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Sleep-disordered breathing and oxidative stress in preclinical chronic mountain sickness (excessive erythrocytosis)

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Abstract

Chronic mountain sickness (CMS) is considered to be a loss of ventilatory acclimatization to high altitude (>2500 m) resulting in marked arterial hypoxemia and polycythemia. This case-control study explores the possibility that sleep-disordered breathing (SBD) and associated oxidative stress contribute to the etiology of CMS. Nocturnal respiratory and Sa_{O2} patterns were measured using standard polysomnography techniques and compared between male high-altitude residents (aged 18–25) with preclinical CMS ([excessive erythrocytosis (EE)], n=20) and controls (n=19). Measures of oxidative stress and antioxidant status included isoprostanes (8-iso-PGF2 $_{alpha}$), superoxide dismutase and ascorbic acid. EE cases had a greater apnea-hypopnea index, a higher frequency of apneas (central and obstructive) and hypopneas during REM sleep, and lower nocturnal Sa_{O2} compared to controls. 8-iso-$PGF2_{alpha}$ was greater in EE than controls, negatively associated with nocturnal Sa_{O2}, and positively associated with hemoglobin concentration. Mild sleep-disordered breathing and oxidative stress are evident in preclinical CMS, suggesting that the resolution of nocturnal hypoxemia or antioxidant treatment may prevent disease progression.

Keywords

polycythemia; sleep-disordered breathing; altitude; oxidative stress

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1. Introduction

Chronic mountain sickness (CMS) is considered to be a loss of ventilatory acclimatization to high altitude (>2500 m) that results in marked arterial hypoxemia and polycythemia. Clinical criteria for its diagnosis are a hemoglobin concentration ≥ 21 g/dl for males or ≥ 19 g/dl for females in the absence of chronic pulmonary disease or other conditions causing erythrocytosis, and three or more of the following symptoms: breathlessness, palpitations, sleep disturbance, cyanosis, dilitation of veins, paresthesia, headache or tinnitus (Leon-Velarde et al., 2005). In the vast majority of CMS cases, arterial hypoxemia worsens over time, and frequently leads to pulmonary hypertension or *cor pulmonale*. CMS affects approximately 10 million persons worldwide, including nearly 10% of male high-altitude residents (Vargas and Spielvogel, 2006). Females are comparatively protected from the condition, however this difference diminishes after menopause (Leon-Velarde et al., 1997). Due to its progressive nature, CMS raises morbidity and mortality rates in highland regions in North America, Asia and, in particular, the Andean Plateau of South America where more than 35 million people reside.

Despite detailed clinical characterization, the fundamental pathophysiologic processes responsible for CMS remain unclear. One factor complicating our understanding of CMS is that the condition is most often diagnosed in older adults, at which point the effects of normal aging on pulmonary, hematologic and ventilatory characteristics obscure the identification of primary causal factors (Leon-Velarde et al., 1993; Monge-C et al., 1989). Helpful in this regard is a report by Vargas and Spielvogel in which they describe a preclinical form of CMS, termed excessive erythrocytosis (EE), defined as a hemoglobin concentration greater than two standard deviations above the altitude and age-specific mean in the absence of established CMS (Vargas and Spielvogel, 2006). In their study, young men with EE showed a progressive rise in hemoglobin concentration over a period of four years that was accompanied by the gradual development of symptoms that define CMS. Importantly, the availability of such preclinical cases provides the opportunity to identify pathophysiologic processes prior to established disease and possibly identify targets for early therapeutic intervention.

Blunted response to acute hypoxia (hypoxic ventilatory response [HVR]) and alveolar hypoventilation occurs in CMS, yet the etiologic importance of such processes remain unclear given that healthy long-term residents of high altitude also show such blunting (Severinghaus et al., 1966; Weil, 1986). We considered that a key etiologic factor for could involve respiratory instability during sleep given that nocturnal breathing patterns are disrupted in patients with established CMS compared to controls (Spicuzza et al., 2004; Sun et al., 1996). Moreover, prior evidence shows that chronic intermittent hypoxia (CIH) experienced during sleep-disordered breathing can act to impair respiratory control during wakefulness (Spicuzza et al., 2004) by enhancing basal and hypoxia-induced chemosensory discharge of the carotid bodies (Peng et al., 2001; Peng et al., 2003). It has been suggested that redox status may play a key role in mediating such impaired ventilatory control (Peng and Prabhakar, 2003; Prabhakar, 2001, 2002; Semenza and Prabhakar, 2007). In CMS, HVR is not enhanced, as would be expected with increased chemosensory discharge, however ventilatory control is impaired leading us to consider the possibility that CIH on the scale of years may have a different, but equally important effect on respiratory control in response to hypoxia. Based on prior studies demonstrating the occurrence of sleep-disordered breathing in CMS and the importance of expanding our knowledge concerning the mechanisms by which CIH impairs respiratory control, we hypothesized that young men with EE compared to age and altitude-matched controls experience more episodes of sleep-disordered breathing and, as a result, greater oxidative stress. Our findings demonstrate that mild sleep-disordered breathing and oxidative stress are evident prior to established CMS, suggesting that the

resolution of nocturnal hypoxemia or antioxidant treatment may prevent disease progression and help reduce the significant disability and mortality associated with CMS.

2. Materials and Methods

2.1. Subjects

A survey of 1149 male high altitude (3600–4100 m) residents between the ages of 18 and 25 was conducted in order to identify young males with EE (cases) and healthy controls. Surveys were conducted at four postsecondary schools in La Paz, Bolivia. After the project was orally presented to students, they were invited to participate in the screening process. Acceptance rates for screening at the four institutions were 50%, 40%, 100% and 60%. To determine eligibility for the physiologic portion of this study, survey participants completed a questionnaire to document age, altitude of birth; residential and medical history; current or past symptoms of dyspnea, exercise tolerance, headache, tinnitus, cyanosis, sleep disturbance, and exposures to substances known to influence pulmonary function (e.g., smoke, paint, dust). At the time of survey, peripheral blood samples were obtained by finger stick for the measurement of hematocrit and hemoglobin. Hematocrit was measured in duplicate using the microcentrifuge technique and hemoglobin in triplicate using the cyanmethemoglobin method. Arterial O_2 saturation (Sa_{O2}) was measured from a warmed digit while the subject was resting for >3 minutes. The survey also included a physical exam to measure heart rate and blood pressure, and a simple evaluation of pulmonary and cardiac function.

Exclusion criteria were female sex, ages <18 or >25 years at the time of first study, history of conditions with increased risk of EE secondary to hypoxemia (e.g., cardiac or pulmonary disease, chronic bronchitis, obesity [BMI > 30]), anemia, having been born at low altitude, having lived at low altitudes for more than 1.5 years, or having spent more than 1 month at low altitude within the 6 months prior to the survey. Polycythemia is extremely uncommon in women prior to menopause. For this reason, we chose to exclude females from study given that our aim was to characterize the preclinical CMS in young adults to avoid the confounding effects of age.

Qualifying survey participants were divided by EE status (i.e., EE or control), age (18 to 21 or 22 to 25 years), and place of residence (i.e., La Paz or El Alto). Dichotomization was based on the mid-range value for age, and the altitude ranges that define La Paz (3100–3800 m) or El Alto (4000 – 4300 m). Given previous studies that demonstrate a positive correlation between age, altitude and CMS (Monge-C et al., 1989; Moore et al., 1998), EE subjects and controls were frequency-matched according to age and place of residence. Within each EE, age and residential category (e.g., control, 22 to 25 years old, El Alto) subjects were randomly selected and invited to participate in subsequent studies. Cases and controls included for study were 22.4 ± 0.6 and 21.5 ± 0.5 years of age, respectively (p=NS). Among all 1149 survey participants, 11.6% had a hemoglobin concentration ≥18.3 g/dl and were considered to be EE as defined by Vargas and Spielvogel (Vargas and Spielvogel, 2006).

All study subjects granted informed consent to study procedures approved by the Colorado Institutional Review Board (COMIRB) and Colegío Medico, the Bolivian equivalent ethics review committee.

2.2. Study protocol

Cases and controls selected for study were asked to come to the Bolivian Institute for High Altitude Biology (IBBA) for study. On the first visit peripheral blood samples were collected from an antecubital vein for a repeat measurement of hemoglobin to confirm EE or

control status, as well as to assess levels of erythropoietic markers and to test for iron deficiency. At this time each subject was interviewed to obtain more detailed information regarding medical and residential history (subject, parental and grandparental), current or past symptoms of CMS, self-reported ancestry, as well as frequency and duration of travel to low altitudes. The second visit consisted of respiratory testing during wakefulness. Sleep studies were conducted on the third and fourth visits, the first of which was a practice session to guard against first-night effects. Although 29% of subjects elected to forgo the practice night, their sleep quality and sleep time were similar to those who completed the practice night; therefore their data were included with the results presented here.

2.3. Blood sampling

All blood samples were collected immediately upon awakening after the polysomnography study. While subjects were seated quietly at rest, venous blood was withdrawn from an antecubital vein into heparinized and EDTA vacutainers by routine venipuncture for the measurement of erythropoetic markers [erythropoietin (EPO), soluble transferrin receptor (sTfR); both in EDTA], hemoglobin and hematocrit to confirm EE or control status. Also assessed were markers of oxidative stress and antioxidant status [isoprostanes (8-iso-PGF2 $_{alpha}$, in EDTA plasma), superoxide dismutase (SOD, in heparin plasma) and ascorbic acid in EDTA plasma]. Samples for ascorbic acid measurements were mixed with 10% meta-phosphoric acid, and stored in amber tubes to minimize exposure to light. Samples for EPO, sTfR, 8-iso-PGF2$_{alpha}$, SOD and ascorbic acid were stored in 500ul aliquots at −80°C until analysis.

Plasma EPO was determined via a double-antibody sandwich ELISA (R&D, US) with a sensitivity of 0.6 mIU·ml $^{-1}$ and intra-assay precision of 4.2%. sTfR, an index of bone marrow erythropoetic activity and for which elevated values denote iron deficiency, was assessed using an enzyme linked immunosorbent assay (R&D, US) with a sensitivity of 0.5 nmol·l^{-1} and intra-assay precision of 4.3%.

8-iso-PGF2$_{alpha}$ concentrations were quantified by HPLC, a linear method for human plasma concentrations between 0.0025 and 80 μg·l^{-1} with an inter-day accuracy and precision of <10% (Haschke et al., 2007). After vortexing (1minute) and centrifuging the sample (13000g, 10 minutes, 4°C), the supernatant was transferred into a HPLC vial and stored at −80°C until being placed in the autosampler at 4°C. The standard curve was generated using 2.5, 5.0, 10.0, 25, 50, 100, 250 and 500 pg·ml^{-1} standards. Total plasma SOD activity (Mn-SOD, Cu,Zn-SOD and Fe-SOD) was determined by detecting superoxide radicals generated by xanthine oxidase and hypoxanthine (Cayman Chemical, US; intra- and inter-assay precision values of 3.2% and 3.7%, respectively). SOD is expressed as units per ml (U·ml ^{1}) where one unit is defined as the amount of enzyme needed to exhibit 50% dismutation of superoxide radical. Ascorbic acid (reduced, total, oxidized, oxidized/reduced ratio, and % oxidized) was measured by high performance liquid chromatography (HPLC) as previously described (Rumelin et al., 1999).

2.4. Pulmonary function and sleep studies

2.4.1. Daytime—Forced vital capacity (FVC) and forced expiratory volume at 1 minute (FEV_1) and forced inspiratory flow at 50% of FVC ($FIF_{50\%}$) were determined by spirometry and flow-volume curves measured for forced expiratory flows (e.g., $FEF_{50–75\%}$) by body plethysmography (Sensor Medics Autobox U6200). To minimize the influence of inter-operator error, the same two technicians conducted all ventilatory studies. Reported values are the best of three repeated efforts. Lung diffusion capacity (DL_{CO}) measured by the single breath method using CO, and corrected for altitude and hemoglobin levels.

2.4.2. Nocturnal—Standard polysomnography studies were conducted using a 20 channel digitized PSG system (Respironics Inc. Alice 4) with wireless monitoring capabilities, a sampling rate up to 200 Hz, 50/60 Hz filters, CMRR >90 db and input impedance at least 1 M ohm. Subjects were directed to an individual sleeping room where electroencephalography (EEG) leads (central and occipital) were placed according to the international 10–20 lead placement system and used, along with electro-oculography (EOG) and chin electromyography (EMG), to score sleep stages based on the Rechtschaffen and Kales criteria (Rechtschaffen, 1968). Electrocardiography (ECG), thoracic and abdominal respiratory effort, airflow and nasal pressure, snore sensors, pulse oximetry, and leg EMG data were also recorded. A sleep technologist monitored each subject from lights “out” to lights “on”.

The numbers and duration of apneas, hypopneas, periods of prolonged hypoventilation and desaturation events were calculated from the polysomnography data. Apneas were defined as an absence of airflow at the nose and mouth >10 seconds which was classified as obstructive when there was an absence of airflow despite the presence of respiratory efforts, central when there was an absence of airflow accompanying an absence of respiratory effort, or mixed when the period of apnea consisted of an initial central part and a terminal obstructive component. Hypopneas were defined as a reduction of airflow or amplitude of thoracoabdominal movement >30% from baseline and a >4% Sa_{O2} fall of >10 seconds), and periods of prolonged hypoventilation (>50% reduction in airflow >2 minutes and a >5% fall in Sa_{O2}). The apnea-hypopnea index (AHI) was calculated as the total number of apnea and hypopnea events. Nadir Sa_{O2} (lowest Sa_{O2} recorded), sleep efficiency (i.e., the ratio of total sleep time to the total length of time spent in bed) as well as total recording time and total sleep episode times were noted. Scoring of sleep stages using established criteria and assessment of sleep-disordered breathing per unit sleep time or sleep stage were also recorded (Rechtschaffen, 1968).

2.5. Statistical Analysis

Data were checked for entry errors prior to analyses and evaluated for normality using Kolmogorov–Smirnov tests. Mean values or frequencies for ventilatory, nocturnal breathing and oxygenation characteristics were compared between EE and controls by ANOVA or chi-squared for continuous or categorical variables, or by Mann-Whitney non-parametric tests, as appropriate. For ordinal data, a measure of central tendency (i.e., the mean), the U-value, sample size and significance are reported. The contribution of ventilatory characteristics during wakefulness, sleep-disordered breathing and nocturnal hypoxic episodes for EE status was assessed using forward inclusion logistic regression models. Pearson correlation coefficients were used to determine the relationship between continuous variables (e.g., sleep-disordered breathing and hemoglobin). A two-sided p-value of <0.05 was considered to be evidence of association or difference in sample means, with p values > 0.05 and <0.10 being reported as trends. All statistical analyses were performed in SPSS (v. 19).

3. Results

3.1. Demographic and symptom characteristics (Table 1A)

By design, groups were similar in age, no subjects were obese, all had been born at high altitude, and all were lifetime high-altitude residents. Height and resting heart rate were also similar in the control and EE groups but EE subjects were heavier and had a higher body mass index than controls (Table 1A). Resting Sa_{O2} was lower in EE than control subjects during wakefulness (91.2 ± 0.6 % *versus* 89.3 ± 0.7 % for controls and EE, respectively; $p<0.05$).

An equivalent proportion of subjects were of Andean (Aymara or Quechua) ancestry (36.8% vs. 40% for controls and EE, respectively); the remainder self reported being Mestizo (i.e., Andean-European mixture). An equivalent and high proportion of subjects reported that their father and mother were also born at high altitude (father: 89.4 % *versus* 90.0% and mother, 94.7% *versus* 85.0% for controls and EE, respectively; all p=NS).

EE subjects experienced dyspnea, palpitations, and dizziness more frequently than controls. The occurrence of headache and fatigue also tended to be more frequent (Figure 1). While a higher percentage of EE subjects experienced cyanosis, difficulty sleeping, and edema than controls, differences were not significant between groups. Reported exposure to substances that may influence respiratory function (e.g., paint, sawdust), and smoking or alcohol use was equivalent between EE and control subjects (data not shown).

3.2. Hematologic and Sa_{O2} characteristics (Table 1B)

By definition, hematocrit and hemoglobin concentration, as well as erythrocyte count were markedly higher in EE than controls (all $p<0.001$; Table 1B). Erythrocyte production, as measured by reticulocyte count, was also greater in EE subjects compared to controls ($p<0.001$) despite similar EPO and sTfR values (Table 1B).

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration fell within normal limits for all subjects (70–100 fl, 27–34 pg/cell and 32–38%, respectively) and none of these variables differed between EE and controls (data not shown).

3.3. Lung function (Table 1C)

FVC, FEV1 and FEV1/FVC % were all within the normal range for all subjects and did not differ between the control and EE groups indicating the absence of overt obstructive, restrictive lung, or peripheral airway disease. FEF measurements ($FEF_{25–75\%}$, [Table 1C], $FEF_{50\%}$ and $FEF_{75\%}$) and FIF 50% (data not shown) were also the same and within normal ranges for both groups, supporting the absence of obstructive lung disease or upper airway obstructions or abnormalities. DL_{CO} corrected for altitude and hemoglobin was lower in EE subjects compared to controls, but similar between groups when expressed as a function of alveolar volume (Table 1C). As assessed by logistic regression, DL_{CO} corrected for altitude and hemoglobin was negatively associated with EE status (β: −0.06; $p<0.01$).

3.4. Sleep studies

Total sleep time, sleep efficiency and the percentage of total sleep time spent in each sleep stage were similar between control and EE subjects (Table 2).

Compared to controls EE subjects had a higher apnea-hyponea index (AHI) due primarily to an increased frequency of hypopneas and also central and obstructive apneas during REM sleep (Table 2). No differences in the frequency of apneas, hypopneas or AHI were identified between EE and controls during NREM sleep (data not shown). The total duration of obstructive apneic or hypopneic events was greater in EE than control subjects, whereas the duration of central apneic events was similar. Maximum and mean hypopnea duration were positively associated with EE status (β: +0.12 and β: +0.15, respectively; both $p<0.01$). In contrast, mixed apneas, expressed as either the number of events or their total duration, were equivalent between control and EE subjects. Figure 2 shows a typical polysomnography trace for an EE and control subject.

Nadir nocturnal Sa_{O2} values were lower in EE subjects compared to controls, and were negatively associated with EE status (β: −0.27, $p<0.05$, Table 2); the same tendency was

apparent for average nocturnal Sa_{O2}. As shown in Figure 3 there was a consistent tendency for EE subjects to have Sa_{O2} values in lower ranges during the night. Desaturation events, expressed as number per hour, did not differ between EE subjects and controls. However maximum desaturation duration was positively associated with EE status (β: +0.06; $p<0.05$).

3.5. Comparison of markers of oxidative stress between EE and control subjects

Plasma 8-iso-$PGF2_{alpha}$ levels were higher in EE than controls, and were positively associated with EE status (10.7 ± 1.1 pg·ml^{-1} *vs.* 7.3 ± 0.2 pg·ml^{-1}, β: +1.14; both $p<0.01$) (Figure 4). Individuals with higher 8-iso-$PGF2_{alpha}$ levels had lower nadir and average nocturnal Sa_{O2}, and higher hemoglobin levels (Figure 5).

Plasma SOD activity was similar between EE and control subjects (5.4 ± 1.1 U·ml^{-1} and 3.5 ± 0.6 U·ml^{-1}, respectively; Figure 4), and was not associated with 8-iso-$PGF2_{alpha}$ levels, hemoglobin, or any measure of sleep-disordered breathing. Likewise, there were no differences in total, reduced or oxidized ascorbic acid between EE subjects and controls; the same was true when data were considered as the ratio of reduced:oxidized or the percentage of oxidized ascorbic acid (Figure 4).

4. Discussion

In support of our hypothesis that sleep-disordered breathing acts as a stimulus for respiratory instability and contributes to the etiology of CMS, we found that young males with elevated hemoglobin levels and lower Sa_{O2} but normal lung function during wakefulness had more episodes of apnea and hypopnea, and mild nocturnal hypoxemia relative to age- and altitude-matched controls. Also consistent with our hypothesis that oxidative stress due to chronic intermittent hypoxia played a role in the development of respiratory instability in CMS, the oxidative stress marker 8-iso-$PGF2_{alpha}$, was elevated in EE subjects compared to controls and associated with sleep-disordered breathing and hemoglobin levels.

To address the hypothesis that sleep-disordered breathing precedes established CMS and the relationship between nocturnal hypoxia, oxidative stress, and EE status, our study had to meet several criteria. To achieve an unbiased study design, we sampled a large number of young men attending post-secondary schools in La Paz and were thereby able to generate a quasi-random sample of young men either with or without EE but matched for age, altitude of residence, ancestry, and socioeconomic status. To protect against the possibility that cases or controls were misclassified due to abnormal hemoglobin or iron status, hemoglobin values at the time of screening were confirmed on the first in-clinic visit and all subjects were assessed for iron deficiency or anemia. Moreover, to minimize the impact of learning effects, practice efforts were included for all respiratory tests and most subjects spent one complete night in the laboratory prior to recorded polysomnography. One limitation of our sleep studies is that we were unable to make direct measurements of PET_{CO2} to assess nocturnal hypoventilation and instead relied on an indirect measurement (prolonged hypoventilation [>50% reduction in airflow >2 minutes and a >5% fall in Sa_{O2}]) (reviewed in (Lee-Chiong, 2003)). With respect to assessing respiratory function, we also lacked residual volume and hence total lung volume measurements.

Previous investigations have been complicated by limitations in the ability to discriminate between the influence of age versus pathology on pulmonary and other physiologic variables thought to play an etiologic role for CMS. For this reason, one of the greatest strengths of this study is our focus on young males with a preclinical form of the disease. Our approach is based on and supported by a study conducted in Bolivia by Vargas and Spielvogel which indicated that EE precedes the onset of the constellation of symptoms that define CMS (Vargas and Spielvogel, 2006). Their study used a survey of 8200 individuals residing in La

Paz, Bolivia and its' surrounding high-altitude communities (3100–4300 m) to establish a mean hemoglobin concentration (17.5 ± 0.4 $g \cdot dl^{-1}$) for males between the ages of 15 and 35, which is close to the mean value we obtained (16.9 ± 1.1 $g \cdot dl^{-1}$); the difference likely being due to the younger age range of our subjects. Defining EE as a hemoglobin level > 18.3 $g \cdot dl^{-1}$ or two standard deviations above the mean for men in this age group (Vargas and Spielvogel, 2006), their longitudinal observations over a 4-year period in 20 young men with EE showed a progressive decline in Sa_{O2} that was paralleled by rising hemoglobin as well as an increased frequency and severity of the symptoms that collectively define CMS (Vargas and Spielvogel, 2006), leading them to conclude that EE was a preclinical form of CMS. Although prospective studies such as this are ideal to illustrate pathophysiologic processes associated with disease onset or progression, there are practical and logistic difficulties associated with their conduct. This is particularly true in developing countries such as Bolivia where, in our experience, patient follow-up over long periods of time is difficult.

Impaired respiratory control during wakefulness and sleep appears to be central to the pathophysiology of CMS. Most notably, alveolar ventilation is reduced in CMS compared to controls, and is inversely proportional to hemoglobin concentration (Vargas and Spielvogel, 2006). Impaired ventilatory sensitivity to hypoxia (HVR) (Kryger et al., 1978b; Leon-Velarde and Richalet, 2006) and hypercapnia (HCVR) (Fatemian et al., 2003; Kryger et al., 1978b) have also been reported in CMS, but are not sufficient to account for the disease given that blunted ventilatory responses are also apparent in healthy long-term high-altitude residents (Kryger et al., 1978b; Severinghaus et al., 1966; Weil et al., 1971). Periodic breathing or mild respiratory disturbance during sleep is also common in lowlanders visiting high altitudes (Nussbaumer-Ochsner et al., 2012) as well as permanent highland residents with or without CMS (Coote et al., 1993b; Coote et al., 1993a; Sun et al., 1996). However, CMS patients have more frequent and severe periods of sleep-disordered breathing, as well as lower and more variable nocturnal Sa_{O2} compared to age-matched controls (Sun et al., 1996). Spicuzza, et al., reported that Sa_{O2} levels in patients with polycythemia were between 81–85% for 50% of the night, and between 76–80% for 38% of the night (Spicuzza et al., 2004). In contrast, controls spent the majority of the night with Sa_{O2} levels >81%. Our results support these findings in that EE subjects tended to be more hypoxic compared to controls during the night as determined by nadir and average nocturnal Sa_{O2}, as well as the duration of time spent in lower Sa_{O2} ranges. Lower Sa_{O2} values in EE subjects resulted largely from a higher frequency of hypopneas, as well as central and obstructive sleep apneas during REM sleep. Notably, the degree of nocturnal hypoxia experienced by EE subjects in our study is markedly less pronounced compared to that of CMS patients reported by Sun et al., likely due to the fact that our subject population was younger and had comparatively mild polycythemia that, by definition, classified them as EE rather than CMS (Sun et al., 1996). Given that an Sa_{O2} of 80% is considered to be the threshold for the stimulation of EPO (Cohen et al., 1981) it may be that even without striking differences in the frequency of apneas, subtle nocturnal desaturation may be sufficient to reduce Sa_{O2} in individuals who are already mildly hypoxemic to levels that become clinically significant in terms of initiating an erythropoietic response. Further underscoring the importance of oxygenation during sleep for CMS, pharmacologic stimulation of ventilation in CMS patients living at 3100 m in Colorado resulted in fewer periods of rapid desaturation, lower hematocrit, and higher alveolar ventilation both at night and during wakefulness (Kryger et al., 1978a; Kryger et al., 1978c).

Chronic intermittent hypoxia (CIH) caused by sleep-disordered breathing not only reduces nocturnal Sa_{O2}, but also impairs control of breathing during wakefulness and increases hemoglobin levels in patients with established CMS (Richalet et al., 2005; Spicuzza et al., 2004). One possible explanation for the connection between abnormal oxygenation during sleep and wakefulness is that CIH elicits a greater reflex response from the peripheral

chemoreceptors (i.e., carotid bodies) compared to sustained hypoxia and impairs ventilatory control by increasing the production of reactive oxygen species during periods of episodic hypoxia and subsequent re-oxygenation, as has been described in ischemia-reperfusion literature (Prabhakar, 2001, 2002). Sensitization of the carotid bodies should theoretically increase ventilation, as illustrated by the fact that acute intermittent hypoxia (IH) exaggerates the progressive rise in minute ventilation following repeated hypoxic exposure (i.e., long-term ventilatory facilitation [vLTF]) in sleep apnea patients compared to controls (Lee et al., 2009). Notably, however, Julien et al (Julien et al., 2008) indicate that CIH during development impairs vLTF, and increases apnea frequency (Julien et al., 2008). Their findings support previous suggestions that vLTF protects against persistent apneas, in part, by increasing respiratory drive (Mitchell and Johnson, 2003). To explore the possibility that redox status was important for sleep-disordered breathing in EE, we assessed markers of oxidative stress and antioxidant capacity in EE subjects and controls. Oxidant production is notoriously difficult to measure directly given the extremely short half-life and instability of oxidant species. Based on its chemical stability and reliability as an indicator of lipid peroxidation resulting from free radical production or antioxidant insufficiency we elected to use circulating levels of 8-iso-$PGF2_{alpha}$ as our principal measure of oxidative stress. We consider that the higher 8-iso-$PGF2_{alpha}$ levels apparent in EE compared to controls may reflect oxidative stress induced by sleep-disordered breathing, and that this may, in turn, contribute to continued unstable breathing patterns during sleep, and eventually a reduction in Sa_{O2} during wakefulness. Our finding that SOD and ascorbic acid measurements were independent of EE status suggests that enhanced oxidant production rather than antioxidant insufficiency is likely responsible, in part, for higher 8-iso-$PGF2_{alpha}$ levels in EE. However, future studies could benefit from the inclusion of a larger panel of endogenous and dietary antioxidants. In support of our findings, markers of lipid peroxidation have also been reported to be elevated in individuals with established CMS relative to their healthy counterparts (Jefferson et al., 2004).

Two studies in particular suggest that resolution of the CIH induced by sleep-disordered breathing may offer novel preventive or therapeutic treatment for CMS. First, correction of CIH reduces the production of reactive oxygen species. Specifically, in patients with obstructive sleep apnea Continuous Positive Airway Pressure (CPAP) treatment reduced levels of the potent reactive oxygen species superoxide (O_2^-) and increased levels of nitric oxide (Ip et al., 2000; Schulz et al., 2000). Moreover, treatment with a O_2^- scavenger (i.e., MTPP) not only prevented the CIH-induced increase in reactive oxygen species but also facilitated long-term carotid body and respiratory motor activity (Semenza and Prabhakar, 2007), and equalized the difference in vLTF between apnea patients and controls. Another study reports that pretreatment with an antioxidant cocktail (coenzyme Q10, SOD, vitamin E, and vitamin C) reduced enhanced vLTF in patients with obstructive sleep apnea (Lee et al., 2009).

Despite normal lung function in our study population, pulmonary diffusion capacity and resting Sa_{O2} were reduced in EE subjects compared to controls. Given that subjects with lower DL_{CO} had greater maximum and mean duration of hypopneas (both $r = -0.37$, $p<0.05$), lower nadir Sa_{O2} values during sleep ($r = +0.33$, $p<0.05$) and reduced Sa_{O2} during wakefulness ($r = +0.5$, $p<0.01$), we consider it likely that an impaired efficiency of gas exchange at high altitude plays a role in decreasing Sa_{O2} in EE. This possibility is in keeping with previous work showing that decreased pulmonary gas exchange efficiency (i.e., increased circulation time and/or “loop gain” in the metabolic control of ventilation) contributes to respiratory instability in central sleep apnea (Szollosi et al., 2008). The mechanisms underlying lower DL_{CO} in EE subjects, and the degree to which this contributes to impaired control of breathing in CMS is unclear, but deserves further exploration.

Despite the public health burden imposed by CMS in highland communities, established treatment options for the condition consist only of periodic phlebotomy and descent to lower altitudes. Notably, the latter is the sole permanent and effective solution. Globally, CMS affects approximately 10 million persons, and as life expectancy increases and infant mortality declines, this figure will only increase. It is important to highlight that the regions most heavily affected by CMS are concentrated in developing countries of South America and Central Asia where economic, familial and cultural constraints not only limit migration to lower altitudes, but also access to health care for diagnosis, treatment and management of complications that accompany the condition. CMS is sufficiently debilitating as to curtail productivity, and severely impair quality of life for affected individuals and the communities in which they live. The lack of treatment options, coupled with the pervasiveness of CMS, underscores the importance of early intervention and preventative therapy. In order to move towards this goal, a shift of focus is required to emphasize the identification of early pathophysiologic processes rather than on the description of established disease.

In summary, our results present novel evidence that sleep-disordered breathing may play an etiologic role for the development of CMS. If this is the case, our findings open up the possibility that identifying and subsequently treating sleep-disordered breathing patterns in young, permanent high-altitude residents may slow or prevent the development of CMS. Further exploration of the relationship between antioxidant insufficiency (endogenous and dietary) and ventilatory instability in EE and CMS may prove valuable, given the elevated 8-iso-PGF2 $_{\text{alpha}}$ levels observed in EE and literature acknowledging that periods of episodic hypoxia, such as that resulting from sleep-disordered breathing, impairs ventilatory control by increasing the production of reactive oxygen species. Moreover, future studies should be developed to determine whether elevated markers of oxidative stress in the peripheral circulation in EE and CMS patients reflect inflammatory processes within the lung, and, if this is the case, how this influences the development of arterial hypoxemia in affected individuals. Finally, given the protracted maturation of the lung in humans, our future goals include studies designed specifically to identify whether hypoxic exposure during key developmental periods such as during intrauterine life or the perinatal period affect pulmonary development in ways that could increase susceptibility to CMS in later life.

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Highlights

- we hypothesized that sleep-disordered breathing and, as a result, greater oxidative stress, contribute to the development of chronic mountain sickness (CMS)
- mild sleep-disordered breathing and oxidative stress are evident prior to established CMS
- this suggests that the resolution of nocturnal hypoxemia or antioxidant treatment may prevent disease progression and help reduce the significant disability and mortality associated with CMS

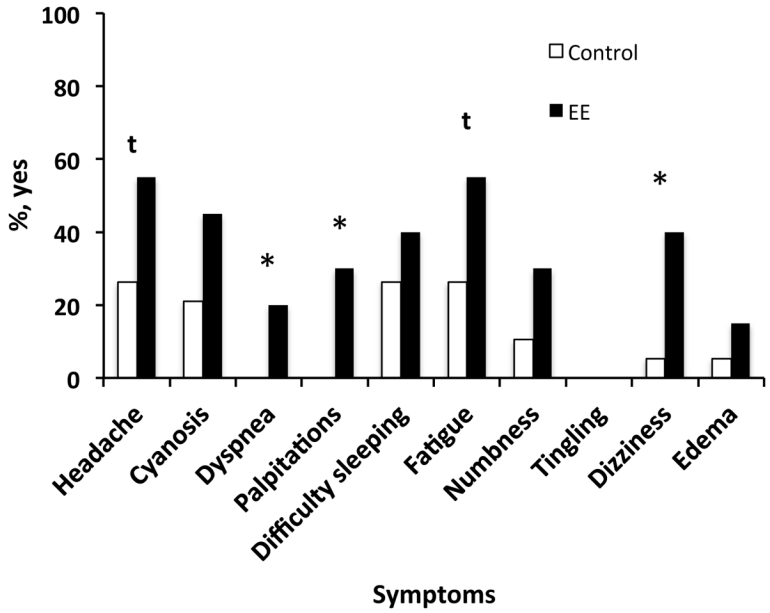

Figure 1.
CMS symptom frequency. Reports of dyspnea, palpitations, and dizziness were more frequent in EE subjects than controls (* $p<0.05$); the same tendency was apparent for headache and fatigue (t, $p<0.10$).

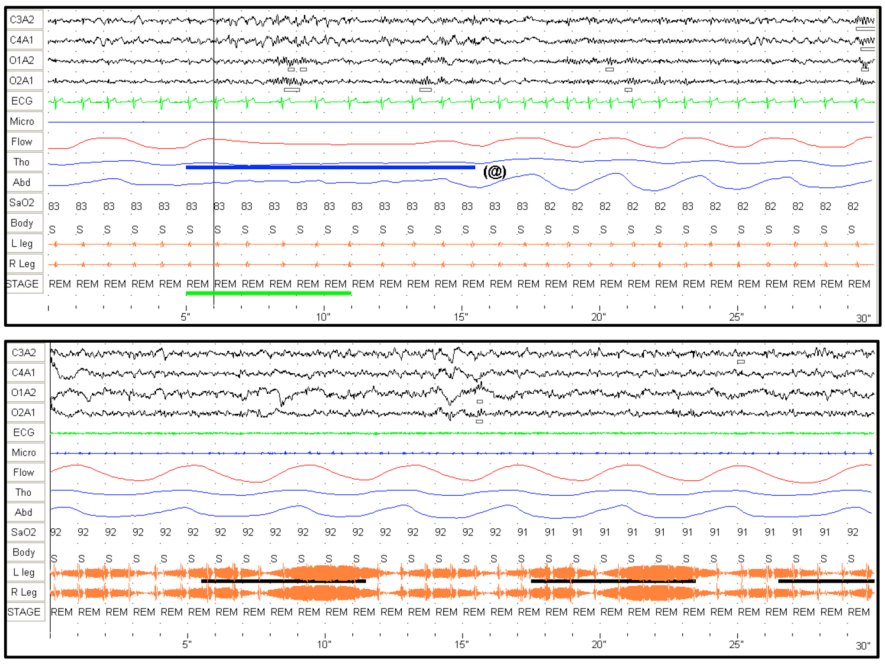

Figure 2.

Example trace obtained from polysomnography studies in EE (top panel) and control subjects (bottom panel). The solid line marked with the @ symbol in the EE trace indicates a period of absent thoracic movement and air flow.

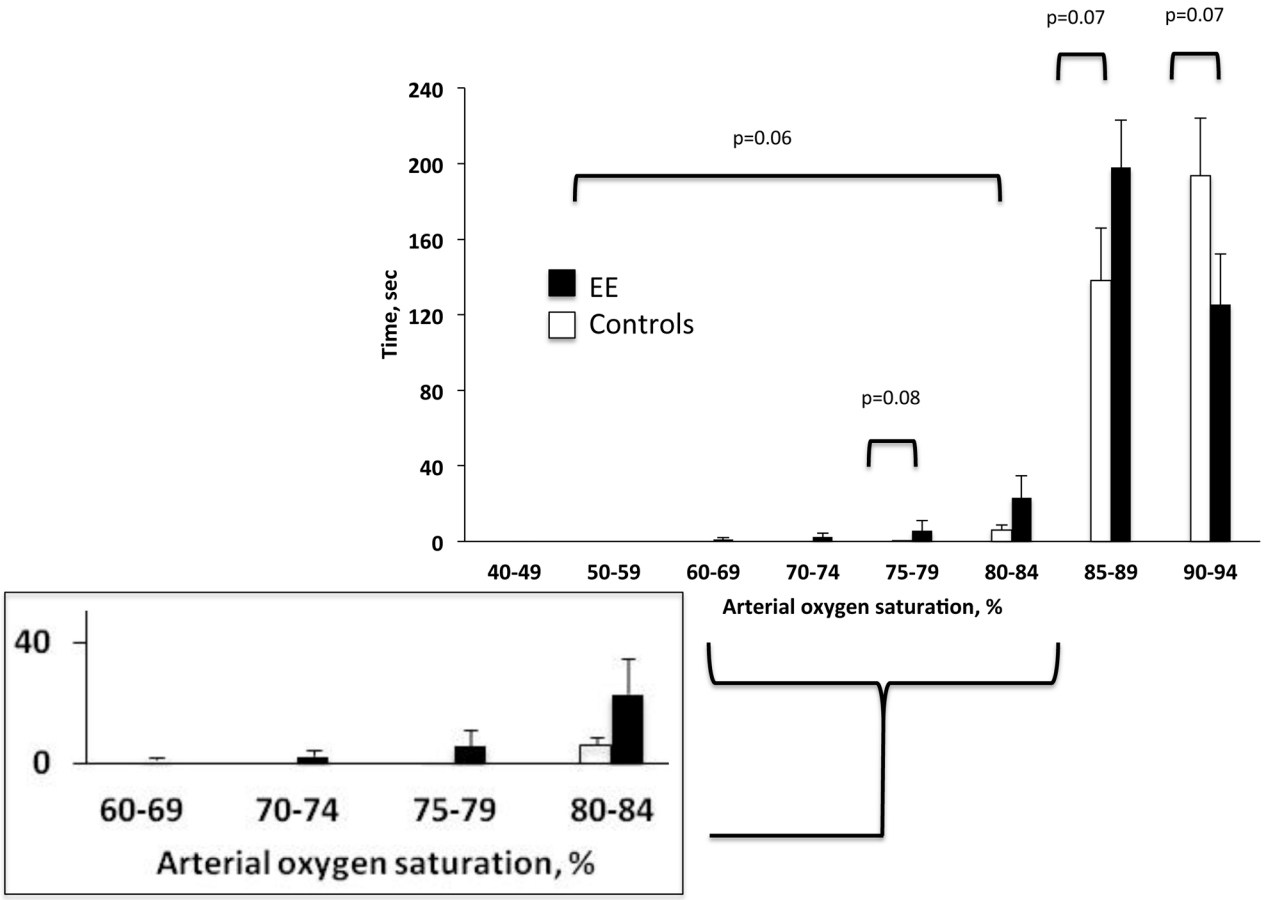

Figure 3.

Nocturnal arterial oxygen saturation (Sa_{O2}) distribution for control (white bar) and EE subjects (black bar). Compared to controls, EE subjects tended to spend more time within lower Sa_{O2} ranges during the night. Specifically, EE subjects spent less time with Sa_{O2} values between 90–94% (p=0.07), and more time with Sa_{O2} values between 70 and 74% (p=0.08), 85–89% as well as < 80%. Data are shown as the mean for each group ± SEM.

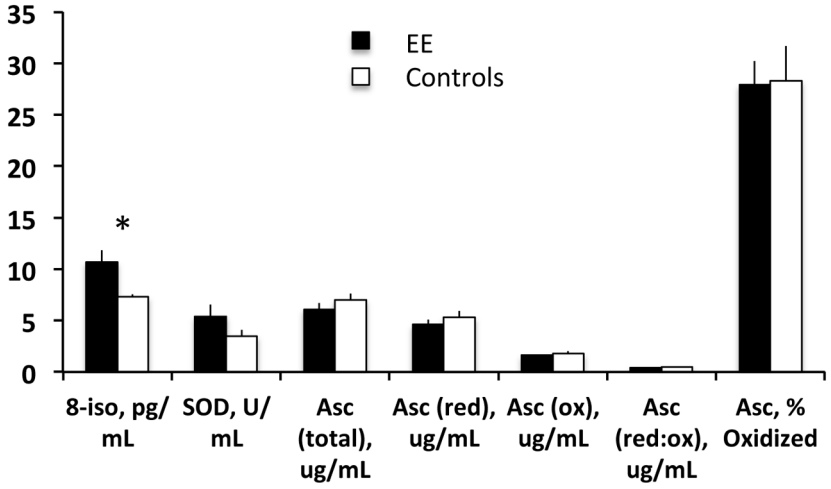

Figure 4.

Markers of oxidative stress and antioxidant capacity in EE subjects and controls. Plasma 8-iso-$PGF2_{alpha}$ levels were greater in EE subjects compared to controls (10.7 ± 1.1 pg/ml *vs.* 7.3 ± 0.2 pg/ml, respectively; $p<0.01$), however both antioxidant status as indicated by superoxide dismutase activity and oxidized ascorbate levels were similar independent of EE status.

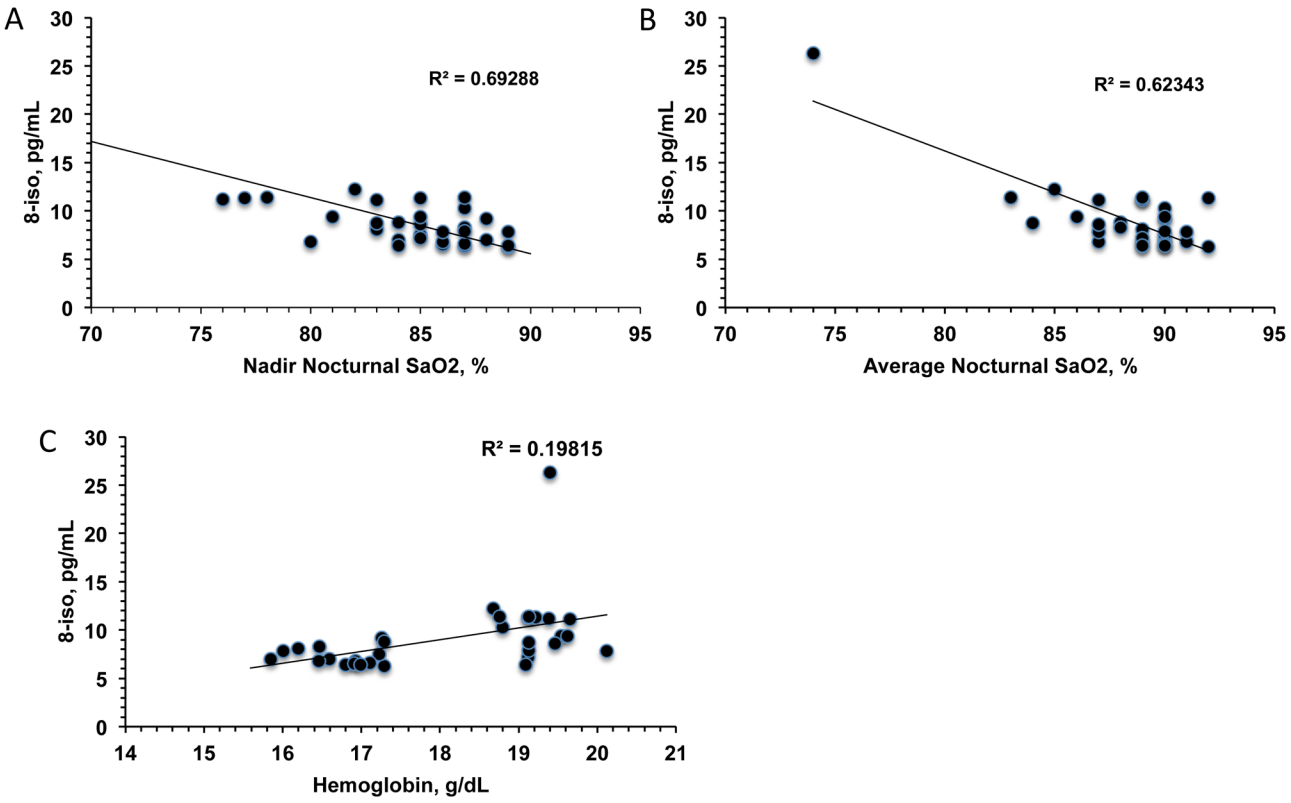

Figure 5.

Relationship between 8-iso-PGF2$_{alpha}$ (A), Sa_{O2} (B), and hemoglobin (C). Among all subjects included for study 8-iso-PGF2$_{alpha}$, a marker of lipid peroxidation is negatively associated with nocturnal Sa_{O2} (nadir [A]) or average Sa_{O2} [B]), but positively associated with hemoglobin concentration. After removing the one individual with the highest 8-iso-PGF2$_{alpha}$ levels R^2 values still demonstrate a similar relationship to average Sa_{O2} and hemoglobin (R^2 = 0.14 and 0.34, respectively).

Table 1

Subject characteristics and hematologic data

1A. Subject characteristics

	EE status		
Variable	**Control (n=19)**	**EE (n=20)**	**p-value**
Demographics			
Age, years	21.5 ± 0.5	22.4 ± 0.6	NS
Weight, kg	61.7 ± 1.6	67.1 ± 1.4	<0.05
BMI, kg/m^2	22.2 ± 0.6	24.0 ± 0.4	<0.05
Height, cm	166.8 ± 1.6	167.0 ± 0.8	NS
Heart rate (rest), bpm	58.1 ± 2.2	64.5 ± 2.7	NS
Oxygen saturation, %	91.2 ± 0.6	89.3 ± 0.7	<0.05

1B. Hematologic data

	EE status		
Variable	**Control (n=19)**	**EE (n=20)**	**p-value**
Hematocrit, %	50.5 ± 0.4	57.5 ± 1.6	<0.001
Hemoglobin, g/dl	16.6 ± 0.1	19.3 ± 0.1	<0.001
Erythrocytes, mm^3 (million)	5.5 ± 0.0	6.4 ± 0.1	<0.001
Reticulocytes, mm^3 (thousands)	181.8 ± 2.8	247.1 ± 2.9	<0.001
EPO, mIU/ml	8.7± 1.1	9.3 ± 1.4	NS
sTfR, pg/ml	19.6 ± 0.9	17.6 ± 1.1	NS

1C. Lung function

	EE status		
Variable	**Control (n=19)**	**EE (n=20)**	**p-value**
FVC, L	5.5 ± 0.2	5.4 ± 0.2	NS
FEV1, L	4.9 ± 0.2	4.7 ± 0.2	NS
FEV1/FVC, %	88.9 ± 1.0	86.4 ± 1.3	NS
FEF 25–75, %	124.5 ± 5.4	112.9 ± 8.9	NS
DL_{CO} *, ml/min/mmHg, %	148.1 ± 6.2	127.4 ± 3.9	<0.01
DL_{CO}/VA, 1/min/mmHg, %	235.5 ± 8.7	251.4 ± 11.0	NS

Values are reported as means ± SEM. Abbreviations: body mass index (BMI); erythropoetin (EPO); soluble transferrin receptor (sTfR); forced vital capacity (FVC); forced expiratory volume (FEV); forced expiratory flow (FEF); lung diffusion capacity (DL_{CO});

* corrected for altitude and hemoglobin.

Table 2

Nocturnal breathing patterns and sleep structure

Variable	EE status		Mann-Whitney	
Sleep structure	**Control (n= 19)**	**EE (n=20)**	**U-value**	**p-value**
Total sleep time (TST), min	330.2 ± 31.4	345.9 ± 21.0	170.0	NS
Sleep efficiency	86.1 ± 1.7	84.5 ± 1.2	156.0	NS
Stage I sleep, % TST	6.0 ± 1.2	4.6 ± 0.7	175.0	NS
Stage II sleep, % TST	59.2 ± 1.7	60.4 ± 2.1	160.5	NS
Stage III or IV sleep, % TST	17.1 ± 2.0	14.4 ± 1.6	158.0	NS
REM, % TST	17.6 ± 1.8	19.1 ± 1.8	159.0	NS
Nocturnal breathing patterns				
Total # of events (AHI)	2.2 ± 0.8	8.6 ± 3.2	108.5	<0.
Hypopneas, REM # of episodes	0.3 ± 0.2	2.9 ± 1.6	132.5	<0.
Duration, total	0.3 ± 0.1	1.2 ± 0.6	102.5	<0.
% TST	0.1 ± 0.0	0.4 ± 0.2	98.0	<0.
Central apneas, REM # of episodes	0.2 ± 0.1	1.0 ± 0.3	126.5	<0.
Duration, total	0.4 ± 0.1	0.3 ± 0.8	174.0	NS
% TST	0.1 ± 0.0	0.1 ± 0.0	183.0	NS
Obstructive apneas, REM # of episodes	0.0 ± 0.0	0.8 ± 0.5	152.0	<0.
Duration, total	0.0 ± 0.0	0.9 ± 0.5	138.0	<0.
% TST	0.0 ± 0.0	0.2 ± 0.1	147.0	NS
Mixed apneas, REM # of episodes	0.0 ± 0.0	0.0 ± 0.0	190.0	NS
Duration, total	0.1 ± 0.0	0.0 ± 0.0	166.0	NS
% TST	0.0 ± 0.0	0.0 ± 0.0	159.0	NS
Nocturnal oxygenation				
Nadir $Sa_{O2,}$ %	86.1 ± 0.6	82.4 ± 1.4	103.5	<0.
Average $Sa_{O2,}$ %	89.6 ± 0.4	87.6 ± 0.9	125.5	NS