DEVELOPMENTAL PHYSIOLOGY AT HIGH ALTITUDE

Alexandra Joachmans-Lemoine, Vincent Joseph.

Centre de Recherche du CHU de Québec, and Université Laval, Quebec, QC, Canada.

Corresponding author contact:

Vincent Joseph

E-mail: vincent.joseph@fmed.ulaval.ca

# Abstract

High altitude is a challenging environment mostly characterized by a low pressure of oxygen, but also by cold temperatures, air dryness, reduced protection against exposure to solar radiations, and more limited resources than at lower altitudes. During postnatal development energy requirements are elevated and reduction of oxygen supply (hypoxia) during this period has profound physiological consequences. Different models of exposure to hypoxia in newborn mammals have been used over the years, and have helped to establish the effects of hypoxia during development on the cardio-respiratory system. Exposure to hypoxia during postnatal development has long-term consequences that manifest throughout the life span. These consequences of neonatal hypoxia might help the adults to better withstand the effects of the reduced O2 pressure, or on the contrary impairs the subsequent responses to hypoxia. Most experimental research on development at high altitude focuses on the hypoxic environment, and the cardio-respiratory system, while only few data are available concerning thermoregulatory processes, and the interactions between cold and hypoxia during postnatal development at high altitude. In summary developmental hypoxia determines the ability of adult mammals to withstand life at high altitude, and the available data indicate that this might be an important driving force in short-term acclimatization, and long-term adaptation to high altitude. Developmental physiology at high altitude should therefore be considered as a central element for physiology and adaptation to this specific environment.

# Introduction

Animals permanently living at high altitude include large and small mammals, birds, reptiles, and amphibian species. These animals are exposed to drastic environmental conditions mainly characterized by reduced availability of oxygen, less abundant resources and cold temperatures. The lower O2 availability is a highly challenging stressor that must be adequately counteracted to sustain the higher energy requirements necessary to survive in this environment. A very large array of research has been conducted to understand how these animals maintain a net flux of O2 from the upper airways to the cells, sustaining metabolic activity despite the reduced O2 availability. This is mainly achieved by physiological responses including enhanced ventilation which helps keeping the high alveolar pressure of O2 (Dempsey et al. 2014; Joseph and Pequignot 2009), larger lungs with extended gas-exchange surface area to maximize O2 diffusion across the alveolo-capillary barrier (Frisancho 2013; Jochmans-Lemoine et al. 2015), high O2 binding affinity of hemoglobin to maintain elevated blood O2 conductance preserving the arterio-venal PO2 gradient (Storz et al. 2010), high hemoglobin to maintain high O2 concentration in arterial blood, dense tissue vascular network for enhanced diffusion of O2, and efficient utilization of O2 at the cellular level (Beall 2007; Monge and Leon-Velarde 1991; Cheviron et al. 2014; Lui et al. 2015).

The physiological systems that govern these responses are eminently "plastic" and subjected to anatomical and functional changes in response to environmental clues. Different time-line of plasticity are classically recognized to determine the resulting phenotype: on one hand *phenotypic plasticity*, or *acclimatization,* allows a gradual improvement of physiological functions after initial exposure to high altitude to optimize O2 flux through the cardio-respiratory system, its delivery to tissues and utilization in cells. Typically, this process is fully reversible upon return to previous conditions and, in the classical view of acclimatization these changes are not heritable from one generation to the next (however this is being increasingly challenged, see below). Ventilatory and hematological acclimatization to chronic hypoxia are well-known responses that increase O2 uptake and transport (Dempsey et al. 2014; Joseph and Pequignot 2009). On the other hand the process of *genetic adaptation* might be the result of natural selection over generations in animals that have been living "high" for thousands to millions years. An additional challenge is encountered by newborn animals at high altitude: their small size enhance the mass-specific O2 consumption, a substantial part of energy is required for growth, and environmental changes during development can lead to long-term effects that persist at adulthood. Indeed, in some cases postnatal hypoxia has an overwhelming impact on the phenotype observed in adults at high altitude, and there is mounting evidences that it is necessary to take into account the responses to postnatal hypoxia and their long-term consequences (also referred to as "*developmental plasticity*") to explain the occurrence of a particular phenotype at high altitude. A more rigorous way to express this is that under a specific environment such as high altitude, the expression of a given phenotype in adults is the result of the interactions between the genetic backgrounds (including limits put on phenotypic plasticity) and the developmental plasticity imposed by the environment (Via et al. 1995; Russell et al. 2008).

While the concepts of phenotypic plasticity, developmental plasticity, and genetic adaptation are typically considered as different fields of research, there is a growing trend to consider that "plasticity" and particularly its developmental aspect is one of the major driving forces of genetic adaptation. Indeed "plastic" responses induced by environmental factors experienced at one generation can lead to permanent morphological changes in the next generation through epigenetic variations affecting DNA methylation in germ-line cells (e.g. non-genetic inheritance) (Dias and Ressler 2014). Whether these processes can affect adaptation to altitude still remains unknown, but there is at least a fertile theoretical framework that helps bringing together developmental physiology and evolution (Danchin and Pocheville 2014) that can pave the way to a better integration of the plethoric data and the apparent large diversity of phenotypic responses and genetic adaptation observed at altitude. While we will not further expand these aspects in the present chapter, it should be kept in mind, that a broader interpretation of these data is clearly possible.

# Why bother about developmental physiology at high altitude?

Without doubt specific responses to high altitude during development are strong enough to have profound consequences on human health, but surprisingly they also have imposed important socio-economics and even cultural changes during the history of colonization in South America (and possibly in other high altitude regions on earth). In fact, the first capital of Peru (Jauja) was located in the high altitude valleys at 3,300 m above sea level. But the mortality rate among newborn animals (pigs, chickens, horses) was so high that the Spanish invaders decided to move their Capital city to Lima, on the harsh environment of the coastal desert boarding the Pacific Ocean (Monge 1948). On the same line of evidence, the first Spaniard settlers in the city of Potosi (4,000m) did not succeed in having children, or their children died "either at birth or in the fortnight thereafter" (Monge 1948). Pregnant women used to go to cities at lower altitude to give birth and raise their children for the 1st year. The first child that was able to survive was born 53 years after the initial establishment of the Spaniards, but his father was considered a fool by relative and friends for wanting his child to be born in Potosi. Regardless, the foolish father dedicated his son to Saint Nicholas of Tolentino, he named his child after the Saint, and raised him in Potosi "curing it miraculously of many sick spells, caused not by the cold but other deadly diseases" (Monge 1948). It is said that a whole generation of children was named Nicholas thereafter, hoping that this would offer miraculous protections against the fatal consequences facing newborn babies at high altitude. During the same time however, the native settlers had no problems in giving birth and raising their child with "customary Indian fertility" (Monge 1948), nobody asked however if this was not a simple reflection of the fact that St Nicholas felt more inclined to protect the babies born from Indian couples rather than from Spanish. Regardless of these considerations, it is clear that postnatal development is a critical aspect of natural history for animals and humans living at high altitude.

# from acclimatization to adaptation across generations.

While being seemingly anecdotal, the above-mentioned historical elements highlight the important fact that throughout history, lowland species have migrated to high altitude with divergent success. On one hand, pigs, horses, or fowls are notably *intolerant* to life at altitude (at least during the first generations of exposure), but on the other hand other species that were introduced in South America by Europeans are now commonly found in high altitude areas (these animals will be characterized as being *tolerant* to life at high altitude), while animals endemic from high altitude are considered as being *genetically* adapted. Most importantly, different phenotypes might be found in these animals (see below). One important question to ask is how to integrate the different time-line of the responses to high altitude in a comprehensive manner?

If adults from a lowland migrant species are able to exhibit adequate acclimatization, they will be able to give birth to a first generation of high-altitude natives. Newborns must adequately respond to the challenge of postnatal hypoxia, which may result in long-term beneficial or detrimental consequences into adulthood. Individuals who survive and adequately develop will give birth to several successive generations, which may ultimately result in genetic adaptation, defined as the result of natural selection of a gene or a group of genes that govern functional change(s) in response to a given environment, and that improve survival and ultimately reproduction. These changes are passed from one generation to the next through genetic material.

This line of events can be schematized as proposed in figure 1 that shows the relationship between individual time scale (from birth to adulthood - horizontal), and the processes of responses, acclimatization, and adaptation (vertical). In this figure, the vertical time line has been modified from (Hochachka et al. 1998), that presented, in adults, “formally defined relationships between time and physiological responses to environmental changes”. It has previously been proposed that developmental events should be added to this drawing to take into account the “programming” effects of postnatal environment (Huicho 2007), and we will use a similar approach to discuss developmental physiology at altitude.

However, it should also be noted that responses that are counter-productive at high altitude are also possible, and in this case the appropriate adaptive response is to avoid or attenuate these responses. For example, in most mammals, pulmonary arterial pressure rises in response to chronic hypoxia, and in species genetically adapted, or tolerant to hypoxia the rise of pulmonary pressure is limited or absent (Jochmans-Lemoine et al. 2015; Storz et al. 2010), this is due to a reduced muscularization of small pulmonary arteries (Tucker and Rhodes 2001). Additionally, elevated hematocrit levels increase blood viscosity with detrimental consequences (Storz et al. 2010), and theoretical elements indicate that the optimum level of hematocrit that ensures the most adequate O2 transport and utilization is very close to the normal sea level hematocrit (Villafuerte et al. 2004).

# Diversity of the phenotypes accounting for adaptation or acclimatization at high altitude

## 4.1 Specific phenotype of animals tolerant to high altitude.

High altitude horses from Columbia have large allelic divergences with a control population at sea level, including in the EPAS1 gene (encoding the HIF-2 protein), and in genes involved in central nervous system function (Hendrickson 2013). Peruvian chickens at high altitude have high hemoglobin oxygen affinity (Velarde et al. 1991), house mice have specific physiological responses at high altitude, including large lungs, with an extended gas exchange area and high ventilation (Jochmans-Lemoine et al. 2015), and dogs present at high altitude (4,300m) have been found to have a marked hyperventilation (Banchero et al. 1975), and accelerated lung development induced by postnatal hypoxia (Johnson et al. 1985). Surprisingly however, this list includes animals that were first described as being *intolerant* to high altitude (horses, chickens - see above), and makes apparently reference to genetic changes supporting their "tolerance" to altitude. One key difference however between animals listed below as being "adapted" to altitude is the time during which this adaptation took place – 4 to 5 centuries vs. 105 to 106 years, and it might be argued that as a typical example where "physiology meets evolution" (Danchin and Pocheville 2014) it is critical to understand the developmental aspect of "*tolerance*" to high altitude.

Humans represent a typical example of a lowland migrant species that have reached high-altitude regions at different times and locations over the past 30,000 or 40,000 years, and the different groups found at high altitude have different physiological and genetic adaptations (Beall 2007; Bigham et al. 2010).

*4.2 Specific phenotype of animals adapted to high altitude*

Among many other examples, Llamas (Llanos et al. 2007), guinea pigs (Bartels et al. 1979), deer mice (Snyder 1985; Storz et al. 2009), Pika (Pichon et al. 2009; Pichon et al. 2013), Andean foxes (Leon-Velarde et al. 1996), and Himalayan gooses (Ivy and Scott 2014), are mainstream examples of animals endemic at high altitude. These animals have been living "high" for thousands to millions of years and possess clear genetic adaptations that ensure efficient hyperventilation (Ivy and Scott 2014; Storz et al. 2010), high hemoglobin oxygen affinity (Snyder 1985; Storz et al. 2009), small elliptical red cells with high hemoglobin concentration, high muscle myoglobin concentration, and/or more efficient O2 extraction at the tissue level (Benavides et al. 1989). Additionally, llamas do not present the typical pulmonary hypertension upon exposure to hypoxia (Llanos et al. 2007) and present only a small increase in hemoglobin concentration at high altitude (Banchero et al. 1971). Deer mice from high altitude regions in North America are considered genetically adapted because of the high O2 affinity of their hemoglobin (Snyder 1985; Storz et al. 2009), but they also demonstrate hematological acclimatization with elevated hematocrit and hemoglobin levels upon exposure to altitude, that decrease upon return to lower altitude (Tufts et al. 2013). In humans, signs of genetic adaptation to high altitude are also present in populations from the Andes, Tibet, and Ethiopia with important phenotypic divergences between these groups, and while high altitude residents from the Andes demonstrate clear sign of adaptation (Bigham et al. 2010), other traits of this population such as their low ventilation level and high hematocrit (Storz et al. 2010; Beall 2007) are considered as being mal-adaptive, and in the most extreme cases related to the development of "chronic mountain sickness" (Leon-Velarde et al. 2005).

While being necessarily limited, this extremely brief overview highlights the diversity of specific adaptations to the high altitude environment, and shed light on the complexity of the physiological, molecular, and genetic mechanisms that underlie these responses. While trying to explain this phenotype based on adult physiology and genetics is without doubt extremely helpful, there is a long tradition of research that takes into account the long-lasting influence of postnatal hypoxia to better understand the unique physiology of animals at high altitude, and there is growing evidence that this is indeed a critical aspect to consider. In the following paragraphs we will present the effects of postnatal hypoxia on each system involved in acclimatisation and adaptation to high altitude. When data are available we will also describe the long-term consequences induced by postnatal hypoxia on these systems.

# Ventilatory responses to chronic hypoxia in adults and newborn.

In adults, exposure to hypoxia evokes an immediate increase of the ventilation mediated by the peripheral chemoreceptors, mainly localized in the carotid body at the bifurcation of the common carotid artery (Kumar and Prabhakar 2012). Upon reduced cellular O2 tension, carotid type I cells are depolarized, leading to the activation of voltage-dependent calcium channels, and the release of excitatory and modulatory transmitters to activate the post-synaptic nerve endings of the carotid sinus nerve, that in turn sends its efferent projections to the nucleus tractus solitarius, a key element of the brainstem dorsal respiratory group. The depolarization of type I cells is mediated by hypoxic-induced inhibition of K+ channels (Lopez-Barneo et al. 1988), and numerous evidences show that this is mediated by gazotransmitters such as carbon monoxyde (CO) and hydrogen sulfide (H2S) (Peng et al. 2010) and by a subunit of the AMP kinase (Ross et al. 2011), but this issue remains controversial (Buckler 2012), and the relative contribution of these systems to the net hypoxic response in-vivo is unclear (Kemp 2006; Peers et al. 2010). This is further complicated by the fact that numerous inhibitory feedback mechanisms are also present in carotid body and activated upon hypoxic exposure, including classical neurotransmitters such as dopamine (Iturriaga et al. 2009; Gonzalez et al. 1994), or nitric oxyde (Prabhakar and Peers 2014).

When exposed to hypoxia for a prolonged period of time, minute ventilation continues to rise gradually (Schmitt et al. 1994; Powell et al. 1998; Dempsey et al. 2014). This process of ventilatory acclimatization to chronic hypoxia has been first described in humans as a gradual decline of the arterial pressure of CO2 showing increased ventilation over the course of a few days after an ascent at high altitude (Rahn and Otis 1949). Because this occurs in parallel with a gradual increase of arterial pressure of O2, it has long been thought that a higher sensitivity of the respiratory system to hypoxia was responsible for this response. Indeed, key experiments in goats have determined that the peripheral chemoreceptors are both necessary and sufficient to induce ventilatory acclimatization (Smith et al. 1986; Busch et al. 1985; Forster et al. 1981), and that the sensitivity of peripheral chemoreceptors to hypoxia increases during chronic exposure (Vizek et al. 1987). This is accompanied by important structural and biochemical changes (He et al. 2006; He et al. 2005; Chen et al. 2002a; Chen et al. 2002b; Joseph and Pequignot 2009), that include neovascularization and proliferation of progenitor stem cells present in the carotid body that will form new chemosensitive type I cells (Platero-Luengo et al. 2014). There is a good agreement that this process depends upon the activation of the HIF system since heterozygous mice KO for HIF-1 (HIF-1 +/-) have a reduced ventilatory acclimatization to hypoxia (Kline et al. 2002).

In newborn the peripheral chemoreceptors are functional in-utero, and they become almost silent at birth due to the transition from the low foetal PaO2 to the relatively hyperoxic ex-utero environment (Blanco et al. 1984). Within a few hours, or a few days, the peripheral chemoreceptors are reset to their new functional PO2, and regain their sensitivity to hypoxia (Blanco et al. 1984). During postnatal development, carotid body are essential to maintain the breathing pattern, and acute suppression of carotid body activity by inhalation of a few breaths of hyperoxic gas reduces minute ventilation by about 40-60% in human neonates (Al-Matary et al. 2004). In newborn mammals, carotid body denervation induces a profound hypoventilation, impairs body growth, and leads to high mortality during the next days or weeks (Bureau et al. 1985; Donnelly and Haddad 1990; Hofer 1984). Thus adequate function of peripheral chemoreceptor is critical for the establishment of normal breathing during postnatal development. Yet, as opposed to the robust and sustained ventilatory response evoked by acute exposure to hypoxia in adults, the typical response in newborn is biphasic with a peak response occurring within a few minutes of exposure to hypoxia, followed by a rapid down-regulation of ventilation, that might even go below the baseline level (Bissonnette 2000). Then a gradual process of development occurs, during which, the ventilatory response to hypoxia reaches its full maturity between the 2nd and 3rd postnatal week (Bissonnette and Knopp 2001; Potvin et al. 2014; Joseph et al. 2000). This is accompanied by an increased sensory response of peripheral chermoreceptors to hypoxia (Blanco et al. 1984; Kholwadwala and Donnelly 1992; Wasicko et al. 1999; Sterni et al. 1995; Niane et al. 2011), and central maturation of the respiratory control system (Simakajornboon and Kuptanon 2005; Bissonnette 2000). Chronic hypoxia delays this pattern of postnatal development (Wasicko et al. 1999; Eden and Hanson 1987; Joseph et al. 2000), and some studies have shown that in rats raised in chronic hypoxia, the sensitivity of the peripheral chemoreceptors to hypoxia is reduced (Hertzberg et al. 1992; Landauer et al. 1995), while other did find a drastic reduction of the hypoxic ventilatory response, without noticeable changes of the carotid sinus nerve response to hypoxia (Eden and Hanson 1987), suggesting that chronic postnatal hypoxia might also alters the integration of peripheral chemoreceptor inputs at the level of the central nervous system. Some studies have shown changes of catecholamine synthesis rate and turnover in the peripheral chemoreceptors of rats raised under chronic hypoxia, which might underlie the delayed maturation (Hertzberg et al. 1992; Joseph et al. 2000), and other have reported that postnatal hypoxia stimulates cellular growth of the carotid body (Wang and Bisgard 2005), but since the net output of the carotid body in response to hypoxia integrates both excitatory neurotransmission and inhibitory feedback mechanisms, the size of this organ does not predict higher sensitivity. Moreover these alterations persist at adulthood, even when the rats are returned to normoxia for a prolonged period of time (Okubo and Mortola 1990), and interfere with the normal process of ventilatory acclimatization to hypoxia (Lumbroso and Joseph 2009). In humans, studies reported a blunted hypoxic ventilatory response in adult men living at high altitude (in South America) (Severinghaus et al. 1966; Lahiri et al. 1969), and studies performed in children of different ages showed that this altered response, rather than being an innate trait, was acquired during development (Lahiri et al. 1976).

Apparently, these developmental delays and long-term alterations of the respiratory control system would have no adaptive values, and might even be detrimental at high altitude. High altitude natives in South America are well known for their sensitivity to develop chronic mountain sickness, characterized by excessive erythrocytosis and reduced alveolar ventilation, and it has been suggested that the occurrence of this high altitude disease could be linked to perinatal hypoxic events (Moore et al. 2007). In adult rats that have been raised under postnatal hypoxia the ventilatory acclimatization to chronic hypoxia is impaired, which is associated with excessive erythrocytosis upon exposure to chronic hypoxia (Lumbroso and Joseph 2009). As far as we are aware, very few studies have evaluated if these long-term effects on respiratory control are also present in species adapted to high altitude. Because this is not an adaptive effect, it is possible to hypothesize that this should not be the case. We recently compared the effect of postnatal hypoxia in rats and mice (that have divergent physiological responses when raised at high altitude - (Jochmans-Lemoine et al. 2015)), and showed that the developmental delay of hypoxic ventilatory response in mice is much less important than in rats (Jochmans-Lemoine, 2015, ms in preparation). Interestingly, a protection against the reduced ventilatory hypoxic sensitivity due to postnatal hypoxia might explain why adult mice living at high altitude have higher ventilation compared to rats (Jochmans-Lemoine et al. 2015), but this remains to be firmly established. In other words, at least for these mice, their tolerance to high altitude could be determined, at least partially, by the reduced sensitivity of their respiratory control system to the detrimental effects of postnatal hypoxia. On the contrary, in rats the delayed development of hypoxic ventilatory response might contribute to their well-known intolerance to high altitude. These data are interesting, but they are not sufficient to conclude that protection against the detrimental effects of postnatal hypoxia is an important feature of genetic adaptation to high altitude. Indeed, while mice are commonly found at high altitude, they do not display genetic markers of adaptation to altitude (Storz et al. 2007) that are found in other rodents, such as the deer-mice (Storz et al. 2009).

In humans the genetic background appears to be very important to determine the effect of developmental hypoxia on respiratory control. Despite similar pattern of life-long exposure to altitude, high altitude natives from the Himalayan plateau don't have the typical blunted ventilatory response to hypoxia observed in Andean natives (Zhuang et al. 1993; Hackett et al. 1980). In south-American subjects that are born and raised at sea level the proportion of native American ancestry established by genetic markers is inversely proportional to ventilation during exercise, the hypoxic ventilatory response, and the ratio of ventilation to VO2 (Brutsaert et al. 2005).

Accordingly, postnatal hypoxia delays the maturation of the peripheral chemoreceptors, drastically reduce the ventilatory response to hypoxia in newborn mammals, and leads to long-lasting reduction of the hypoxic ventilatory response and acclimatization. It is clear that in rats this contributes to their well-know intolerance to hypoxia, and it also affects the respiratory control in human high altitude natives. Human studies suggest that the genetic background determines the sensitivity, and long-term consequences of postnatal hypoxia on the respiratory control system. It remains to be determined if this resistance to the effects of postnatal hypoxia observed in some species or human group is a widespread strategy that differentiates species that are tolerant (or adapted) and species that are intolerant to high altitude. The underlying mechanisms likely involve genes that govern the hypoxic responses in peripheral chemoreceptors and in the central nervous system.

# Effects of crhonic hypoxia on lung morphology and oxygen diffusion in adults and newborn

During postnatal development, intense structural and functional changes occur in the lungs. In rats, alveolar formation occurs during a critical period starting around postnatal day 4 (Burri et al. 1974). At this age, the lungs are made of a relatively small number of large, thick-walled saccules. This stage is followed by progressive projection of ridges into the airspaces, forming the contours of the future alveoli (septation phase). During the second postnatal week, the alveolar walls become thinner, and alveolar capillary are properly organized allowing the formation of a fully mature lung (alveolarization phase) (Blanco et al. 1991; Bruce et al. 1999; Burri 1984; Burri et al. 1974). This process allows the formation of alveoli, which are more numerous, smaller, and thin-walled compared to the saccules seen in newborn. The development of pulmonary vessels occurs in parallel to these events. In humans, septation and alveolarization begin shortly before birth and develop at least until the end of the 1st or 2nd year of life (Burri 1984).

Human natives from altitude regions have higher diffusing capacity, lung volume, and functional residual capacity than low altitude subjects (Droma et al. 1991; Frisancho 2013), lower maximum expiratory flow-rate per lung volume, and lower upstream airway conductance, see (Massaro and Massaro 2002) for review. These traits suggest that at high altitude there is an excess growth of the gas exchange region compared with conducting airways (Massaro and Massaro 2002). Because the gas exchange regions develop mainly after birth, while the conductive zone develops in-utero, this suggest that exposure to altitude or hypoxia during postnatal development accelerates the maturation of the gas exchange surface in the lungs (Massaro and Massaro 2002). Indeed subjects that are acclimatized to altitude since childhood (in Peru), or that were born at altitude, have a higher forced vital capacity, a VO2 max roughly 15-20% higher and arterial O2 saturation during exercise 5% higher compared to subjects that have migrated to altitude as adults (Brutsaert et al. 2004). Yet, in these subjects the hypoxic ventilatory response is notably lower (Lahiri et al. 1976; Lahiri et al. 1969; Severinghaus et al. 1966) and ventilation during exercise is reduced by about 16% which indicates that their blunted ventilation is not limiting for O2 transport, and in fact these subjects extract more O2 despite their lower pulmonary ventilation (Frisancho 2013; Frisancho et al. 1973). Remarkably, the net effect of developmental exposure to high altitude persists at sea level: adult Peruvian Quechas from Lima that have been born and raised at high altitude have higher aerobic capacity than subjects born and raised at sea level, after controlling for their level of activity (Kiyamu et al. 2015). Finally, infants between 1 and 24 months of age born at the altitude of 3,440 meters (La Quiaca, Jujuy, Argentina) have higher lung volume than infants at sea level (Llapur et al. 2013). Therefore, these are permanent anatomical changes imposed by exposure to high altitude during the critical period of postnatal development.

Animal studies consistent with these findings are available. In dogs, exposure at altitude (3,100m) increases the distensibility, diffusion capacity, and volume of the lungs if the exposure starts at 2.5 months of age: early during development, but these effects are not present if dogs are raised at high altitude as adults (Johnson et al. 1985). Rats that has been raised at the high altitude station of the Jungefraujoch (3,450 m in the Swiss Alps) between the 23rd and the 44th postnatal days have larger lungs, higher alveolar and capillary surface areas than control rats raised at low altitude (Burri and Weibel 1971). However, if rats raised at sea level are exposed to 13% O2 throughout gestation and postnatal development then the rate of postnatal alveolar formation is reduced between postnatal days 2-40 (Blanco et al. 1991), leading to reduced alveolar surface area, while it is effectively increased if exposure to hypoxia is restricted to postnatal days 23-44 as in the previous mentioned study (Burri and Weibel 1971). In guinea pigs born at sea level and raised at high altitude (3,800m, Barcroft Laboratory of the University of California White Mountain Research Station) for up to 6 months starting from the 3rd postnatal week, lung growth is accelerated, resulting in increased lung volume, alveolar surface, capillary surface area, and higher membrane and lung diffusing capacities (Hsia et al. 2005). On the same line of evidence, high altitude living deer mice have larger lungs than deer mice from sea level (Hammond et al. 2001; Shirkey and Hammond 2014). Therefore, the effects of high altitude residence on lungs appear to be mostly due to a developmental influence that result in increased lung volume, with enhanced diffusion capacity and higher alveolar surfaces.

On the other hand, some data clearly indicate that postnatal hypoxia might delay alveolar maturation, and permanently reduce the gas exchange surface. In adult rats living at high altitude, lung volume and the alveolar surface area are apparently sub-optimal (Lumbroso et al. 2012), and much lower than in mice living at the same altitude (Jochmans-Lemoine et al. 2015). However, high altitude rats that have been exposed to 32% O2 (similar to sea level PO2) during the first 2 postnatal weeks, and raised thereafter under ambient hypoxic conditions until adulthood, have a reduced hematocrit level, lower right ventricular hypertrophy, and lung structure with smaller airspace to tissue ratio that most probably reflect higher alveolar exchange surface area (Lumbroso et al. 2012). When we exposed sea level rats to the low O2 pressure found in La Paz during postnatal days 4-14 (period of alveolar development), the alveolar surface area was reduced and arterial oxygen saturation was lower in response to acute hypoxia compared to control rats (Jochmans-Lemoine 2015, ms under review), much like previously reported when hypoxic exposure is restricted to this early postnatal period (Blanco et al. 1991). Thus, the impaired gas exchange function of the lungs found in rats at high altitude is induced by postnatal hypoxia and appears to play a key role to determine the mal-adaptive phenotype of rats living at 3,600 m above sea level (La Paz, Bolivia) that have an excessive hematocrit, extremely high right ventricular hypertrophy, low ventilation and low metabolic rate (Lumbroso et al. 2012; Jochmans-Lemoine et al. 2015). This is a striking difference compared to species that are endemic and show genetic adaptation at high altitude (deer mice), or to species that are tolerant to high altitude (house mice - (Jochmans-Lemoine et al. 2015) and Jochmans-Lemoine 2015, ms under review), probably reflecting differences on the cellular and molecular interactions between hypoxia and the processes governing postnatal lung development. Interestingly, it has been suggested that because postnatal hypoxic exposure reduces O2 consumption rate (see below), and because there is a very tight relationship between O2 consumption and gas exchange surface in the lungs, the impaired alveolar development in rats raised under chronic hypoxia might simply reflect the reduced O2 needs (Massaro and Massaro 2002).

# Metabolic effects of hypoxia in adults and newborn.

Apart from increasing lung ventilation and optimizing the lung exchange surface to maintain a high flux of O2 at high altitude, another response to hypoxic exposure is to reduce the rate of O2 consumption. This response might be highly efficient, is conserved across evolution, and under extreme conditions of hypoxia it ensures survival in species that show stunningly long-lasting resistance to anoxia such as aquatic turtles (Hochachka and Lutz 2001; Hochachka et al. 1996). While in mammals this extreme response is not possible, a certain degree of metabolic rate reduction is observed upon exposure to acute hypoxia, particularly in newborn or in small adults, in which metabolic rate is high relatively to their body mass (Mortola 1999, 2004; Singer 2004). The decrease of metabolic rate might be accompanied by a reduction of core body temperature, due to a reduction of the thermoregulatory set point. Current models are consistent with the hypothesis that hypoxia activates cAMP dependent pathways in the hypothalamic pre-optic area, causing an elevation of the thermal sensitivity of pre-optic warm-sensitive neurons, in turn leading to an inhibition of thermogenesis and activation of heat loss (Steiner and Branco 2002; Branco et al. 2006; Bicego et al. 2006): in other words, this response is a decrease of the thermoregulatory set point initiated in the pre-optic hypothalamic area. Gasotramsmitters such as Nitric Oxide, Carbon Monoxyde and Hydrogen Sulfide appear as critical mediators of this response (Branco et al. 2014; Steiner and Branco 2002; Branco et al. 1997; Paro et al. 2001). Interestingly, blockade of NMDA receptors amplifies the hypoxic-induced fall of body temperature and this effect is present in developing rats between the 4th and the 20th post-natal day (Baig and Joseph 2008). These data clearly illustrates that this process is tightly regulated by the nervous system rather than a passive response to O2 limitation (Branco et al. 2014; Barros et al. 2001; Steiner and Branco 2002; Gordon and Fogelson 1991).

However, under prolonged hypoxia this effect does not persist, and after only 1 day of hypoxic exposure body temperature in rats reaches pre-hypoxic levels, but with a blunted circadian variations (Mortola and Seifert 2000; Bishop et al. 2000). After 4 weeks of hypoxic exposure (in mice), body temperature and its circadian variations returned to a normoxic pattern (Beaudry and McClelland 2010). Furthermore, the evidence indicate that reduction of metabolic rate is not a valuable strategy for animals that should respond to the every-day challenges of hunting or foraging for their food, escaping predators, responding to period of cold exposures, and engaging into social behaviours. In fact, animals that are tolerant or adapted to hypoxia appear to have a normal metabolic rate (Jochmans-Lemoine et al. 2015), and if anything adaptation at high altitude favours the maintenance of high aerobic capacity (Brutsaert et al. 2004; Cheviron et al. 2014; Hochachka et al. 1998; Cheviron et al. 2012). In rats raised at high altitude (3,600 m – La Paz, Bolivia) we have found a low metabolic rate that seems mainly to be a response to impaired gas exchange function, in order to maintain arterial oxygen saturation (Jochmans-Lemoine et al. 2015). The basal metabolic rate in these animals was indeed so low that when they were challenged with exposure to severe hypoxia (12% O2 - or a PO2 of 60 mmHg, that would be less than 8% O2 at a sea level atmospheric pressure) they were not able to further reduce O2 consumption or rectal temperature, and exhibited high mortality. On the other hand, under similar conditions, mice had a normal metabolic rate, were able to reduce O2 consumption and rectal temperature, and tolerated the most sever level of hypoxia without mortality.

As a general rule, body size appears as an important determinant of the ability to decrease metabolic rate in hypoxia, and this is best explained by the higher mass-specific O2 consumption in small animals compared to large animals (Hill 1959; Frappell et al. 1992; Mortola 2004; Singer 2004), thus the hypo-metabolic response is increased in newborn mammals compared to adults of the same species. There are species-specific differences in the magnitude of the hypometabolic response to hypoxia, that are inversely related to the hypoxic ventilatory response (Mortola et al. 1989), leading to the concept that the ratio of minute ventilation to O2 consumption (Ve/VO2) is the best estimate of the hypoxic ventilatory response. During postnatal development the hypo-metabolic response to hypoxia becomes gradually less important, and this evolves in parallel with the maturation of the hypoxic ventilatory response (Mortola 1999; Baig and Joseph 2008). It might be hypothesized that because maturation of the hypoxic ventilatory response is delayed at high altitude, the hypo-metabolic response to hypoxia should be maintained for a longer period of time. It might also be questioned whether there are differences between adapted (or tolerant) species and intolerant species in the maturation of these responses at high altitude. As far as we are aware however, only a limited number of studies have addressed these questions. Hamsters are an interesting example because they live underground, in burrows that are notable for being hypoxic and hypercapnic environment. Compared to rats raised under postnatal hypoxia from birth to postnatal days 21-22, hamsters do not demonstrate a reduced metabolic rate, and they maintain higher minute ventilation (Frappell and Mortola 1994). Because rats demonstrate a blunted postnatal growth in these conditions, while the hamsters had a normal postnatal body growth, the specific response of the hamster to postnatal hypoxia has clearly an adaptive value (Frappell and Mortola 1994). Interestingly, in 1 day-old human infants that are born in La Paz (Bolivia - 3,600m) minute ventilation and metabolic rate are both similar than in sea level infants, implying that the hypo-metabolic response to hypoxia is not present, and that higher O2 extraction from the lungs compensates for the lower inspired PO2 at altitude (Mortola et al. 1992). As noted above it has been suggested that O2 consumption rate and lung growth under chronic postnatal hypoxia are tightly linked (Massaro and Massaro 2002), if this is indeed the case, then a systematic exploration of metabolic rate during postnatal development on animals showing divergent lung growth at high altitude could help establish whether this hypothesis is relevant.

# Interaction between multiple stressors: responses to cold exposure at high altitude.

Exposure to cold temperatures at high altitude poses an additional challenge. In endotherms animals (birds and mammals) the physiological response to cold under normoxic conditions is to enhance heat production, which requires enhanced O2 consumption, and is necessary to maintain body temperature at its high optimal level. Under intense cold exposure, the regulatory mechanisms ensuring heat production are overridden by heat losses, and internal temperature drops (hypothermia), which might lead to respiratory and cardiac arrest, and ultimately to death (Fong 2010).

Despite their immature thermoregulatory responses and their small size (that increases body surface-volume ratio), newborn mammals are more resistant to cold exposure than adults. Indeed, body temperature decreases more rapidly and more profoundly in newborn compared to adult mammals (Mortola 2005), but in parallel, cardio-respiratory arrest occurs at much lower body temperature in newborn than in adults (Fong 2010; Tattersall and Milsom 2003). In the absence of systematic literature studying thermoregulatory responses during postnatal development at high altitude, it is only possible to have some hints on the interaction between hypoxia and cold exposure during postnatal development from two type of experimental paradigms that can be more easily used in sea level laboratory settings: some studies asked what are the effects of cold exposure on hypoxic responses, other studies asked what are the effects of hypoxia on responses to cold exposure.

## 8.1 Effects of cold exposure on hypoxic responses:

The intricate interactions between cold and hypoxic exposure on metabolic control has been investigated in the late 50's (Hill 1959), and since these initial studies, all available evidence are in the same direction: compared to thermo neutral conditions, the effects of hypoxic exposure on metabolic rate are much more important under cold conditions. In 6-days old mice, maintained under thermoneutral conditions at 33°C, brief exposure to hypoxia (10% O2 – 3 minutes) almost double minute ventilation with only a marginal reduction of O2 consumption (-10 to -15%) (Bollen et al. 2009), which is consistent with the notion that metabolic and respiratory depression require longer exposure time to be apparent. When mice are placed at 26°C, basal O2 consumption and minute ventilation are increased (which is the normal thermogenic response), but hypoxic exposure caused only a small increase of minute ventilation, followed by a profound ventilatory and metabolic depression that extended for a few minutes after the return to normoxia, accompanied by a small reduction of body temperature. In this study, hypoxic exposure at 26°C also induced an important behavioural response characterized by body movements and vocalization of the pups that were not present at 33°C. This behavioural response has been interpreted as reflecting a more stressful effect of hypoxia under cold exposure, when O2 consumption is increased (Bollen et al. 2009). In newborn dogs (1-2 weeks) exposed to cold conditions (20°C) and normoxia, O2 consumption and minute ventilation increase compared to dogs maintain at 30°C. Both values decreased to a much greater extend when the dogs are exposed gradually to hypoxia in cold conditions compare to warm conditions but below 10% O2, metabolic rate is no longer different no matter what the temperature is (Rohlicek et al. 1998). Remarkably, the arterial pressure of CO2 and the arterial content of O2 were maintained at similar levels under cold and warm conditions, for all levels of hypoxia (Rohlicek et al. 1998), while the PaO2 was only slightly lower in cold hypoxic compared to warm hypoxic conditions. This highlight that there is a highly efficient matching between minute ventilation and metabolic rate to adjust the hyperventilatory response under cold and warm conditions (leading to the similar PaCO2), and, as noted above, it is necessary to take into account both minute ventilation and O2 consumption (or CO2 production rate) to have a reliable estimate of the hypoxic ventilatory response under cold exposure.

*8.2 Effects of hypoxia on responses to cold exposure:*

Studies conducted in newborn rats (2 day-old) have shown that in hypoxia the typical increase of metabolic rate observed in response to cold exposure is suppressed (Mortola and Dotta 1992), a similar effect was reported in newborn infants (1 day-old) born at the altitude of 4,300 m (Cerro de Pasco – Peru) (Frappell et al. 1998). Therefore, cold-induced thermogenesis is supressed during exposure to acute hypoxia at sea level and in 1-day old human at high altitude. However, studies performed in newborn rats tend to indicate that longer exposure to hypoxia might, on the contrary, increase the thermogenic response to hypoxia (Mortola and Naso 1998). Indeed, when 8 day-old rats are raised under chronic hypoxia between postnatal days 2-8 (at an inspired PO2 level of 92 mmHg, equivalent to 12% O2), the thermogenic response evoked by cold exposure is more elevated than in control rats. In 2 day-old rats whose mother have been exposed to hypoxia throughout gestation, the thermogenic response to cold exposure was not different from control rats. Surprisingly however there was no signs of tissue or cellular responses that could explain the findings in the 8 day-old hypoxic pups, on the contrary, the content of uncoupling protein (UCP – a major determinant of thermogenesis capacity) was decreased in the interscapular brown adipose tissue of rat pups raised in postnatal hypoxia (Mortola and Naso 1998). As far as we are aware, there are no report of the developmental profile of thermogenic capacity and cold responses during postnatal development at high altitude. In adult deer-mice that have been captured at high altitude and thereafter maintained at low altitude for 6 weeks, the thermogenic capacity measured as maximum O2 consumption induced by cold exposure under hypoxia was about 60% higher than in lowland deer-mice (Cheviron et al. 2012), but it seems that this effect is determined by genetics rather than by development at high altitude. In high-altitude mole-rats (3,200m) the thermogenic response to cold exposure was less important than in low-altitude control mole-rats (Broekman et al. 2006), but high-altitude animals had lower thermal conductance (higher isolation or efficient behavioural response to limit heat loss during cold exposure), and their response to stimulation of non-shivering thermogensis by noradrenaline was more important (higher increased of metabolic rate and body temperature), showing overall greater thermoregulatory capabilities (Broekman et al. 2006).

# Conclusion

The data reviewed in this chapter highlight the critical importance of the period of postnatal development for physiological responses at high altitude. It is evident that for newborn animals living under the constant exposure to reduced O2 availability there is a delay of maturation of the respiratory control system leading to a blunted hypoxic ventilatory response in adults, but this long-term detrimental effect might be, at least in some cases, compensated by accelerated lung growth, thus leading to enhanced aerobic performances despite the reduced minute ventilation. However, some data tend to indicate that genetic adaptations to altitude might contribute to eliminate the effects of postnatal hypoxia on the respiratory control system, maintain high O2 consumption rate, therefore ensuring accelerated lung growth. There are also strong evidences showing interactions between cold exposure and hypoxia, both factors (cold or hypoxia) modulating the expected physiological response to the other factor. We've only been able to identify a limited number of studies describing thermoregulation in human newborn at high altitude, or on the effects of postnatal hypoxia on thermoregulation in newborn rats, but the postnatal maturation of thermoregulation at high altitude remains poorly known. Similarly it remains to establish whether there are specific adaptations during postnatal development, which is without doubt an intriguing line of research to pursue. Overall, this field of research is important to better understand the natural history, and the adaptation of animals living at high altitude, and it might also provide relevant information for human health at high altitude.

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**Figure Legend:**

*Figure 1*: Relationship between individual time scale (from birth to adulthood - horizontal), and the processes of responses, acclimatization, and adaptation to high altitude (vertical arrows). During postnatal life physiological functions such as respiratory control, lung anatomy, or thermoregulation follow a gradual process of development leading to a mature system. Postnatal hypoxia might either accelerates (1), have no effect (2), or delays (3) this development. These different pathways of development lead to a mature system optimized (1'), normal (2'), or dysfunctional (3') relatively to the requirements of the high altitude environment. In adults, phenotypic plasticity also contributes to adjust the phenotype (1''), but non-adaptive responses might also occur (3''). At each stage (adult or postnatal development), responses to environmental clues are orchestrated by specific sensors. Depending on these pathways, individuals might either be tolerant, or intolerant to life at high altitude. Genetic adaptation is thought to result from selection of the most advantageous designs. Figure adapted from (Hochachka et al. 1998) and (Huicho 2007).