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Acclimatization to chronic intermittent hypoxia in mine workers: a challenge to mountain medicine in Chile

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ABSTRACT

In the past two decades, Chile has developed intense mining activity in the Andes mountain range, whose altitude is over 4,000 meters above sea level. It is estimated that a workforce population of over 55,000 is exposed to high altitude hypobaric hypoxia. The miners work under shift systems which vary from 4 to 20 days at the worksite followed by rest days at sea level, in a cycle repeated for several years. This Chronic Intermittent Hypoxia (CIH) constitutes an unusual condition for workers involving a series of changes at the physiological, cellular and molecular levels attempting to compensate for the decrease in the environmental partial pressure of oxygen (PO_2). The mine worker must become acclimatized to CIH, and consequently undergoes an acute acclimatization process when he reaches the worksite and an acute reverse process when he reaches sea level. We have observed that after a period of 3 to 8 years of CIH exposure workers acclimatize well, and evidence from our studies and those of others indicates that CIH induces acute and chronic multisystem adjustments which are effective in offsetting the reduced availability of oxygen at high altitudes. The aims of this review are to summarize findings of the physiological responses to CIH exposure, highlighting outstanding issues in the field.

Key words: High Altitude, Mine Workers, Intermittent Hypoxia, Acclimatization, Chilean Model.

1. GENERAL CONCEPTS

High altitude-induced hypobaric hypoxia involves a series of adaptive changes in multiple physiological systems in exposed individuals. Based in our findings and those of others, in this review we summarize the main physiological responses observed in mine workers intermittently exposed to hypobaric hypoxia. The literature on the occupational health of high-altitude exposed workers has been recently reviewed by Vearrier and Greenberg, 2011.

Hypobaric hypoxia occurs as a consequence of the low partial pressure of oxygen (PO_2) in the inspired air, resulting from the low barometric pressure found at high altitudes. A lower arterial PO_2 in turn initiates a physiological response attempting to maintain tissue oxygenation. One parameter used to determine respiratory response and the transport of oxygen in the blood to the tissues is percent hemoglobin saturation ($\%SaO_2$), which can be estimated with a portable device. At sea level values fluctuate between 95 and 97%; values below 90% SaO_2 are associated with pathological situations such as respiratory failure (Farias *et al*., 2006). An effect produced by exposure to hypobaric hypoxia is the well known Acute Mountain Sickness (AMS), whose symptoms include headaches, vomiting, fatigue, loss of appetite and sleep disturbances (Hackett *et al*., 1976, León-Velarde *et al*., 2010).

The acute response to hypoxia depends mainly on 4 factors: a) the altitude reached, i.e. the degree of hypobaric hypoxia, b) the rate of climb, c) individual susceptibility, and d) physical and environmental requirements in the ascent and arrival. It is known that upon exposure to 2,500 m altitude the problems associated with hypobaric hypoxia begin, with the consequent challenges for the individuals exposed (León-Velarde *et al*., 2005).

The lower availability of oxygen at high altitudes triggers physiological mechanisms that first seek to protect oxygen transport to the tissues, inducing mainly respiratory and cardiovascular adjustments. The increase in ventilation depends on the activity of the peripheral chemo receptors, particularly those located inside the carotid bodies that detect changes in arterial PO_2 and transmit sensorial information to regulate breathing (Prabhakar *et al*., 2000). Some data indicate that enzymes present in all mammalian cells, especially hydroxylases, are sensitive to moderate hypoxia and their activity may be controlled at several other levels, which provides flexibility to the physiological responses to hypoxia (Schofield and Ratcliff, 2004).

Important factors in personal susceptibility to hypoxia are the presence of sensitive oxygen sensors and effective physiological adaptations. Based on susceptibility to hypoxia, exposed individuals may be classified as good responders, who tolerate and acclimate without symptoms; a group with a low response and temporary minor symptoms that may limit performance; and poor responders, who display low sensitivity to hypoxia and ineffective compensatory physiological responses.

Residency in high altitude locations exposes humans to ambient hypobaric hypoxia, thus Chronic Hypoxia exposure

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refers to groups of people who live for generations at high altitudes or are occasionally exposed to short periods (days) of normoxia. Acute Hypoxia refers to individuals who live at sea level and are exposed to high altitudes for only minutes, hours or days, as in the case of mountain climbers. Periodic intermittent exposure to high altitudes, as in the case of the Chilean miners who work at altitudes over 4,000 m is called Chronic Intermittent Hypoxia (CIH) (Richalet *et al*., 2002; Vargas *et al*, 1989; Farías *et al*., 2006). Mining workers at high altitudes must adjust to the requirements of hypobaric hypoxia for some days and then return to sea level, where they lose some of the acclimatization to hypoxia, depending on the time in normoxia. This model of exposure to hypobaric hypoxia, named the Chilean model or CIH exposure, has received increased attention in recent years (Casanegra *et al*., 1993; Chamorro *et al*., 1993; Jalil *et al*., 1994; Jimenez 1995; Richalet *et al*., 2002; Farias *et al*., 2006; Brito *et al*., 2007).

2. ACCLIMATIZATION TO INTERMITTENT EXPOSURE TO HIGH ALTITUDES

The term 'altitude acclimatization' describes the processes whereby lowland humans and animals respond to reduced PO_2 in the inspired air. It refers only to the changes in response to hypoxia seen as beneficial, as opposed to changes that are pathological and result in illness such as AMS (West *et al.*, 2007; Vearrier and Greenberg, 2011). Altitude acclimatization involves a series of adaptive physiological adjustments that compensate for the reduction in ambient PO_2 (see Figure 1) and is the best strategy for the prevention of AMS (Forgey, 2006) Altitude acclimatization allows people to achieve their maximum physical and cognitive work performance possible for the altitude once they are acclimatized (Fulco *et al*., 2000).

2.1.. Physiological effects of intermittent hypobaric hypoxia.

Cyclical exposure to high altitude triggers responses throughout the route of oxygen, beginning with the carotid chemoreceptors and including ventilatory responses, pulmonary circulatory adjustments, the alveolar/capillary barrier, erythropoietin, hemoglobin, adjustments in the distribution of intra and extravascular fluid, changes in acid-base conditions, PO_2, PCO_2 and O_2 delivery at the cellular level, with modifications in the peripheral capillaries and mitochondrial enzyme optimization.

One type of intermittent exposure to hypobaric hypoxia occurs with exercise training at high altitude; endurance athletes have evolved different models of exposure with the ultimate goal of increasing athletic performance at sea level. The most used models are Live High (at altitude) and Training Low (at sea level) LH-TL, Live High-Train High LH-TH, Intermittent Hypoxic Exposure (IHE) and Intermittent Hypoxic Training (IHT) (Wilber, 2007). With the aim of using altitude acclimatization to enhance aerobic capacity via hypoxia-induced increases in serum erythropoietin and hemoglobin concentrations, athletes either live and/or sleep in hypoxia (natural or artificial). Also, by stimulating ventilation and better oxygen saturation, the increased aerobic capacity improves muscle oxygenation and increases exercise tolerance. Based on studies of maximum oxygen uptake (VO_2max) and

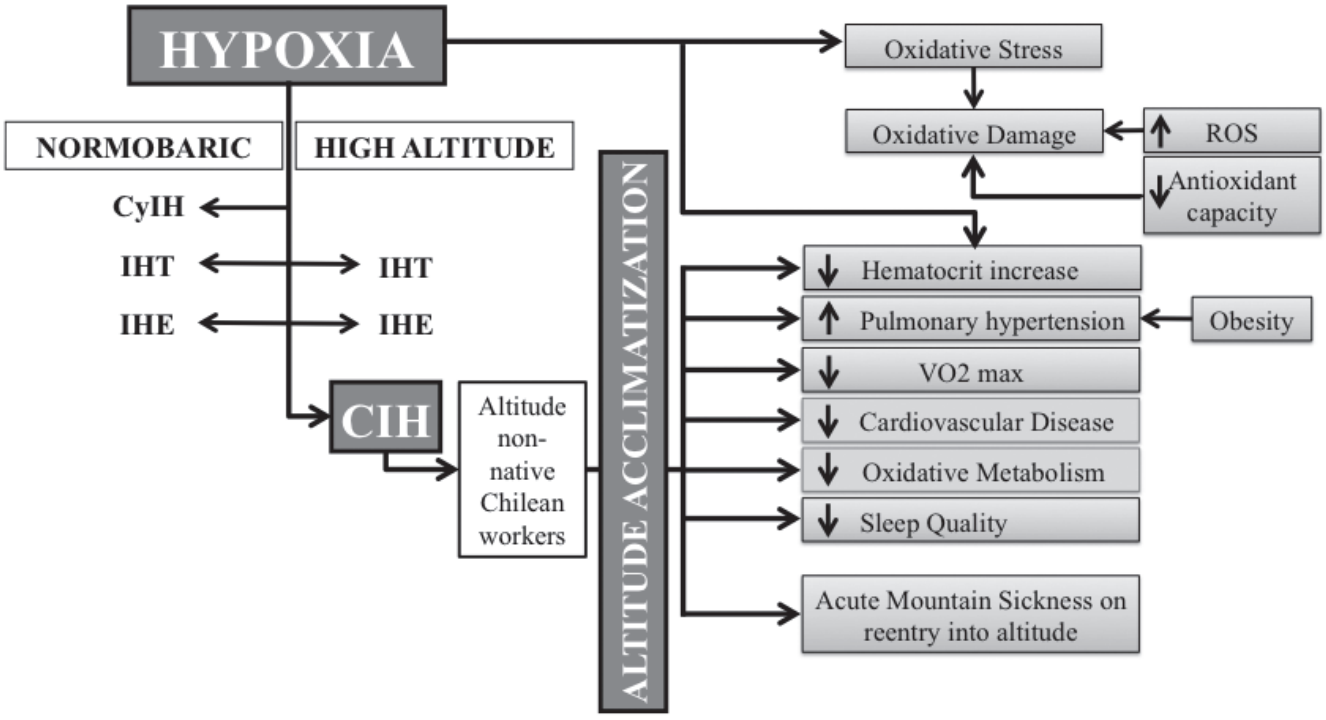

Figure 1. Physiological responses to hypoxia and effects of high altitude acclimatization. CyIH: Cyclic Intermittent Hypoxia. IHT: Intermittent Hypoxic Training. IHE: Intermittent Hypoxic Exposure. CIH: Chronic Intermittent Hypoxia. ROS: Reactive Oxygen Species. See text for details.

(maximum) power output, training at high altitude does not consistently result in increased exercise performance at sea level, with some evidence indicating benefits of training at high altitude on exercise performance at altitude (Vogt and Hoppeler, 2010). A great individual variability in responses has been observed and although some debate still exists in terms of the efficacy of the different models, combinations of them are being recently explored to improve results (see Millet *et al.*, 2010 for review). From a research standpoint this experience has served to learn more about how hematological, aerobic and muscle responses are modulated by intermittent hypoxia (de Paula and Niebauer, 2012).

Other forms of intermittent exposure to hypoxia occur in several diseases such as chronic obstructive pulmonary disease, congestive heart failure, obesity-hypoventilation syndrome and obstructive sleep apnea, where hypoxia and normoxia occur at the same barometric pressure. This intermittent isobaric hypoxia is called recurrent or cyclic intermittent hypoxia (CyIH) (Gilmartin *et al.*, 2008). The study of the relationships between CyIH and cardiovascular disease has seen significant progress; however the physiological mechanisms linking them remain unknown. Both animal (Fletcher *et al.*, 1992; Brooks *et al.*, 1997) and human (Peppard *et al.*, 2002) models showed increases in arterial blood pressure after CIH exposure with enhanced chemosensitivity being invoked as a contributor to the CIH-induced increased blood pressure (Tamisier *et al.*, 2009).

CIH exposure in no altitude native Chilean miners differs from the IHT/IHE and CyIH because it involves alternating between normobaric normoxia and hypobaric hypoxia. The CIH model has gathered interesting information about the characteristics of acclimatization to CIH, particularly in relation to the effects of hypoxia, but there is also evidence that hypobaria is playing a role. For example, studies showing different heart rate variability responses in normobaric hypoxia *versus* hypobaric hypoxia suggest that these two exposure conditions are clearly not equal stimuli to the cardiovascular and respiratory systems (Basualto-Alarcón *et al.*, 2012).

The long term adjustments in CIH tend to resemble those in chronic hypoxia at the level of ventilatory and cardiovascular responses, red cell mass and cardiac β-adrenergic receptors, among others. Considering exposure to the same altitude however, there is a difference in the time needed to complete acclimatization. Whereas acclimatization to chronic hypoxia is achieved in few months, CIH acclimatization is achieved in years, with stabilization of biomedical variables being observed after 18 months of exposure (Richalet *et al.*, 2002; Jimenez, 2003).

2.2. *Polycythemia and Pulmonary Hypertension*

Some of the body's first strategies when man or other animals not genetically adapted to high altitudes are subjected to hypoxia are expressed as metabolic, respiratory and cardiovascular adjustments. The increased production of red blood cells that improves oxygen transport capacity from the lungs to the tissues is one of them (Richalet, 1990). In the kidney, hypoxia stimulates the secretion of the hormone erythropoietin (after 2 to 3 hours of exposure to high altitude), which in turn stimulates the production of red blood cells by the bone marrow. This increase in erythropoiesis becomes the essential mechanism of long term acclimatization (Eckardt *et al.*, 1989).

In a study performed in Chilean mine workers, Richalet *et al.*, 2002 showed that hematocrit increased both at sea level and at high altitude after 12 and 19 months of CIH, and returned to pre-exposure values after 31 months of CIH exposure. The risk of polycythemia was lower than in chronic highlanders (Richalet *et al.*, 2002). In another study of subjects exposed to CIH for 12 years or more to a 4 x 3 commuting system (4 days at altitude and 3 days at sea level) at 3,550 m altitude, hematocrit and hemoglobin reached lower values than for residents at altitude over 4,000 m (Brito *et al.*, 2007).

According to the Venice Symposium Consensus, pulmonary hypertension (PH) is defined as a mean pulmonary artery pressure (PAP) > 25mm Hg in conjunction with right ventricle (RV) and right atrium (RA) enlargement (McLaughlin, 2004). PH is described as a marker of chronic exposure to high altitude-induced hypoxia; a direct relationship exists between PAP and the altitude reached (Peñaloza and Arias-Stella, 2007). The development of high-altitude PH is one of the main physiological changes observed in a poor response to hypoxia, and in people who permanently live at high altitudes the prevalence of PH increases from 5 to 10% (Leon-Velarde *et al.*, 2005).

A 32-month prospective study performed in Chilean miners exposed to CIH in a 7 x 7 commuting system at 4,500 m a.s.l. showed that PAP increased in hypoxia, but further evidence of PH was not observed (Antezana *et al.*, 2003). Moreover, values of PAP were lower than in subjects living permanently at high altitude (Richalet *et al.*, 2002; Hultgren, 1997). Similar results were observed in a 24-month follow-up of workers exposed to CIH in a 28 x 28 commuting system at 3,700 m a.s.l. at Kumtor, Kyrgyzstan (Saryvaeb *et al.*, 2003). However in subjects with more than 12 years of exposure to CIH, a 4% prevalence of PH was observed (Brito *et al.*, 2007). Thus polycythemia and PH are some of the main chronic changes during exposure to chronic hypoxia and CIH at high altitude (Pasha and Newman, 2010; Leon-Velarde *et al.*, 2010; Brito *et al.*, 2007).

Obesity, defined as a body mass index above 30 kg/m^2, has been reported to be a risk factor for the development of PH at high altitude, and obesity-related hypoventilation has been reported to be the primary mediator of the increased risk for this disorder (Valencia *et al.*, 2004).

2.3. *Physiological response to Exercise*

Exercise tests are often used to elucidate the effects of high altitude hypoxia on aerobic capacity. We used two different protocols of exercise to test the degree of acclimatization and the response to exercise during high altitude induced-CIH. The response to sub-maximal exercise (workload of 100 watts in a stationary bike) was used as a test of acclimatization. In mine workers exposed to CIH in a 7 x 7 commuting system at 4,500 m for a period of 2.5 years, blood pressure, heart rate and artery oxygen saturation during resting and during exercise were measured. CIH resulted in a useful degree of acclimatization that was maintained over a period of three years (Farías *et al.*, 2006). Conversely, another group of workers exposed intermittently to high altitudes showed that the cardiovascular response to a sub-maximal exercise load does not change between the first and fourth day of shifts at high altitudes (Jalil *et al.*, 1994). In order to test long term effects of CIH exposure

on exercise performance we used Aerobox sessions. Aerobox is a high-energy exercise regime that combines moves from a range of martial arts disciplines. Methodologically, the phases of a 45 min Aerobox training session are warm up, main phase (with the highest cardiovascular impact), localized phase (such as pushups and abdominal exercises) and recovery phase (this phase allows time for the blood vessels and the heart to resume their normal status).

Studies performed by Osorio in 2005 (unpublished) on 12 workers acclimatized to CIH performing Aerobox sessions at 4,000 m altitude indicated that the lowest level of oxygen saturation appears in the cardiovascular phase, with 83% SaO_2 (Table 1). Heart rate (HR) reached a higher level in the cardiovascular phase with 159 bpm. Considering that the heart rate at maximum exercise (HRmax) for this group is 172 bpm (Astrand, 1952; Fox *et al*., 1971; Richalet *et al*., 2002), HR during the cardiovascular phase reached 92%HRmax, a value considered adequate for training at maximum aerobic capacity (American College of Sports Medicine, 2000; Skinner *et al*., 2003). In the localized phase 59% HRmax was observed, indicating that the cardiac rhythm is within the physiological limits recommended for aerobic training (American College of Sports Medicine, 2000); Skinner *et al*., 2003). The high level of HR observed during exercise corresponds to a group trained and acclimatized to high altitudes.

Richalet *et al*., (2002) found that maximum oxygen consumption (VO_2max) decreased significantly with time of CIH exposure. Only part of this decrease can be attributed to the decrease in maximum heart rate induced by the downregulation of β-adrenergic and upregulation of muscarinic receptors (Richalet *et al*., 1992). A detraining effect of CIH exposure and/or sedentary living during the resting periods at sea level are probably also responsible for this effect (Richalet *et al*., 2002). A lack of physical activity during mining work could be another reason to explain the reduction in VO_2max.

2.4. Cardiovascular response

Andean mountain medicine has repeatedly shown that populations chronically exposed to altitude have low incidence of hypertension, atherosclerosis and myocardial infarction (Mortimer *et al*., 1977; Naeije, 2010). To what extent these characteristics depend on chronic hypoxia exposure, racial or nutritional factors has not yet been elucidated (Gamboa, 2003). Similarly, it has been recognized that long-term high-altitude hypoxia exposure protects the heart against ischemia / hypoxia injury, inducing a reduction of infarct size during acute ischemia. The relaxing effects of hypoxia on arterial smooth muscle cells may be proposed as contributors to this protection, as this vasodilatation tends to counteract polycythemia-induced blood viscosity (Hurtado, 1960).

Overall, the initial cardiovascular effects of altitude exposure involve an acute response associated with increased HR, blood pressure, cardiac output and myocardial contractility. Over time, during chronic hypoxia cardiac output decreases at levels lower than pre-exposure, accompanied by a decrease in sympathetic activity secondary to cardiac β-adrenergic receptor desensitization (Richalet, 1990).

Multiple mechanisms are involved in the cardioprotection induced by CIH. Hypobaric CIH preserves myocardial contractility and prevents apoptosis of cardiomyocytes (Beguin *et al*., 2007, Zhu *et al*., 2006, Dong et al., 2003, Zhang et al., 2004), increases coronary flow and myocardial capillary angiogenesis, activates ATP-sensitive K^+ channels and inhibits mitochondrial permeability transition pores (Zhong *et al*., 2002, Zhu *et al*., 2003). A rat model of CIH has also provided evidence that CIH attenuates β-adrenergic receptor activity by decreasing β-adrenergic receptor density and affinity in the right ventricle, and these alterations in the β-adrenergic receptor may contribute to cardiac protection in CIH (Guan *et al*., 2010).

In miners exposed to CIH for 31 months, blood pressure initially increased followed by a reduction, but remained slightly elevated compared to blood pressure measured at sea level; also a reduction in pulse and a slight dilation of the right ventricle were observed (Richalet *et al*., 2002; Farias *et al*., 2006). A study performed on Chilean soldiers exposed to CIH for more than 12 years revealed an increase in the amount of triglycerides and a reduction in LDL cholesterol (Brito *et al*., 2007).

Autonomic control in high altitude-exposed subjects has been studied using the non-invasive technique of power spectral analysis of HR (heart rate variability; HRV). Variables usually determined included power in the low (LF, 0.04-.0.15 Hz) and high (HF, 0.15-0.4 Hz) frequency ranges of the heart period spectrum, among others. Analyses showed an increase in the LH/HF ratio during acclimatization (Sevre *et al*., 2001). Since HF power is assumed to be a marker of parasympathetic activity and LF power to be a combination of both parasympathetic and sympathetic tonic activity, it has been concluded that the sympathetic tone is less reduced than the parasympathetic control of HR upon high-altitude exposure (Sevre *et al*., 2001; Cornolo *et al*., 2004).

2.5. Metabolic response and metabolic rate

High altitude-induced hypoxia alters the regulation of substrate metabolism favoring carbohydrate oxidation,

TABLE 1

Hemoglobin saturation (SaO_2, %) and heart rate (HR, bpm) values during Aerobox sessions at 4,000 m altitude in workers acclimatized to CIH.

		Aerobox Sessions (phases)			
	Resting	**Warming up**	**Main**	**Localized**	**Recovery**
SaO_2, %	90 ± 3	89 ± 2	83 ± 2	90 ± 6	92 ± 4
HR, bpm	83.5 ± 4.3	89.2 ± 5.6	159 ± 10	101 ± 9.6	90.6 ± 8.8

(mean ± SD, n=12)

as an adaptive mechanism to the limited ATP supply due to a diminished oxidative phosphorylation caused by the reduction in the available oxygen. Thus cells increase anaerobic glycolysis through positive regulation of the glycolytic enzymes and decrease the activity of some ATP consumers, e.g., Na^+-K^+ ATPase. Invariably acclimatization to high altitudes results in the increased use of blood sugar (Brooks *et al*., 1991). It has also been demonstrated that acclimatization to hypobaric hypoxia selectively reduces key enzymes responsible for lipid oxidation in heart, liver and skeletal muscle. Therefore, the greater dependency on blood sugar than on lipid metabolism probably contributes to the maintenance of homeostasis by optimizing the energy performance per unit of oxygen (Kennedy *et al*., 2001). We have studied the relationships between oxidative mitochondrial phosphorylation and oxygen consumption during chronic or intermittent hypobaric hypoxia exposure. Using a rat model of exposure to simulated conditions of continuous or intermittent high altitude (Farías *et al*. 2005a, Farías *et al*., 2005b) we observed a greater inhibition of oxygen consumption in spermatid cells in chronic versus intermittent hypobaric hypoxia (inhibition of 80% *vs*. 57%, respectively), in the presence of the mitochondrial H^+-ATPase inhibitor oligomycin. Similar oxygen consumption in both hypoxic treatments was observed after uncoupling of the oxidative phosphorylation, suggesting that continuous chronic hypobaric hypoxia is associated with an uncoupling of oxidative phosphorylation.

Basal metabolic rate (BMR) increases during the first days of exposure to high altitudes, apparently depending on the altitude reached. Increases of 6% and 10% have been found at altitudes of 3,650 and 3,800 m, respectively. As the days progress BMR decreases, but does not reach sea level values. For mountaineers who climb for 10 to 18 hours a day in high altitudes, the largest proportion of their energy comes from fats, and glycogen is only used for short and high-intensity exercises. However, after 18 days at the moderate altitude of 4,300 m, Young *et al.* (1982) found that the muscular glycogen at rest was lower than at sea level. Apparently, there are no data available about the deposits of muscular and hepatic glycogen at extreme altitudes, but with many more days of intensive work and adequate caloric consumption, it would not be surprising to find this reduced in liver and muscle (Young *et al.*, 1982). These findings suggest that in humans from sea level chronically exposed to high altitudes, fat is the principal fuel for exercise and the re-synthesis of the muscular glycogen can be reduced. It is not clear whether this is an effect of altitude or lower food consumption during hypoxia exposure. An elevated metabolism in rats (Rennie *et al.*, 1977) and humans (Costill *et al.*, 1977) has also been observed, indicating a saving of muscular glycogen, probably through the inhibition of the enzymes phosphofructokinase and pyruvate dehydrogenase.

2.6. Sleep quality

Travel to altitude is associated with a reduction in sleep quality (Anholm *et al*., 1992). These symptoms can be relieved with descent, simulated descent in a hyperbaric chamber or by enhancing room air with oxygen (West, 2002). In addition, travel to altitude results in a temporary reduction of rapid eye movement (REM) sleep that appears to improve with acclimatization (Anholm *et al*., 1992; Przybylowski *et al*., 2003). In the study performed by Richalet *et al*., (2002) with Chilean miners, sleep quality was altered during the first two nights at high altitude (worse on the second night) and did not ameliorate with time of exposure. In workers acclimatized to CIH a poorer sleep quality at 3,800 m than at sea level was observed. In this study some of the changes observed between sea level and altitude were: greater number of arousals (10.4 *vs.* 28.7), higher apneas/hypopneas index (5.8 *vs.* 10.1), and greater oxygen desaturation and periodic breathing (zero *vs.* 9.8) (Vargas *et al*., 2002). Supplemental oxygen partially reduced these differences between sea level and high altitude.

There is great concern regarding exposure to altitude of obstructive sleep apnea (OSA) syndrome carriers, since their cyclical reduction in $\%SaO_2$ during sleep is exacerbated by environmental hypobaric hypoxia. $\%SaO_2$ is low during sleep in altitude, and whereas the development of AMS has been shown to be partially associated with low mean $\%SaO_2$ in sleep (Burgess *et al*., 2004), positive airway pressure has been shown to prevent the occurrence of AMS (Johnson *et al*., 2010). Thus it is considered that untreated obstructive sleep apnea is a condition incompatible with altitude. In turn, the effects observed, including excessive daytime sleepiness and impaired daytime function, can affect job performance.

In terms of the respiratory mechanisms contributing to AMS, it has been long recognized that the ventilatory response is reduced in people susceptible to AMS (Hackett et al., 1982). Recently, in a group of people exposed to experimental hypoxia (FIO_2 = 80% SaO_2), the acute hypoxic ventilatory response at 5 min (HVR5min) was greater in individuals not susceptible to AMS compared to susceptible individuals (Nespoulet *et al*., 2012). These results support the hypothesis of low chemoreceptor sensitivity as a marker of AMS predisposition (Moore *et al*., 1986), suggesting high chemo sensitivity as a protective factor for AMS.

2.7. Oxidative stress

Reactive oxygen species (ROS) are produced by diverse cellular processes and are considered to have beneficial and harmful effects. Moderate concentrations of ROS mediate their beneficial effects such as cellular defense against infections, control of vascular tone, ventilation and erythropoietin production, the induction of mitogenic responses and modulation of several transduction signaling pathways. Overproduction of ROS leads to their harmful effects due to oxidative stress (for reviews see Dröge, 2002 and Valko *et al*., 2007).

Several reports indicate that exposure to high altitude hypobaric hypoxia causes oxidative cellular damage. This cellular oxidative stress appears directly related to the altitude level and an increased production of ROS seems to be responsible for these effects (Dosek *et al*., 2007). Concomitantly, oxygen enrichment of room air is increasingly being used in work stations at high altitude (West, 2002, 2003) and since the production of ROS is favored at higher oxygen supply (Dosek *et al*., 2007; González *et al*., 2002), oxidative stress may be an even more important factor in high altitude exposed workers.

The main cause of oxidative stress is the lower availability of O_2 to be reduced to H_2O by the enzyme cytochrome oxidase in the mitochondrial respiratory chain. This produces an accumulation of electrons that in the absence of sufficient oxygen as final acceptor will form superoxide anion (O_2^-) which in turn produces hydrogen peroxide (H_2O_2) and the

hydroxyl radical (OH^-) after reacting with water (Maiti *et al.*, 2006). The accumulation of these reduced equivalents formed principally in complexes I and III of the electron transport chain is known as "reductive stress" and can favor self-oxidation of one or more mitochondrial complexes, such as the redox pair ubiquinone–biquinol, as well as increasing the $NADH/NAD^+$ ratio (Dosek *et al.*, 2007). The accumulation of free radicals of coenzyme Q, called ubisemiquinone, causes the transfer of its unpaired electron to oxygen, generating the superoxide radical anion ($\cdot O_2^-$). Although ($\cdot O_2^-$) by itself is not particularly harmful, it is a precursor of the highly reactive hydroxyl radical (OH^-) and, through irreversible condensation with nitric acid, forms peroxynitrite (ONOO) (Manukhina *et al*., 2006). Oxidative damage may also be produced by reductions in the antioxidant capacity, and it has been established that during hypoxia the cellular systems of redox defense are affected. Antioxidant enzyme activities such as superoxide dismutase (SOD), glutathione reductase (GSR) and glutathione peroxidase (GPX) are reduced (Maiti *et al.*, 2006). Other molecular events that also favor oxidative stress induced by hypobaric hypoxia are the xanthine dehydrogenase/oxidase system and the inducible isoform of nitric acid synthase (iNOS). The former has been described as a strong ROS generator in high altitude conditions, as hypoxic cells generate greater amounts of ATP and cAMP by the action of adenylyl kinase from two ADP. iNOS is up-regulated during acclimatization, thus altering the balance of ROS/NO, which recovers as time elapses (Dosek *et al*., 2007). The alteration in this balance may be related to the microcirculatory changes caused by hypobaric hypoxia expressed in AMS and cerebral and pulmonary edemas (Dosek *et al.*, 2007). Finally, other factors that contribute to the development of oxidative stress are exercise, UV radiation (which penetrates the epidermis with greater aggressiveness in high-altitude zones), lack of antioxidant supplements in the diet and the oxidation of catecholamines (adrenaline, noradrenaline and dopamine), which increases with altitude (Askew, 2002). The high reactivity and oxidant properties characteristic of ROS and free radicals result in overall damage that affects main cell components including carbohydrates, proteins, lipids and even DNA (Blokhina *et al.*, 2003).

Little is known about the level of real oxidative damage suffered by cell structures of the organs and tissues exposed to a situation of high altitude. Moller *et al*., (2001) exposed 12 healthy individuals to an altitude of 4,559 m, which caused a significant increase in the rupture of the DNA chain measured in the urine. The damage was prominent in endonuclease-III sites. Also, when a group of humans were exposed simultaneously to an altitude of 2,700 m and cold conditions, the peroxide lipid levels and the DNA damaged chain in the urine increased up to 23% at 6,000 m and up to 79% at 8,848 m, indicating that the oxidative stress increases with escalating altitude (Joanny *et al*., 2001). Thus, studies performed on humans consistently describe that high altitude hypoxia causes oxidative damage to lipids, proteins, and DNA chains. This damage may be due to the increase in the production of ROS and/or to the reduced antioxidant capacity. Given their high content in unsaturated fatty acids, cell membranes constitute a main target of ROS, with lipoperoxidation being usually observed upon exposure to hypoxia (Behn *et al*., 2007). In animal models, CIH (12 hrs per day, simulated 4,000 m altitude for 6 months) increases lipoperoxidation and carbonyl derivatives in skeletal muscle (Radak *et al*., 1994, 1997). However, short exposure for 5 days to 7,576 m elevation causes an increase in lipoperoxidation in plasma in rats (Kumar *et al*., 1999). Maiti *et al*. (2006) also reported that exposure of 3 and 7 days to 6,100 m significantly increases ROS levels and lipoperoxidation in various brain regions. It appears that the effects of oxidative stress are systemic, as suggested by Nakanishi *et al*. (1995), who reported that exposure to 5,000 m resulted in increased serum levels of malonydialdehyde in lungs, liver, heart and kidneys in rats, whereas exposition to a simulated altitude of 9,000 m causes increases in lipid peroxidation of selected rat brain membranes (Rauchová *et al*., 2012).

As previously stated, exposure to high altitudes reduces activity and expression levels of antioxidant enzymes; consequently the disruption in the efficiency of the antioxidant systems due to the increase in ROS production by hypobaric hypoxia leads to oxidative damage of macromolecules (Dosek *et al*., 2007). A reduction in the activity of superoxide dismutase (SOD) content in skeletal muscle has been reported in rats exposed to intermittent hypobaric hypoxia (Radak *et al*., 1994). Reduction in the activity of glutathione peroxidase (GPX) has also been shown in the liver of rats exposed to high altitude (Nakanishi *et al*., 1995), whereas Imai *et al*. (1995) compared GPX activity of high altitude residents (4,000 m) to individuals from sea level, finding that high-altitude dwellers had lower levels of GPX activity. Nevertheless, it has been observed that catalase (CAT), SOD and also the heat shock proteins lead to the stabilization of cell membranes and restriction of apoptosis, alleviating the oxidative effect when subjected to hypobaric hypoxia (Manukhina *et al*., 2006). Rat myocardia cells, for example, presented high levels of SOD and CAT after CIH exposure to 3,500 m (Ning *et al*., 2000). Additionally, our studies of expression and activity of glutathione reductase (GR) in CIH found no differences in GR expression, but lower activity in testes and epididymis in CIH exposed rats (Farias *et al*. 2010). However, we observed that melatonin decreased lipid peroxidation in heart, kidneys and lung under CIH conditions, but melatonin did not exhibit any protective effect in liver, testis, epididymis sperm count (Farias *et al*., 2012). It is generally accepted that testicular and seminal ROS levels are important in terms of the deleterious effects of hypoxia on male fertility (Reyes *et al*., 2012).

In view of the pathological roles played by increased ROS generation, the supplementation of antioxidants seems a good detoxification strategy to improve tissue function under hypobaric hypoxia. In our rat model we observed that exogenous administration of ascorbic acid and blueberry extract restored GR activity, reduced lipoperoxidation and hypospermatogenesis typical of hypobaric hypoxic exposure (Farias *et al*., 2010; Zepeda *et al*., 2012). However, the clinical translation of antioxidant therapies has proven difficult, as few benefits and even harmful effects (Dotan *et al*., 2009) have been observed in clinical trials aimed to reduce oxidative stress by antioxidant supplementation (Armitage *et al*., 2009; Villanueva and Kross, 2012).

3. FINAL CONSIDERATIONS

Exposure to chronic and intermittent hypobaric hypoxia is a biomedical condition unique in the world and represents a great challenge for miners such as Chilean mine workers.

Studies leading to the elaboration of strategies to prevent and revert the negative physiological effects brought about by exposure to high altitudes are highly desirable. Specifically, urgent new challenges for the future are: a) Acute Mountain Sickness on the first day of the re-ascent after 3-7 days of rest; this condition affects people with signs of good acclimatization, oxygen saturation, without polycythemia or hypertension, despite years of exposure to CIH, b) Prevention or control of disturbance of quality of sleep, periodic breathing, oxygen desaturation, sleep fragmentation and /or total sleep, c) Causes of polycythemia in CIH, d) Biological parameters that define a suitable physiological intermittent hypoxia acclimatization to altitudes between 3,000 and 5,500 m, e) Long-term monitoring of pulmonary artery pressure, f) Aging biomarkers of CIH, g) Hypoxia tolerance test to identify, at sea level, before the climb, the good responders to hypoxia and susceptibility to severe AMS, and h) Best strategies to mitigate the effects of CIH, based on nutrients, sleep hygiene, supplemental oxygenation, commuting patterns, ergonomic jobs adjustments, etc. The determination of markers that can identify in advance the good and bad responders to this environmental condition (Richalet *et al*., 2012; Burtscher *et al*., 2008), as well as markers of health condition of workers chronically exposed will greatly contribute to the health of miners who have to work intermittently under different shift schemes at work sites over 4,000 m altitude. in the Andes mountain range.

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