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Altitude effect on sleep quality and serum melatonin levels in Chilean mining workers

Efecto de la Altitud en la calidad de sueño y niveles séricos de melatonina en trabajadores mineros en Chile

Rodrigo Calderón-Jofré[1,2] & Fernando A. Moraga[1]

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ABSTRACT: High altitude (HA) mining operations are a very important business in Chile, but reduced availability of oxygen affects the sleep quality, increasing the risk of accidents. An important regulator of sleep-wake cycle is the hormone Melatonin, produced by pineal gland as a sleep inductor. The aim of this study is to evaluate the effect of high altitude (4,500 m) on the quality of sleep of workers undergoing to Chronic Intermittent Hypobaric Hypoxia (CIHH) using self-reported surveys of sleepiness and sleep quality, measurement of sleep apnea (using nocturnal oximetry) and serum levels of melatonin. The Desaturation index (ID4) results revealed higher HA scores compared to sea level (SL). Regarding melatonin levels, the results show that it is increased in HA versus SL and this increase would be related to oxygen saturation during sleep. These data link sleep quality in HA to its melatonin levels, suggesting that melatonin may be a potential biomarker for sleep quality.

KEY WORDS: Melatonin, chronic intermittent hypoxia, sleep quality, mining workers, oxygen saturation.

INTRODUCTION

In Chile, one of the most important economic activities is high-altitude mining, where the facilities are located mainly in the north of the country at more than 3,000 m, reaching up to 5,200 m. The need for workers (about 120,000 people related to the large mining industry) and the geographical situation in Chile has allowed people to travel from the lowlands where they used to live, to the high altitude where the mines are located, in a few hours (Consejo de Competencias Mineras, 2017). This rapid relocation is associated with a type of altitude exposure known as chronic intermittent hypobaric hypoxia (CIHH), where people work at high altitude for a period of time and return to sea level to rest (Richalet *et al.*, 2001; Moraga *et al.*, 2014). Previous studies have referred to the potential risk of this type of CIHH occupational exposure in people's health, mainly in the cardiovascular and pulmonary system in the long term (Richalet *et al.*; Brito *et al.*, 2007; Vinnikov *et al.*, 2011).

A major effect of high altitude exposure is associated with decreased sleep quality. Previous work focusing primarily on climbers exposed to acute hypoxia has shown sleep disturbances due to high altitude (Wickramasinghe and Anholm, 1999). A report using simulated acute hypoxia equivalent to 4,500 m showed that poor sleep quality is related to altered mood and cognitive function (De Aquino Lemos *et al.*, 2012). In the mining population with prolonged exposure to CIHH, self-reported sleep quality is altered in the first two nights at high altitude without significant relief with exposure time (Richalet *et al.*, 2001), indicating lack of acclimatization to sleep at high altitude. Furthermore, our previous study showed the presence of reduced oxygen and sleep disturbance at 4200 m (Moraga *et al.*, 2014). These alterations affect the quality of sleep in high-altitude miners, being an important factor related to the occurrence of accidents during work shifts and threatening life expectancy due to the development of other health problems related

[1] Laboratorio de Fisiología, Hipoxia y Función Vascular, Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte, Coquimbo, Chile
[2] Departamento de Ciencias Básicas, Universidad Santo Tomás, La Serena, Chile

to metabolic and cardiovascular functions. (Spiegel *et al.*, 1999; Newman *et al.*, 2001; Shahar *et al.*, 2001).

Melatonin is the most important product of the pineal gland, the release of which is controlled by the suprachiasmatic nuclei (SCN) of the hypothalamus, a key regulator of the circadian rhythm (Arendt and Skene, 2005). Mammalian melatonin levels rise in the late afternoon in response to darkness, peaking between 3 a.m. and 5 a.m., and returning to baseline before awakening (Arendt, 1995). In humans, melatonin plays an important role as a sleep promoting hormone, inducing changes such as sedation, reducing metabolism and body core temperature, associated with sleep time (Dijk and Cajochen, 1997; Kräuchi and Wirz-Justice, 2001). In addition, exogenous melatonin has been used as a sleep promoter in older people and to adjust the circadian cycle in people who work night shifts or after long flights (Arendt and Skene, 2005). In addition to its sleep inducing properties, evidence points to additional benefits of melatonin as a free radical scavenger, which protects against oxidative stress (Kędziora-Kornatowska *et al.*, 2007; Torres *et al.*, 2015).

Previous works have related acute hypoxia in the alteration of the circadian rhythm and the sleep cycle (Vargas *et al.*, 2001; Bosco *et al.*, 2003). Our previous work demonstrated that oxygen supplementation during sleep improves oxygen saturation and sleep quality (Moraga *et al.* 2014). However, information on melatonin levels in the human population with this type of intermittent hypoxia is limited. Furthermore, considering the bioavailability of melatonin and its derivatives, it is possible to use them as a marker of circadian alteration (Van Cauter *et al.*, 1994; Deacon & Arendt, 2010). The objective of this study is to correlate the effect of two different altitudes on sleep quality, using different surveys and pulse oximetry at night, and to correlate these data with serum melatonin levels.

MATERIAL AND METHOD

Subjects: 2 groups of 30 male volunteers working at sea level (SL) or 4,500 m (HA) for at least 3 months were included in the study. All the volunteers were working for 7 days on a day shift at the indicated altitude and resting 7 days at lower altitudes. All the people gave their written consent in agreement with the ethics committee of the Faculty.

Anthropometric measurement: age (years), weight (kg), height (m), waist circumference (cm) and skinfolds of biceps, triceps, subscapular and suprailiac areas were recorded. The body mass index (BMI) was estimated using the relationship: BMI = weight/height^2 (expressed in kg/m^2). Body fat percentage was calculated from skinfolds using the formula of Durnin and Womersley (1973).

Nocturnal Pulse Oximetry: Oximetry was performed on the third night of the work shift, using a Nonin WristOx 3100™ oximeter (Nonin, Plymouth, MN, USA). To determine the presence of sleep apnea/hypopnea events, parameters for Total number and Time of events, ID4 (desaturation events equal to or greater than 4%) and Oxygen saturation (SpO2) were obtained using the nVision program provided by the manufacturer (Nonin).

Sleep Quality and Sleepiness. Sleep quality and sleepiness were evaluated using self-assessed questionnaires the morning after evaluation of nocturnal pulse oximetry. For the quality of sleep, a translated 6-item Spiegel Sleep Quality Questionnaire was used (Spiegel *et al.*, 1999). Sleepiness was evaluated by a translation of Epworth Sleepiness Score (Johns, 1991).

Blood samples and melatonin detection: Blood samples were collected from brachial vein before entering the shift (between 7 and 9 am) on day 3 at the indicated altitude. The separated serum was frozen at -80ºC until analysis. For determination of melatonin levels, ELISA test was performed in duplicate using a commercial kit for serum and plasma immunoenzyme assay, following manufacturer instructions (catalogue number RE54021; IBL-Hamburg GmbH, Hamburg, Germany).

Statistical Analysis: All data were analyzed using GraphPad Prism 6.1 software and presented as Mean ± Standard Error (SE). Comparisons between SL and HA were performed using Mann–Whitney–Wilcoxon Test, and $p < 0.05$ was considered statistically significant.

RESULTS

Of the initial 60 volunteers, only 25 in SL and 18 in HA completed the study. Table I summarizes the characteristics of the study population, wi-

Table I: Characterization of population in this study. Data are presented as Mean±SE.

Altitude	N	Age (yr)	Weight (kg)	Height (m)	BMI	Waist circumference (cm)	Body Fat %
SL	25	39.8±2.1	80.6±2.2	1.71±0.012	27.4±0.5	96.9±1.4	26.3±1.1
HA	18	40.0±1.9	81.8±3.1	1.72±0.019	27.3±0.7	94.8±3.7	27.4±0.7

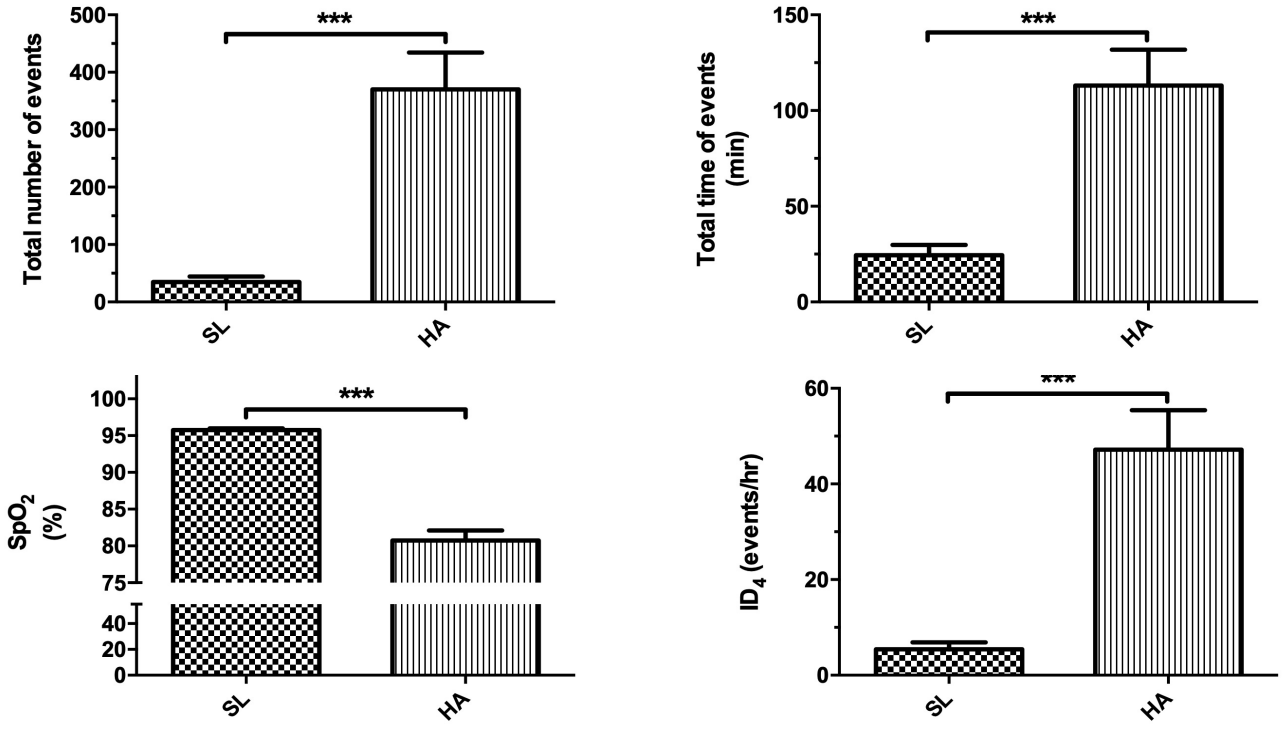

Figure 1: Nocturnal oximetry parameters at SL and HA. *** p<0.001

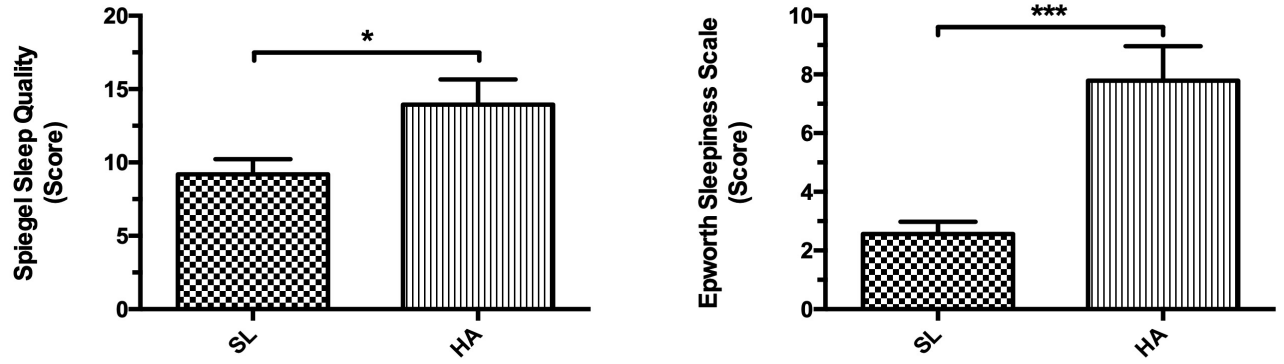

Figure 2: Sleep Quality (Spiegel) and Sleepiness (Epworth) scores, according to self-reported questionnaires.
* p<0,05, *** p<0.001.

thout showing significant differences between both groups. Most of the volunteers were characterized as overweight according to BMI and body fat percentage measurements, and were recognized as sedentary. No cardiac, respiratory or metabolic problems were reported.

The results of nocturnal oximetry are shown in Figure 1. As expected, oxygen saturation decreases, reaching a saturation of 80.7%±1.4 in HA (compared with 95.8±0.2 in SL). Additionally, the number and duration of desaturation events were significantly higher in HA comparing to SL. Desaturation

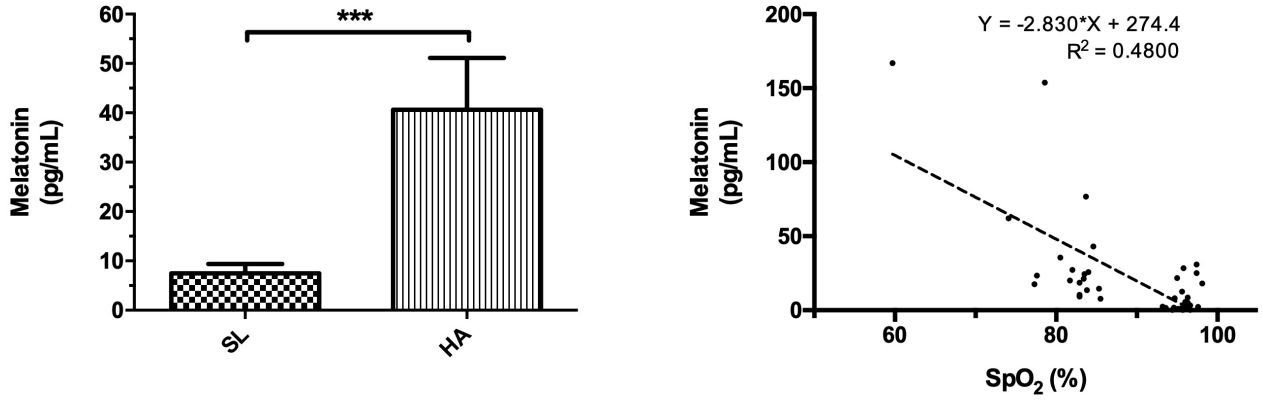

Figure 3: Melatonin levels at SL and HA, and relationship with SpO2. *** p<0.001

events (indicated as ID4) were significantly higher in HA (47.2±8,3 versus 5.4±1.4 in SL).

The self-reported sleep quality (Spiegel) and sleepiness (Epworth) questionnaires are shown in Figure 2. Increased scores can be seen in both questionnaires in HA, indicating an altered sleep pattern in people at HA.

Melatonin levels are shown in Figure 3. We observed an increased value of Melatonin in HA (40.6±10.5 pg/mL) compared to SL (7.5±1.9 pg/mL). Furthermore, melatonin levels can be correlated with nocturnal oxygen saturation, demonstrating that higher melatonin levels occurred in volunteers with lower saturation (Y= - 2.830X + 274.4, R2=0.4800).

DISCUSSION

This study provides the first report of altitude blood melatonin level in mining subjects undergoing CIHH. Our results for plasma melatonin levels in the SL population are in good agreement with the levels reported in previous studies, where the values obtained at the time of sampling are less than 25% of the maximum peak of melatonin (Arendt *et al.*, 1982; Wehr, 1991; Benloucif *et al.*, 2005). However, it was not possible to conduct a larger study to find out an entire night or 24-hour melatonin profile, due to technical limitations and concerns of the worker´s unions and the company's reasons.

Altitude is known to significantly affect sleep quality, primarily due to hypoxia. As seen previously in a prospective study, people working a 7-day shift at high altitude exhibited altered sleep quality for the first 2 nights, and did not improve for at least 18 months of exposure (Richalet *et al.*, 2011). In addition, the alteration in the production of melatonin associated with sleep disorders was previously described, in a study with patients suffering from chronic insomnia, where the onset of melatonin was earlier than the control people and in a longer period of time, but to a lesser extent at night (Hajak *et al.*, 1995).

Control of melatonin production is regulated by SCN and is highly dependent on light exposure. Evidence indicates that light suppresses melatonin production (Lewy *et al.*, 1980), delaying the onset of melatonin secretion. Specifically, exposure to certain wavelengths of light (in the light spectrum range between 446 and 477 nm) during the dark phase suppresses the production of melatonin (Brainard *et al.*, 2001). Furthermore, the duration of light exposure is necessary to synchronize the circadian rhythm, which affects the duration of the sleep / wake cycle according to the seasonal variation of the duration of the day and night (Wehr, 1991). However, the effect of light exposure apparently does not change the plasma level of melatonin, but rather prolongs the production period when light exposure is reduced (as in winter), discarding the idea that high melatonin in HA could be due to exposure to artificial light or a seasonal difference during sampling. Taking into account that most of the miners in this study had samples taken during the day shift and were exposed to the same artificial light in the mining complex, it is possible to consider that most of them should have a similar photoperiod and the observed differences could be the result of differences in individual habits before sleep or personal differences that affect the quality of sleep. Other studies have established that a constant activity routine could minimize changes in the circadian

rhythm (Duffy and Dijk, 2002; Gunn *et al.*, 2016), but this could not be reflected in the production of melatonin as seen in this work.

Despite the fact that altitude affects sleep, studies of melatonin levels in humans living or working at high altitudes are scarce, and most studies have been conducted on climbers. In a rat model simulating an altitude of 8,000 m, an elevation of melatonin was observed during the first 7 days of exposure, but decreased after that, returning to control levels at 14 to 21 days (Kaur *et al.*, 2002). In another work, melatonin appears to play a protective role against oxidative stress in the heart, lungs, and kidneys in rats exposed to 96-hour intervals of intermittent hypoxia (equivalent to 4,500 m) for 32 days (Farías *et al.*, 2012).

However, most of the evidence on the effects of melatonin and intermittent exposure to hypoxia comes from studies of obstructive sleep apnea syndrome (OSA), a model of episodic intermittent hypoxia associated with upper airway obstruction. Previous studies suggested that OSAS stimulates cardiac damage in animal models, which could be improved by treatment with melatonin (Xie *et al.*, 2015). Melatonin also stimulates the expression of antioxidant enzymes and reduces oxidative stress markers, alleviating hippocampal injury associated with intermittent hypoxia (5%) for 7 and 14 days (Hung *et al.*, 2008). In hamsters, melatonin increases vasodilation and capillary perfusion, reducing oxidative stress and microvascular damage associated with insulin resistance (Bertuglia and Reiter, 2009). Administration of melatonin to rats exposed to chronic hypoxia (10% oxygen) could attenuate right ventricular pressure and reduce oxidative damage and the expression of proinflammatory substances, leading to reduced pulmonary hypertension (Hung *et al.*, 2017) .

Taken together, it can be assumed that the endogenous elevation of plasma melatonin during sleep in people exposed to CIHH could be used as a potential biomarker to analyze sleep quality, functioning as a protective mechanism for various health problems related to disorders of sleep due to high altitude exposure. Further analysis should consider studying a long-term effect of high altitude exposure on melatonin levels and whether exogenous melatonin treatment could improve the negative effects of high altitude on sleep.

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Corresponding Author:
Fernando A. Moraga.
Laboratorio de Fisiología, Hipoxia y Función Vascular
Departamento de Ciencias Biomédicas
Facultad de Medicina
Universidad Católica del Norte
Coquimbo
CHILE

Email address: fmoraga@ucn.cl

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