, acetazolamide 29%, blood pressure medications 18%, non-steroidal anti-inflammatory medications 17%, aspirin 15%, Synthroid 13%, and asthma medications 9%. There were over 100 different medications being taken by individuals enrolled. Conclusion: We found that the current trekker population is older; less fit, may have significant medical problems and take numerous medications. These findings have implications for guides, expedition medical providers and local physicians. Acknowledgements: Funding: Wilderness Medical Society, Nepal International Clinic. 2015.

WEIGHT LOSS AND LIPID METABOLISM UNDER HYPOXIA. Nikolaus Netzer. Hermann Buhl Institute, Paracelsus Medical University, Salzburg, Austria. *Email: nikinetzer@yahoo.com*. Physiologic data in humans and animals from the last three decades shows that in a greater percentage of the populations (non altitude natives) weight loss occurs under moderate hypoxia at levels around 15% and less oxygen in the air [1-4]. The AMAS 2000 project, bringing patients with metabolic syndrome to moderate altitude [5], previous observations at high altitude [6], and recent investigations in simulated moderate altitude have shown positive effects in humans with reduction of insulin resistance (Homa Index) and in glucose metabolism with reduced HbA1c and increased glucose tolerance. Hypoxia obviously leads to reduced fat intake and resorption [7]. Preliminary research in our own institution has shown that low intense training of obese people in moderate hypoxia changes the lipid levels to the better with reduced low density lipoproteins (LDL) and relatively increased high density lipoprotein levels (HDL) accompanied by significantly more weight loss over a short period of time in hypoxia than normal air [8]. So far, the reasons for the physiologic reactions of hypoxia on fat and glucose metabolism remain not totally understood. Also it remains unclear why not all humans and animals react with weight loss to the same levels of hypoxia. Among the mediators which have been studied to be the reasons for the initiation of a change in fat and glucose metabolism under hypoxia, leptin is one of the most promising. In adipose fat tissue cells leptin might initiate reduced fat utilisation and consequently cause reduced fat intake under moderate hypoxia. However, this does not occur in all fat tissue and might be influenced by the genetic background of the individual. Recent promising studies of high scientific quality have shown that in obese Zucker rats under hypoxia the expression of leptin in adipose fat tissue cells

is increased and probably leads to weight loss and reduced food intake [9]. The same mechanism seems to be true for humans [10, 11]. The hypoxia inducible factor 1 alpha (HIF-1 alpha) seems to be the promoter for the leptin gene in human and rodent adipocytes and other cells [12]. Whereas former hope in leptin as an obesity killer has been destroyed by the finding of grelin as its counterpart in a biofeedback loop, upcoming research looks for new pathways in these biofeedback mechanisms when leptin is generated during physical exercise or under hypoxaemia.[13, 14] Upregulated leptin levels and downregulated inflammation as well as grehlin levels under hypoxia for several days might be they key to understand weight loss under continuous hypoxia. 1. Kayser B. (1992) Int J Sports Med; 13: 129-132; 2. Rose MS et al. J Appl Physiol; 65: 2545-2551; 3. Hannon JP, Klain GJ, Sudman DM. (1976). AM J Clin Nutr; 29: 604-13; 4. Kayser B. (1994). Sports Med; 17: 309-32; 5. Schobersberger W., Schmid P, Lechleitner M. (2003) Austrian Moderate Altitude Study 2000 (AMAS 2000). Eur J Appl Physiol 88: 506-514 ; 6. Armelini, F. (1997). Horm Metabol Res; 29: p. 458-461; 7. Boyer J and Blume FD. (1984). J Appl Physiol; 57: 1580-1585; 8. Netzer NC, Chytra R, Küpper T. (2008). Sleep Breath; 12 (2): 129-134 ; 9. Simler N, Grosfeld A, Peinequin A. (2006). Am J. Physiol Endocrinol Metab.; 290 (3): 591-597; 10. Tschopp, M. (1998). Lancet; 352: 1119-1120.; 11. Yingzhong Y, DromaY, Rili G, Kubo K. (2006). Intern Med; 45(16): 941-946; 12. Grosfeld A, Andre J. (2002). J Biol Chem; 277 (45): 42953-7; 13. Sifiakas NM, Anthonisen NR, Georgopoulos D (eds.). (2003). Informa Health Care; p: 292; 14. Boussaida A, et al. (2006). J Sports Science Med; 172-181. 2011.

WHY AND HOW ANOXIC CRUCIAN CARP MAINTAIN CARDIAC PUMPING. Jonathan Stecyk, Kære-Olav Stenslokken, Linda M Hanson, Bent C Larsen, Anthony P Farrell, Göran E Nilsson. University of Oslo, University of British Columbia, University of Oslo. *Email: jonathan.stecyk@imbv.uio.no.* The crucian carp (Carassius carassius) seems unique among vertebrates in its ability to maintain cardiac performance during prolonged anoxia. We have shown that cardiac output (Q) remains unchanged for 5 days of anoxia. This remarkable feat seems possible because of interlinked ultimate and proximate determinants. The ultimate basis is the evolution of two anoxia-survival strategies. Firstly, carp have evolved a solution to avoid self-poisoning by lactate and H during prolonged periods of anaerobic metabolism with the exotic ability to ferment lactate to ethanol. Then, to avoid intoxication, ethanol must be excreted at the gills. It seems the blood flow requirement for ethanol management is high. Measurement of ethanol excretion from anoxic fish (3 days at 12°C) while manipulating Q suggested that it is perfusion-limited. Thus, ethanol excretion likely necessitates a sustained Q. The second anoxia-survival strategy is that the normal cardiac activity involves a low routine cardiac ATP demand. Estimates of in vivo anoxic cardiac power output (a proxy for cardiac ATP demand) and direct measurement with in situ perfused carp hearts during normoxia and anoxia revealed that the cardiac ATP demand of anoxic crucian carp lies within its maximum cardiac glycolytic potential. Consequently, the evolution of relatively low cardiac work means that there is no need to reduce it during anoxia to match ATP supply and demand. At the proximate level, extracellular aci-

dosis can be avoided due to ethanol production (blood plasma pH remained above 7.4 even after 7 days of anoxia at 8°C). Avoiding acidosis is critical for long-term cardiac viability since anoxia and acidosis below pH 7.4 impaired performance of isolated, spontaneous contracting carp hearts at 6.5°C. Thus, the maintained cardiac pumping of anoxic crucian carp, which is permitted by a low routine cardiac ATP demand and absence of severe extracellular acidosis, is seemingly necessary for management of ethanol excretion. 2009.

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