

Cardiac autonomic activity during sleep in high altitude resident children compared to lowland residents

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For Review Only

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3 **Title: Cardiac autonomic activity during sleep in high altitude resident children**
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5 **compared to lowland residents.**
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45 **Running title: Sleep and heart rate variability in high altitude children**
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Abstract

Study Objectives: We aimed to characterize heart rate variability (HRV) during sleep in Andean children native to high altitude (HA) compared to age, gender and genetic ancestry-similar low altitude (LA) children. We hypothesized that the hypoxic burden of sleep at HA could induce variation in HRV. As children have otherwise healthy cardiovascular systems, such alterations could provide early markers of later cardiovascular disease.

Methods: Twenty-six LA (14F) and 18 HA (8F) children underwent a single night of attended polysomnography. Sleep parameters and HRV indices were measured. Linear mixed models were used to assess HRV differences across sleep stage and altitude group.

Results: All children showed marked fluctuations in HRV parameters across sleep stages, with higher vagal activity during NREM sleep and greater variability of the heart rate during REM. Moreover, HA children showed higher very low frequency HRV in REM sleep and, after adjusting for heart rate, higher low to high frequency ratio in REM sleep compared to children living at lower altitude.

Conclusion: We confirmed previous findings of a stage-dependent modulation of HRV in Andean children living at both high and low altitudes. Moreover, we showed subtle alteration of HRV in sleep in HA children, with intriguing differences in the very low frequency domain during REM sleep. Whether these differences are the results of an adaptation to high altitude living, or an indirect effect of differences oxyhaemoglobin saturation remain unclear, and further research is required to address these questions.

Keywords: apnea, autonomic nervous system, children, high altitude, hypobaric hypoxia, heart rate variability, REM sleep, very low frequency power.

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3 **Statement of Significance.** People living in the high Andes have reduced life expectancy compared
4 to lowlanders. Sleep related hypoxia is a putative cause, with morbidity mediated by sympathetic
5 nervous system activation. We report heart rate variability, a measure of autonomic regulation, during
6 sleep in high and low altitude dwelling children. Differences in heart rate variability spectral power
7 between sleep stages support previous research. Novel findings in REM sleep at high altitude of
8 greater very low frequency power and higher low to high frequency ratio (adjusting for heart rate)
9 may be a marker of exposure to 'hidden' hypoxia. Exposure to chronic intermittent hypoxia in high
10 altitude dwelling children could recalibrate the autonomic nervous system underpinning reduced life
11 expectancy in this population.
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Introduction

Heart rate variability (HRV), the beat-beat variation in heart rate identified from the R-R interval recorded on an electrocardiogram (ECG), reflects cardiac autonomic regulation. Power spectral density analysis generates high-frequency (HF), low-frequency (LF) and very low frequency (VLF) components (0.15 – 0.4 Hz; 0.04 – 0.15 Hz and 0 - 0.04 Hz respectively). HF power is understood to reflect parasympathetic activity. LF power, while less well understood, is likely to reflect both aspects of the autonomic nervous system¹. The meaning of VLF is still debated, but it has been suggested that it may reflect the function of the renin-angiotensin system and/or thermoregulatory processes². A chronic imbalance of the autonomic nervous system, characterized by an overactive sympathetic drive and correspondingly under-active parasympathetic drive, is a risk factor for cardiovascular morbidity and mortality³. This is potentially relevant to populations living at high altitude (HA), that is at 2500m or more above sea-level, who are exposed to chronic hypobaric hypoxia. Hypoxia is a potent stimulus of the sympathetic nervous system and healthy mountaineers exposed to HA experience an increase in sympathetic output⁴ leading to increased heart rate, systemic vascular resistance, blood pressure, and metabolic rate.

Over 140 million people permanently live at HA and unlike mountaineers have adapted to their hypoxic environment. However, people living in the high Andes may be poorly adapted to chronic hypoxia due to steady influx of non-adapted lowlanders over time facilitated by the Andean topography, that connects high plains to low lying land. Colonization by Europeans following the Spanish conquest 400 years ago⁵ resulted in further mixing of the gene pool with contemporary populations displaying significant European genetic admixture. Chronic mountain sickness (CMS)⁶, characterized by excessive erythropoiesis, severe hypoxemia and early death^{7,8}, affects around 15% of the older HA population and may be a hallmark of incomplete adaptation to hypobaric hypoxia in this population. Of note, Andean adults with CMS have higher noradrenaline levels as well as higher sympatho-vagal tone on HRV measures compared to controls at the same altitude⁹. Furthermore, a correlation between severity of CMS and decreased baroreflex suggests that alteration in the autonomic nervous system may be a biomarker of CMS.

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3 We have reported that Andean HA native children experience additional hypoxic challenge
4 during sleep compared to the waking state¹⁰. This intermittent hypoxic ‘load’ during sleep could, in
5 theory, mimic the chronic intermittent hypoxia seen in conditions such as obstructive sleep apnea,
6 leading to increased sympathetic tone^{11,12} and, in turn, increased the risk of hypertension, stroke,
7 myocardial infarction^{13,14} and, possibly, CMS.

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12 Heart rate variability has a circadian pattern¹⁵⁻¹⁷ and there are marked differences in power spectral
13 components between the waking state, Rapid Eye Movement (REM) and Non-REM sleep stages¹⁸⁻²¹
14 in both adults and children. Specifically, during sleep, cardiac autonomic activity is characterized by
15 altered sympatho-vagal balance with higher normalized high frequency power (indicating a
16 predominant parasympathetic drive) during NREM stages compared to wakefulness and a reduction
17 in REM sleep^{22,23}. Also, the total power, which represents the total variability of the heart rate, shows
18 a dramatic reduction during N3 and a strong increase (often higher than wakefulness) in REM sleep.
19 This reliable pattern of autonomic fluctuations during sleep, which has been observed during both
20 nighttime and daytime sleeping periods^{19,24,25}, coupled with the advantage that confounding
21 environmental stimuli are minimised (e.g., movements are reduced) and that the ‘hypoxic load’ is
22 maximal during sleep, make sleep the optimal context in which to investigate the cardiac autonomic
23 activity of individuals living in different altitudes. We aimed to characterize heart rate variability
24 during sleep in Andean children native to HA and children of similar age and genetic ethnic ancestry
25 native to lowlands. We hypothesized that any subtle increase in sympathetic nervous system tone
26 between altitude populations would be more apparent during REM sleep when parasympathetic drive
27 is lowered.

28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 **Methods**

47 48 **Subjects**

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50 As part of a larger study (Development and Sleep at Altitude - DeSAT) investigating the
51 effects of altitude on sleep disordered breathing²⁶ and neurocognitive performance²⁷, 59 children and
52 adolescents from low altitude (LA) Santa Cruz, 500m above sea-level ($n_L=32$) and La Paz, 3700m
53 above sea-level ($n_H=27$), Bolivia underwent a single night of attended polysomnography. Subjects

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3 were aged from 7.00 to 17.15 years. Inclusion criteria required participants to have been born at term,
4 have no chronic health conditions (including no history of known obstructive sleep apnea), learning
5 difficulty or developmental disorder and have been born and lived at their resident altitude for at least
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7 five years prior to participation. The study was approved by the institutional ethics committees of the
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9 University of Western Australia and the Universidad Privada de Santa Cruz de la Sierra, Bolivia.
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14 **Measures**

15 *Socio-demographic data.*

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17 Data were collected for mothers of participant children on age of completion of full-time
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19 education.
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22 *Physical examination*

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24 All children underwent a detailed, physical examination supervised by a pediatric
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26 somnologist (CMH) or otolaryngologist (KH) including: cardiorespiratory examination, resting blood
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28 pressure (Microlife, Zurich) and height and weight. Body mass index (BMI) centiles were derived
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30 from standard Control and Prevention growth charts.
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32 *Genetics*

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34 DNA were extracted from saliva samples (Western Australia DNA Bank, University of
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36 Western Australia) and the whole gene amplified (K BioSciences, Hoddesdon, UK). Individual
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38 European, Native American, and African admixture proportions were estimated using a panel of 28
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40 ancestry informative markers (AIMs) previously noted to demonstrate high-frequency differences in
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42 allele frequency between these different ancestry groups. The admixture modeling program
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44 admixmap²⁸ was used to model the distribution of admixture in the cohort
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46 (<http://homepages.ed.ac.uk/pmckeigu/admixmap/index.html>) and to generate individual ancestry
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48 estimates. AIM ancestry-specific allele frequencies were estimated from their reported counts in
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50 modern European, African, and Native American populations^{26,27}.
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52 *Polysomnography*

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54 Attended polysomnography was carried out in an established sleep laboratory setting (Santa
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56 Cruz) and in a temporarily adapted facility (La Paz) using computerized ambulatory systems
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3 (Compumedics PS2 system, Melbourne, Australia) according to accepted guidelines²⁹. All studies
4 were performed by an experienced polysomnographic technologist (AC). A standard ambulatory sleep
5 montage included electroencephalography (C3/A2, C4/A1) with electrode placement according to the
6 international 10-20 system³⁰, electromyography at submental and anterior tibialis, bipolar
7 electrooculography, electrocardiography and oxyhaemoglobin saturation monitoring (SpO₂).
8 Respiratory inductance plethysmography bands were used to measure abdominal and thoracic
9 excursions and nasal thermistors provided a constant flow monitor. Polysomnographs were scored
10 (AC) based on the established sleep staging³¹ and respiratory³² criteria for paediatrics and all studies
11 were peer reviewed (CMH). All PSG included continuous ECG recording and standard
12 neurophysiological sleep stage scoring based on EEG, EOG, and EMG. Only stages N2, N3, and
13 REM sleep are reported in this report as N1 is a transition state comprising less than 5% of the night.
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27 **Data Pre-processing**

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29 All polysomnography studies were initially visually assessed to ensure presence of adequate
30 quality ECG and the nasal airflow signals for a minimum of 4 hours of the overnight recording.
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33 Heart-beats were automatically detected in the ECG, sampled at 256 Hz. R-waves were
34 detected after band-pass filtering (0.9-36 Hz, 349th order FIR filter, applied in the forward and reverse
35 direction to avoid phase-shift) using a peak-detector, and then visually checked. Segments of signal
36 where no clear R-waves could be identified were marked as bad data and removed from further
37 analysis (marked as gaps in the data). Automatically identified R-waves were replaced by nearby
38 peaks when deemed incorrect, based on regularity of RR intervals and the expected shape of the QRS
39 complex. The resulting RR-interval time-series was then linearly interpolated to obtain a regularly
40 sampled signal with a sampling rate of 5 Hz. This signal was then high-pass filtered (in the forward
41 and reverse directions) at 0.003 Hz using a first-order Butterworth filter (applied in the forward and
42 reverse directions) to remove its DC component and slow trends.
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53 To account for any potential influence of respiration on HRV, all polysomnograms were
54 automatically inspected for abnormal respiration. For regular (normal) respiratory cycle detection, the
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3 thermistor generated nasal flow signal was first band-pass filtered between 0.08 and 0.8 Hz using a 5th
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5 order Butterworth filter to remove the DC component of signal and slow trends and avoid spectral
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7 components which are well above the average fundamental frequency of respiration in children (0.23
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9 Hz to 0.36 Hz in children from 4 to 16³³ . Local maxima and minima of the flow signal corresponding
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11 to the start and end of expiration/inspiration were identified separately using envelope detectors. The
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13 initial sets of extrema detected were refined using amplitude thresholds. This was to avoid peaks and
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15 trough with undesirable amplitudes (e.g. a local peak with negative amplitude and troughs with
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17 positive amplitudes) and ensure normal respiratory cycle detection. Duration thresholds were then
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19 applied to the refined extrema to eliminate those which are abnormally close or distant from one
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21 another. Undergoing this procedure resulted in regular (normal) respiratory cycle detection and hence
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23 regular respiratory rate quantification. Finally, the editing on both respiratory and ECG signals was
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25 combined (logic AND operator) to produce a final edit that ensured that the HRV parameters were
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27 extracted from portions of polysomnograms with both normal cardiac patterns and regular respiration.
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30 *HRV calculation*

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33 Heart rate variability was calculated from the previously processed RR interval signal using
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35 the Welch power spectral density estimation method (rectangular window of 100 seconds, 50%
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37 overlap). The Welch Power Spectral Density (PSD) estimation operator was then applied to the
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39 filtered RR signal to produce a PSD. Very low frequency (VLF), LF and HF powers were then
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41 calculated as the area under the PSD curve from 0 - 0.04 Hz, 0.04 – 0.15 Hz and 0.15 – 0.4 Hz
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43 respectively. Total power was calculated as the sum of all three powers. Normalized HF power was
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45 calculated by dividing the power in each band by the sum of LF and HF power, in accordance with
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47 recommendations³⁴. For all the selected subjects, at least 5 minutes (300s) of continuous RR interval
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49 data were available for each sleep stage, other than for REM where 2 participants (one LA and one
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51 HA) had 226s and 295s of recording respectively. For wake recordings 7 participants (5 at LA and 2
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53 at HA) had less than 5 minutes recording – minimum 135seconds. (see Table 1).
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Variables were generated for each sleep stage N2, N3 and REM as well as for pre-sleep wakefulness, as follows:

- Average RR intervals (RR; ms, intervals between consecutive R-peaks)
- Total power (ms^2)
- Very low frequency (VLF, ms^2) power
- Low frequency (LF, ms^2) power
- High frequency (HF, ms^2) power and normalised HF power [HFnu: $\text{HF}/(\text{LF}+\text{HF})$]
- LF/HF ratio
- Respiratory interval (s)

Note that normalised LF power [$\text{LF}/(\text{LF}+\text{HF})$] are not reported since this variable is the complement of normalized HF (normalized LF = 1 - normalized HF). A similar redundancy also exists with LF/HF³⁵, but due to the nonlinear relationship between HF or LF normalized with LF/HF, discrepancies in statistical testing may arise. Nevertheless, we decided to report both HFnu and LF/HF for descriptive purposes.

Statistical methods

Data were checked for normality using the Shapiro Wilk test. If they were not suitable for parametric descriptive and inferential statistics, non-parametric analyses were used, where available. The HRV frequency-domain parameters (total power, VLF, LF, and HF) were transformed using a natural logarithm before statistical analysis. LF/HF ratio was computed on log-transformed LF and HF data. Between-altitude differences were analyzed using Mann Whitney U or using t-tests and chi-square tests, as appropriate. To investigate differences in sleep architecture, we ran several ANCOVA with sleep parameters as the dependent variable, altitude group as independent variable and age as a covariate. To assess the cardiac autonomic pattern across sleep stages in the two groups, we employed linear mixed models (LMM), which permit analysis of an unbalanced number of observations per participant. Moreover, LMM allow the influence of factors whose levels are randomly extracted from a population (i.e., participants) to be taken into account, thus yielding more generalizable results³⁶.

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3 For each HRV parameter, we built a separate model, using *Participant* as crossed random effects and
4 *Stage* (Wake, N2, N3, REM) and *Group* (LA, HA) as fixed effects. Bonferroni's test was used for
5 post-hoc comparisons.
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10 **Results**

12 *Sample*

15 Of the 59 children and adolescents who had an overnight polysomnographic study, 5 were
16 excluded as they failed to meet inclusion criteria and 10 were excluded as ECG failed to meet quality
17 criteria throughout the study, leaving a sample size of 44: 26 living at LA (12 male) and 18 living at
18 HA (10 male).
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25 *Demographic and daytime systolic blood pressure*

27 Table 1 shows the main demographic and clinical characteristics of the sample. There was no
28 significant difference in age ($t_{42}=-1.69$, $p=.10$), gender distribution ($\chi^2=0.38$, $p=.54$), or genetic
29 ancestry differences of children between altitude locations. Daytime resting systolic blood pressure
30 was significantly higher (Table 1) at high altitude ($t_{42}=2.73$, $p=.009$). However, blood pressure is
31 influenced by age, gender and height centile during childhood. Given the absence of normative data
32 for this population the contribution of altitude to blood pressure was explored through regression
33 analysis. Controlling for age and height, altitude contributed significantly to systolic blood pressure
34 ($r^2=.13$, $p=.018$) but BMI may be a partial mediator of the effect of altitude on BP as this altitude
35 difference was only at trend level when BMI was controlled for ($r^2=.07$, $p=.059$).
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47 *Polysomnographic results*

49 We observed some differences in sleep architecture between HA and LA children (Table 2).
50 The ANCOVAs showed that HA children spent more time in N2 (%) than LA children ($F(1,41)=$
51 4.740 , $p=.035$), to the detriment of N3 ($F(1,41)= 4.013$, $p=.052$). No other significant differences were
52 observed in sleep architecture. The ANCOVAs also highlighted the expected differences in mean
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3 nocturnal oxyhaemoglobin saturation (SpO_2) and respiratory polysomnographic variables at HA.
4 Specifically, HA children had lower mean overnight SpO_2 levels ($F(1,41)=383.61, p<.001$), higher 3%
5 desaturation index ($F(1,38)=19.63, p<.001$) and higher hypopnea index ($F(1,41)=8.455, p=.006$),
6 compared to LA children. (see Table 2 and see Hill et al., 2016 for discussion of these results).
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10 11 12 *Cardiac Autonomic Activity across Sleep Stages and altitude settings*

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14 Descriptive values for all the cardiac parameters across the different stages are presented in
15 Table 3. Note that we have a limited number of participants with data available from pre-sleep
16 wakefulness as some children fell asleep during, or shortly after, the polysomnography set up before
17 quality data acquisition was established. Nevertheless, our linear mixed model analysis takes into
18 account missing data and provides a corrected estimate of the mean and standard error of the Wake
19 period
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26 Summary results for RR interval analysis are shown in Fig. 1. LMM on RR showed a main
27 effect of sleep stage ($F(3,100.29)=23.504, p<.001$), with a significant lengthening of RR during N2,
28 N3 and REM sleep compared to Wake (all $p's<.001$), and a shortening of RR in REM compared to
29 N2 ($p=.008$). We also observed a Group \times Sleep Stage interaction ($F(3, 100.29)=3.60, p=.016$), with a
30 nominal lengthening of RR during Wake and REM sleep in HA compared to LA, but these contrasts
31 did not reach significance ($p=.096$ and $p=.133$, respectively, Figure 1a).
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38 Analysis of the respiratory intervals showed only a main effect of sleep stage
39 ($F(3,101.25)=48.36, p<.0001$), with shorter respiratory intervals in Wake compared to all sleep stages
40 (all $p's<.0001$), and a reduction of respiratory intervals from N2 to N3 ($p=.011$; Figure 1b). No effect
41 of altitude was observed.
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46 A similar result was observed for total power, with a main effect of sleep stage ($F(3,$
47 $100.41)=12.520, p<.001$), with a significant total power reduction during N3 relative to N2 ($p=.001$),
48 and increased total power during REM sleep relative to W ($p<.001$), N2 ($p=.019$), and N3 ($p<.001$),
49 indicating an increase in heart rate variability during REM sleep. We also observed increased total
50 power during N2 relative to W ($p=.026$). We also found a trend for Group \times Stage interaction ($F(3,$
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3 100.41)=2.923, $p=.056$), with a nominal power increase during Wake and REM in HA compared to
4 LA, but these contrasts did not reach the significance ($p=.279$ and $p=.317$, respectively, Figure 2a).

5
6 The VLF showed a significant main effect of sleep stage $F(3, 101.31)=45.702$, $p<.001$), with
7 a marked power reduction during N3 compared to the other stages (all p 's<.1. 0001). We also
8 observed a nominal greater VLF in the HA compared to the LA group, but this effect did not reach
9 statistical significance ($F(1, 42.678)=2.329$, $p=.134$). Although the interaction Group \times Stage was not
10 significant ($F(3, 101.35)=1.466$, $p=.228$), we observed an higher VLF during REM sleep in the HA
11 group ($p = .026$; Figure 2b).

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13 A similar main effect of sleep stage was found in LF $F(3,101.22)=22.562$, $p<.001$), with
14 increased LF during REM sleep relative to Wake, N2 and N3 ($p=.002$, $p=.009$, and $p<.001$
15 respectively), and a trend-level reduction of LF in N3 compared to N2 ($p<.001$; Figure 2c). No effect
16 of altitude was observed.

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18 Similarly, we found a significant effect of sleep ($F(3,100.36)=15.111$, $p<.001$) for HF, with
19 increased vagal activity in all sleep stages compared to Wake (all p 's<.001), and increased power in
20 N2 compared to REM ($p=.040$, Figure 2d). Again, there were no effects of altitude.

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22 A main effect of sleep stage on HFnu ($F(3,101.25)=42.969$, $p<.001$) with a marked increase
23 during N3 compared to W ($p<.0001$), N2 ($p=.007$), and REM sleep ($p<.001$; Figure 2e), indicating a
24 strong vagal modulation of N3. HFnu was also higher in LA compared to HA, but this comparison did
25 not reach a significant level ($F(1,42.72)=2.254$, $p=.141$).

26
27 Lastly, the LF/HF was different across sleep stages ($F(3, 101.61)=34.30$, $p<.001$), reflecting
28 marked reduction during N2 and N3 compared to W and REM (all p 's <.001; Figure 2f). LF/HF was
29 also higher in N2 compared to N3 ($p=.019$). Nominally, LF/HF ratio was higher in HA compared to
30 LA, but this comparison did not reach a significant level ($F(1, 42.92)=2.689$, $p=.112$).

31
32 As we observed an increased RR interval in all sleep stages in the HA group (possibly related
33 to the slightly older age of this group), and it has previously been shown that there is a strong
34 dependency of frequency-domain parameters on heart rate³⁷⁻³⁹, we repeated the analysis described
35 above including the mean RR interval for each individual and each sleep period as covariates in the
36 models, using raw values for each HRV parameters. We also repeated the analysis on RR intervals
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3 including age as a covariate. The direction of the results was similar even with this correction (see full
4 analysis in the supplemental material). The previous trend level for total power, Group \times Stage
5 interaction was lost ($F(3, 100.88)=.553, p=.647$), while conversely the Group effect of LF/HF,
6 became significant ($F(1,42.73)=5.05, p=.030$), showing higher LF/HF ratio at HA compared to LA.
7
8 Importantly, although the main Group effect for VLF was trending, but still not significant ($F(1,$
9 38.77)=3.68, $p=.062$), the greater VLF power during REM sleep in the HA group remained significant
10 (p = .006).
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18 Discussion

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22 We aimed to investigate cardiac autonomic activity during sleep in Andean children native to
23 high altitude (HA) compared to age, gender and genetic ancestry-similar low altitude (LA) children.
24 We hypothesized that the hypoxic burden of sleep at HA could provoke increased sympathetic
25 activity. Furthermore, altitude differences in REM sleep, with characteristic high sympatho-vagal
26 tone, could reveal subtle changes in autonomic regulation. As children have otherwise healthy
27 cardiovascular systems, such alterations could provide early markers of later cardiovascular disease.
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34 Regardless of altitude, children showed marked fluctuations in HRV parameters across sleep
35 stages consistent with previous research^{19,20,24} indicating that sleep-dependent modulation of
36 autonomic activity is reliable across altitudes. Specifically, mean RR intervals lengthened during all
37 sleep stages compared to wake, with a peak during N2 sleep and a shortening during REM sleep. On
38 the other hand, total power decreased during N3 and increased dramatically during REM sleep,
39 mainly driven by LF power, which showed a marked decrease in N3 followed by a strong increase in
40 REM. The LF fluctuations also influenced the LF/HF ratio. Indeed, LF/HF was also at its lowest
41 during N3 sleep, consistently with previous reports⁴⁰.
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50 Although we filtered our data to exclude periods of irregular breathing, it is possible that
51 respiration might have had a confounding influence⁴¹ on HRV indices, when respiratory rate
52 approaches the boundary between the LF and HF band (0.15 Hz). Respiratory rate is generally higher
53 in children than adults⁴², and our participants exhibited a respiration cycle length of 3.60s to 3.80s
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3 across sleep stages. With this range (0.26-0.28 Hz), therefore, confounding in the interpretation of HF
4 during sleep is not expected to occur⁴³. Nonetheless, we cannot completely exclude the possibility
5 that HA may have altered cardiopulmonary coupling due to hypopnea episodes, but this was
6 minimized by only including periods of regular breathing in the analysis. This could be assessed in the
7 future using coherence or cross-power analysis⁴⁴.

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12 Our principle aim was to explore altitude related differences in HRV during sleep. We report
13 for the first time a nominally higher LF/HF ratio across sleep stages in children living at HA
14 compared to LA after adjusting for R-R interval. Post-hoc comparisons showed that this was
15 significantly higher in REM sleep. This finding should be interpreted with caution. While historically
16 this would have been interpreted as representing an increase in cardiac sympathetic activation in REM
17 sleep at HA⁴⁵ this assumption has been challenged⁴⁶. Experimental studies show that LF power
18 correlates poorly with cardiac sympathetic nerve activation and that the relationship between
19 sympathetic and parasympathetic activity is non-linear⁴⁶. Some authors have proposed that LF may
20 represent the vagally-mediated function of the baroreflex⁴⁷⁻⁴⁹. HA children in this study had higher
21 age and height adjusted daytime blood pressure than LA children. It is possible that higher LF/HF
22 ratio may reflect a greater baroreflex adaptive response to the increased blood pressure that is
23 generally observed during REM sleep⁵⁰. Future study of autonomic function in sleep, using non-
24 invasive technologies such as peripheral arterial tonometry⁵¹ alongside HRV measures, could provide
25 further insights.

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40 However, intriguingly, we also found an increased VLF power in children living at HA
41 compared to those at LA during REM sleep. VLF power is rarely reported in research on sleep and
42 HRV, mostly because its interpretation is still debated. There are several possible explanations for this
43 novel finding.

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48 Firstly, VLF power may be associated with increased metabolic demand and higher energy
49 expenditure⁵² at HA as a response to decreased oxygen availability^{53,54}. This is likely to be more
50 marked in REM sleep when metabolic demands are higher compared to NREM sleep⁵⁵.

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54 VLF power is also affected by the renin-angiotensin system with an inverse relationship
55 between angiotensin-converting enzyme and VLF power². In the current study, the higher VLF may

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3 be the result of lower renin-angiotensin system activity, in response to the higher systolic blood
4 pressures observed in HA participants. This effect would be predicted to be more prominent during
5 waking states and REM sleep, when the blood pressure is higher⁵⁶.

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8 VLF may also be related to thermoregulatory processes². Specifically, core hypothermia
9 increases VLF power⁵⁷. Importantly, during REM sleep body temperature regulation mechanisms are
10 less sensitive⁵⁸ as thermoregulatory centers in the pre-optic hypothalamic nuclei are switched off. This
11 creates a poikilothermic state with absence of shivering in response to cold and lack of peripheral
12 vasodilation in warm environments⁵⁹. As the HA children were studied in a cooler climate
13 (average November temperature 9°C) than LA children (average October temperature 26°C), it is
14 possible that increased VLF activity reflected a compensatory thermoregulatory effect during the
15 recording. However, it should be noted that children were studied in a modern building and slept in
16 appropriate warm bedding so core hypothermia would have been unlikely. Future studies in this field
17 should further explore the relationship between VLF, thermoregulation, and REM sleep.

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29 Finally, it is possible that the high VLF power in REM relates to low SpO₂ during sleep at
30 HA. Several studies have shown that VLF power is higher in patients with mild and severe obstructive
31 sleep apnea syndrome (OSAS) compared to controls, and that OSAS severity is associated with
32 increased VLF power⁶⁰⁻⁶³. Montemurro et al.⁶⁴ suggested that the increased VLF activity in OSAS
33 patients may be due to increased sympathetic activity. Specifically, that during obstructive apneas, the
34 spectral peak usually observed in the LF range shifts into the VLF range. They suggested that this
35 shift is due to the entrainment of the sympathetic discharge by the longer apnea-hyperpnea cycle. HA
36 children in this study had more obstructive hypopnea and central apnea, but not obstructive apnea,
37 than LA children. This is likely to represent a scoring anomaly driven by sleep-related fluctuations in
38 ventilation at HA causing larger drops in SpO₂ due to the steep slope of the oxyhaemoglobin
39 dissociation curve at low oxygen tensions^{10,26}. These exaggerated swings in SpO₂ may also stimulate
40 the sympathetic nervous system. The trend for higher systolic blood pressure readings in HA children
41 lends further support to the possibility of early subtle sympathetic activation that could presage
42 cardiac autonomic activity during wakefulness noted in adult Andean highlanders^{65,66}. While we are
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3 not aware of any publications reporting HRV in Andean children, it has been also reported that
4 Himalayan HA resident children have lower heart rates and higher HRV than lowlanders⁶⁷ – as also
5
6 generally observed in this study.
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10 **Conclusions**

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12 Here we confirm sleep stage-dependent modulation of HRV in children living at different
13 altitudes, with higher vagal activity during NREM sleep and greater variability of the heart rate during
14 REM. Moreover, we observed that children living at high altitude show higher VLF power during
15 REM sleep compared to children living in lower altitude. Whether these differences are the results of
16 an adaptation to high altitude living, or an indirect effect of differences in body temperature,
17 respiration and oxyhaemoglobin saturation remain unclear, and further research is required to address
18 these questions.
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Abbreviations

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5	AIM	Ancestry informative marker
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7	ANOVA	Analysis of variance
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9	BMI	Body mass index
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11	CMS	Chronic mountain sickness
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13	DNA	Deoxyribonucleic acid
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15		
16	HA	High altitude
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18	HF	High frequency
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20	HRV	Heart rate variability
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23	LA	Low altitude
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25	LF	Low frequency
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27	LMM	Liner mixed model
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29	PSD	Power Spectral Density
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32	NREM	Non rapid eye movement sleep
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34	REM	Rapid eye movement
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36	SpO ₂	Oxyhaemoglobin saturation
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39	VLF	Very low frequency
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10
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3 **Title: Cardiac autonomic activity during sleep in high altitude resident children**
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5 **compared to lowland residents.**
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45 **Running title: Sleep and heart rate variability in high altitude children**
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Abstract

Study Objectives: We aimed to characterize heart rate variability (HRV) during sleep in Andean children native to high altitude (HA) compared to age, gender and genetic ancestry-similar low altitude (LA) children. We hypothesized that the hypoxic burden of sleep at HA could induce variation in HRV. As children have otherwise healthy cardiovascular systems, such alterations could provide early markers of later cardiovascular disease.

Methods: Twenty-six LA (14F) and 18 HA (8F) children underwent a single night of attended polysomnography. Sleep parameters and HRV indices were measured. Linear mixed models were used to assess HRV differences across sleep stage and altitude group.

Results: All children showed marked fluctuations in HRV parameters across sleep stages, with higher vagal activity during NREM sleep and greater variability of the heart rate during REM. Moreover, HA children showed higher very low frequency HRV in REM sleep and, after adjusting for heart rate, higher low to high frequency ratio in REM sleep compared to children living at lower altitude.

Conclusion: We confirmed previous findings of a stage-dependent modulation of HRV in Andean children living at both high and low altitudes. Moreover, we showed subtle alteration of HRV in sleep in HA children, with intriguing differences in the very low frequency domain during REM sleep. Whether these differences are the results of an adaptation to high altitude living, or an indirect effect of differences oxyhaemoglobin saturation remain unclear, and further research is required to address these questions.

Keywords: apnea, autonomic nervous system, children, high altitude, hypobaric hypoxia, heart rate variability, REM sleep, very low frequency power.

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3 **Statement of Significance.** People living in the high Andes have reduced life expectancy compared
4 to lowlanders. Sleep related hypoxia is a putative cause, with morbidity mediated by sympathetic
5 nervous system activation. We report heart rate variability, a measure of autonomic regulation, during
6 sleep in high and low altitude dwelling children. Differences in heart rate variability spectral power
7 between sleep stages support previous research. Novel findings in REM sleep at high altitude of
8 greater very low frequency power and higher low to high frequency ratio (adjusting for heart rate)
9 may be a marker of exposure to 'hidden' hypoxia. Exposure to chronic intermittent hypoxia in high
10 altitude dwelling children could recalibrate the autonomic nervous system underpinning reduced life
11 expectancy in this population.
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Introduction

Heart rate variability (HRV), the beat-beat variation in heart rate identified from the R-R interval recorded on an electrocardiogram (ECG), reflects cardiac autonomic regulation. Power spectral density analysis generates high-frequency (HF), low-frequency (LF) and very low frequency (VLF) components (0.15 – 0.4 Hz; 0.04 – 0.15 Hz and 0 - 0.04 Hz respectively). HF power is understood to reflect parasympathetic activity. LF power, while less well understood, is likely to reflect both aspects of the autonomic nervous system¹. The meaning of VLF is still debated, but it has been suggested that it may reflect the function of the renin-angiotensin system and/or thermoregulatory processes². A chronic imbalance of the autonomic nervous system, characterized by an overactive sympathetic drive and correspondingly under-active parasympathetic drive, is a risk factor for cardiovascular morbidity and mortality³. This is potentially relevant to populations living at high altitude (HA), that is at 2500m or more above sea-level, who are exposed to chronic hypobaric hypoxia. Hypoxia is a potent stimulus of the sympathetic nervous system and healthy mountaineers exposed to HA experience an increase in sympathetic output⁴ leading to increased heart rate, systemic vascular resistance, blood pressure, and metabolic rate.

Over 140 million people permanently live at HA and unlike mountaineers have adapted to their hypoxic environment. However, people living in the high Andes may be poorly adapted to chronic hypoxia due to steady influx of non-adapted lowlanders over time facilitated by the Andean topography, that connects high plains to low lying land. Colonization by Europeans following the Spanish conquest 400 years ago⁵ resulted in further mixing of the gene pool with contemporary populations displaying significant European genetic admixture. Chronic mountain sickness (CMS)⁶, characterized by excessive erythropoiesis, severe hypoxemia and early death^{7,8}, affects around 15% of the older HA population and may be a hallmark of incomplete adaptation to hypobaric hypoxia in this population. Of note, Andean adults with CMS have higher noradrenaline levels as well as higher sympatho-vagal tone on HRV measures compared to controls at the same altitude⁹. Furthermore, a correlation between severity of CMS and decreased baroreflex suggests that alteration in the autonomic nervous system may be a biomarker of CMS.

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3 We have reported that Andean HA native children experience additional hypoxic challenge
4 during sleep compared to the waking state¹⁰. This intermittent hypoxic ‘load’ during sleep could, in
5 theory, mimic the chronic intermittent hypoxia seen in conditions such as obstructive sleep apnea,
6 leading to increased sympathetic tone^{11,12} and, in turn, increased the risk of hypertension, stroke,
7 myocardial infarction^{13,14} and, possibly, CMS.

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12 Heart rate variability has a circadian pattern¹⁵⁻¹⁷ and there are marked differences in power spectral
13 components between the waking state, Rapid Eye Movement (REM) and Non-REM sleep stages¹⁸⁻²¹
14 in both adults and children. Specifically, during sleep, cardiac autonomic activity is characterized by
15 altered sympatho-vagal balance with higher normalized high frequency power (indicating a
16 predominant parasympathetic drive) during NREM stages compared to wakefulness and a reduction
17 in REM sleep^{22,23}. Also, the total power, which represents the total variability of the heart rate, shows
18 a dramatic reduction during N3 and a strong increase (often higher than wakefulness) in REM sleep.
19 This reliable pattern of autonomic fluctuations during sleep, which has been observed during both
20 nighttime and daytime sleeping periods^{19,24,25}, coupled with the advantage that confounding
21 environmental stimuli are minimised (e.g., movements are reduced) and that the ‘hypoxic load’ is
22 maximal during sleep, make sleep the optimal context in which to investigate the cardiac autonomic
23 activity of individuals living in different altitudes. We aimed to characterize heart rate variability
24 during sleep in Andean children native to HA and children of similar age and genetic ethnic ancestry
25 native to lowlands. We hypothesized that any subtle increase in sympathetic nervous system tone
26 between altitude populations would be more apparent during REM sleep when parasympathetic drive
27 is lowered.

28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 **Methods**

47 48 **Subjects**

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50 As part of a larger study (Development and Sleep at Altitude - DeSAT) investigating the
51 effects of altitude on sleep disordered breathing²⁶ and neurocognitive performance²⁷, 59 children and
52 adolescents from low altitude (LA) Santa Cruz, 500m above sea-level ($n_L=32$) and La Paz, 3700m
53 above sea-level ($n_H=27$), Bolivia underwent a single night of attended polysomnography. Subjects

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3 were aged from 7.00 to 17.15 years. Inclusion criteria required participants to have been born at term,
4 have no chronic health conditions (including no history of known obstructive sleep apnea), learning
5 difficulty or developmental disorder and have been born and lived at their resident altitude for at least
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7 five years prior to participation. The study was approved by the institutional ethics committees of the
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9 University of Western Australia and the Universidad Privada de Santa Cruz de la Sierra, Bolivia.
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14 **Measures**

15 *Socio-demographic data.*

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17 Data were collected for mothers of participant children on age of completion of full-time
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19 education.
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22 *Physical examination*

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24 All children underwent a detailed, physical examination supervised by a pediatric
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26 somnologist (CMH) or otolaryngologist (KH) including: cardiorespiratory examination, resting blood
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28 pressure (Microlife, Zurich) and height and weight. Body mass index (BMI) centiles were derived
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30 from standard Control and Prevention growth charts.
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32 *Genetics*

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34 DNA were extracted from saliva samples (Western Australia DNA Bank, University of
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36 Western Australia) and the whole gene amplified (K BioSciences, Hoddesdon, UK). Individual
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38 European, Native American, and African admixture proportions were estimated using a panel of 28
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40 ancestry informative markers (AIMs) previously noted to demonstrate high-frequency differences in
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42 allele frequency between these different ancestry groups. The admixture modeling program
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44 admixmap²⁸ was used to model the distribution of admixture in the cohort
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46 (<http://homepages.ed.ac.uk/pmckeigu/admixmap/index.html>) and to generate individual ancestry
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48 estimates. AIM ancestry-specific allele frequencies were estimated from their reported counts in
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50 modern European, African, and Native American populations^{26,27}.
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52 *Polysomnography*

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54 Attended polysomnography was carried out in an established sleep laboratory setting (Santa
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56 Cruz) and in a temporarily adapted facility (La Paz) using computerized ambulatory systems
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3 (Compumedics PS2 system, Melbourne, Australia) according to accepted guidelines²⁹. All studies
4 were performed by an experienced polysomnographic technologist (AC). A standard ambulatory sleep
5 montage included electroencephalography (C3/A2, C4/A1) with electrode placement according to the
6 international 10-20 system³⁰, electromyography at submental and anterior tibialis, bipolar
7 electrooculography, electrocardiography and oxyhaemoglobin saturation monitoring (SpO₂).
8 Respiratory inductance plethysmography bands were used to measure abdominal and thoracic
9 excursions and nasal thermistors provided a constant flow monitor. Polysomnographs were scored
10 (AC) based on the established sleep staging³¹ and respiratory³² criteria for paediatrics and all studies
11 were peer reviewed (CMH). All PSG included continuous ECG recording and standard
12 neurophysiological sleep stage scoring based on EEG, EOG, and EMG. Only stages N2, N3, and
13 REM sleep are reported in this report as N1 is a transition state comprising less than 5% of the night.
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27 **Data Pre-processing**

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29 All polysomnography studies were initially visually assessed to ensure presence of adequate
30 quality ECG and the nasal airflow signals for a minimum of 4 hours of the overnight recording.
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33 Heart-beats were automatically detected in the ECG, sampled at 256 Hz. R-waves were
34 detected after band-pass filtering (0.9-36 Hz, 349th order FIR filter, applied in the forward and reverse
35 direction to avoid phase-shift) using a peak-detector, and then visually checked. Segments of signal
36 where no clear R-waves could be identified were marked as bad data and removed from further
37 analysis (marked as gaps in the data). Automatically identified R-waves were replaced by nearby
38 peaks when deemed incorrect, based on regularity of RR intervals and the expected shape of the QRS
39 complex. The resulting RR-interval time-series was then linearly interpolated to obtain a regularly
40 sampled signal with a sampling rate of 5 Hz. This signal was then high-pass filtered (in the forward
41 and reverse directions) at 0.003 Hz using a first-order Butterworth filter (applied in the forward and
42 reverse directions) to remove its DC component and slow trends.
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53 To account for any potential influence of respiration on HRV, all polysomnograms were
54 automatically inspected for abnormal respiration. For regular (normal) respiratory cycle detection, the
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3 thermistor generated nasal flow signal was first band-pass filtered between 0.08 and 0.8 Hz using a 5th
4 order Butterworth filter to remove the DC component of signal and slow trends and avoid spectral
5 components which are well above the average fundamental frequency of respiration in children (0.23
6 Hz to 0.36 Hz in children from 4 to 16³³ . Local maxima and minima of the flow signal corresponding
7 to the start and end of expiration/inspiration were identified separately using envelope detectors. The
8 initial sets of extrema detected were refined using amplitude thresholds. This was to avoid peaks and
9 trough with undesirable amplitudes (e.g. a local peak with negative amplitude and troughs with
10 positive amplitudes) and ensure normal respiratory cycle detection. Duration thresholds were then
11 applied to the refined extrema to eliminate those which are abnormally close or distant from one
12 another. Undergoing this procedure resulted in regular (normal) respiratory cycle detection and hence
13 regular respiratory rate quantification. Finally, the editing on both respiratory and ECG signals was
14 combined (logic AND operator) to produce a final edit that ensured that the HRV parameters were
15 extracted from portions of polysomnograms with both normal cardiac patterns and regular respiration.
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30 *HRV calculation*

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33 Heart rate variability was calculated from the previously processed RR interval signal using
34 the Welch power spectral density estimation method (rectangular window of 100 seconds, 50%
35 overlap). The Welch Power Spectral Density (PSD) estimation operator was then applied to the
36 filtered RR signal to produce a PSD. Very low frequency (VLF), LF and HF powers were then
37 calculated as the area under the PSD curve from 0 - 0.04 Hz, 0.04 – 0.15 Hz and 0.15 – 0.4 Hz
38 respectively. Total power was calculated as the sum of all three powers. Normalized HF power was
39 calculated by dividing the power in each band by the sum of LF and HF power, in accordance with
40 recommendations³⁴. For all the selected subjects, at least 5 minutes (300s) of continuous RR interval
41 data were available for each sleep stage, other than for REM where 2 participants (one LA and one
42 HA) had 226s and 295s of recording respectively. For wake recordings 7 participants (5 at LA and 2
43 at HA) had less than 5 minutes recording – minimum 135seconds. (see Table 1).
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Variables were generated for each sleep stage N2, N3 and REM as well as for pre-sleep wakefulness, as follows:

- Average RR intervals (RR; ms, intervals between consecutive R-peaks)
- Total power (ms^2)
- Very low frequency (VLF, ms^2) power
- Low frequency (LF, ms^2) power
- High frequency (HF, ms^2) power and normalised HF power [HFnu: $\text{HF}/(\text{LF}+\text{HF})$]
- LF/HF ratio
- Respiratory interval (s)

Note that normalised LF power [$\text{LF}/(\text{LF}+\text{HF})$] are not reported since this variable is the complement of normalized HF (normalized LF = 1 - normalized HF). A similar redundancy also exists with LF/HF³⁵, but due to the nonlinear relationship between HF or LF normalized with LF/HF, discrepancies in statistical testing may arise. Nevertheless, we decided to report both HFnu and LF/HF for descriptive purposes.

Statistical methods

Data were checked for normality using the Shapiro Wilk test. If they were not suitable for parametric descriptive and inferential statistics, non-parametric analyses were used, where available. The HRV frequency-domain parameters (total power, VLF, LF, and HF) were transformed using a natural logarithm before statistical analysis. LF/HF ratio was computed on log-transformed LF and HF data. Between-altitude differences were analyzed using Mann Whitney U or using t-tests and chi-square tests, as appropriate. To investigate differences in sleep architecture, we ran several ANCOVA with sleep parameters as the dependent variable, altitude group as independent variable and age as a covariate. To assess the cardiac autonomic pattern across sleep stages in the two groups, we employed linear mixed models (LMM), which permit analysis of an unbalanced number of observations per participant. Moreover, LMM allow the influence of factors whose levels are randomly extracted from a population (i.e., participants) to be taken into account, thus yielding more generalizable results³⁶.

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3 For each HRV parameter, we built a separate model, using *Participant* as crossed random effects and
4 *Stage* (Wake, N2, N3, REM) and *Group* (LA, HA) as fixed effects. Bonferroni's test was used for
5 post-hoc comparisons.
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10 **Results**

12 *Sample*

15 Of the 59 children and adolescents who had an overnight polysomnographic study, 5 were
16 excluded as they failed to meet inclusion criteria and 10 were excluded as ECG failed to meet quality
17 criteria throughout the study, leaving a sample size of 44: 26 living at LA (12 male) and 18 living at
18 HA (10 male).
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25 *Demographic and daytime systolic blood pressure*

27 Table 1 shows the main demographic and clinical characteristics of the sample. There was no
28 significant difference in age ($t_{42}=-1.69$, $p=.10$), gender distribution ($\chi^2=0.38$, $p=.54$), or genetic
29 ancestry differences of children between altitude locations. Daytime resting systolic blood pressure
30 was significantly higher (Table 1) at high altitude ($t_{42}=2.73$, $p=.009$). However, blood pressure is
31 influenced by age, gender and height centile during childhood. Given the absence of normative data
32 for this population the contribution of altitude to blood pressure was explored through regression
33 analysis. Controlling for age and height, altitude contributed significantly to systolic blood pressure
34 ($r^2=.13$, $p=.018$) but BMI may be a partial mediator of the effect of altitude on BP as this altitude
35 difference was only at trend level when BMI was controlled for ($r^2=.07$, $p=.059$).
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47 *Polysomnographic results*

49 We observed some differences in sleep architecture between HA and LA children (Table 2).
50 The ANCOVAs showed that HA children spent more time in N2 (%) than LA children ($F(1,41)=$
51 4.740 , $p=.035$), to the detriment of N3 ($F(1,41)= 4.013$, $p=.052$). No other significant differences were
52 observed in sleep architecture. The ANCOVAs also highlighted the expected differences in mean
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3 nocturnal oxyhaemoglobin saturation (SpO_2) and respiratory polysomnographic variables at HA.
4 Specifically, HA children had lower mean overnight SpO_2 levels ($F(1,41)=383.61, p<.001$), higher 3%
5 desaturation index ($F(1,38)=19.63, p<.001$) and higher hypopnea index ($F(1,41)=8.455, p=.006$),
6 compared to LA children. (see Table 2 and see Hill et al., 2016 for discussion of these results).
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10 11 12 *Cardiac Autonomic Activity across Sleep Stages and altitude settings*

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14 Descriptive values for all the cardiac parameters across the different stages are presented in
15 Table 3. Note that we have a limited number of participants with data available from pre-sleep
16 wakefulness as some children fell asleep during, or shortly after, the polysomnography set up before
17 quality data acquisition was established. Nevertheless, our linear mixed model analysis takes into
18 account missing data and provides a corrected estimate of the mean and standard error of the Wake
19 period
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26 Summary results for RR interval analysis are shown in Fig. 1. LMM on RR showed a main
27 effect of sleep stage ($F(3,100.29)=23.504, p<.001$), with a significant lengthening of RR during N2,
28 N3 and REM sleep compared to Wake (all $p's<.001$), and a shortening of RR in REM compared to
29 N2 ($p=.008$). We also observed a Group \times Sleep Stage interaction ($F(3, 100.29)=3.60, p=.016$), with a
30 nominal lengthening of RR during Wake and REM sleep in HA compared to LA, but these contrasts
31 did not reach significance ($p=.096$ and $p=.133$, respectively, Figure 1a).
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38 Analysis of the respiratory intervals showed only a main effect of sleep stage
39 ($F(3,101.25)=48.36, p<.0001$), with shorter respiratory intervals in Wake compared to all sleep stages
40 (all $p's<.0001$), and a reduction of respiratory intervals from N2 to N3 ($p=.011$; Figure 1b). No effect
41 of altitude was observed.
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46 A similar result was observed for total power, with a main effect of sleep stage ($F(3,$
47 $100.41)=12.520, p<.001$), with a significant total power reduction during N3 relative to N2 ($p=.001$),
48 and increased total power during REM sleep relative to W ($p<.001$), N2 ($p=.019$), and N3 ($p<.001$),
49 indicating an increase in heart rate variability during REM sleep. We also observed increased total
50 power during N2 relative to W ($p=.026$). We also found a trend for Group \times Stage interaction ($F(3,$
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3 100.41)=2.923, $p=.056$), with a nominal power increase during Wake and REM in HA compared to
4 LA, but these contrasts did not reach the significance ($p=.279$ and $p=.317$, respectively, Figure 2a).

5
6 The VLF showed a significant main effect of sleep stage $F(3, 101.31)=45.702, p<.001$), with
7 a marked power reduction during N3 compared to the other stages (all $p's<.1. 0001$). We also
8 observed a nominal greater VLF in the HA compared to the LA group, but this effect did not reach
9 statistical significance ($F(1, 42.678)=2.329, p=.134$). Although the interaction Group \times Stage was not
10 significant ($F(3, 101.35)=1.466, p=.228$), we observed an higher VLF during REM sleep in the HA
11 group ($p = .026$; Figure 2b).

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13 A similar main effect of sleep stage was found in LF $F(3,101.22)=22.562, p<.001$), with
14 increased LF during REM sleep relative to Wake, N2 and N3 ($p=.002, p=.009, \text{ and } p<.001$
15 respectively), and a trend-level reduction of LF in N3 compared to N2 ($p<.001$; Figure 2c). No effect
16 of altitude was observed.

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18 Similarly, we found a significant effect of sleep ($F(3,100.36)=15.111, p<.001$) for HF, with
19 increased vagal activity in all sleep stages compared to Wake (all $p's<.001$), and increased power in
20 N2 compared to REM ($p=.040$, Figure 2d). Again, there were no effects of altitude.

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22 A main effect of sleep stage on HFnu ($F(3,101.25)=42.969, p<.001$) with a marked increase
23 during N3 compared to W ($p<.0001$), N2 ($p=.007$), and REM sleep ($p<.001$; Figure 2e), indicating a
24 strong vagal modulation of N3. HFnu was also higher in LA compared to HA, but this comparison did
25 not reach a significant level ($F(1,42.72)=2.254, p=.141$).

26
27 Lastly, the LF/HF was different across sleep stages ($F(3, 101.61)=34.30, p<.001$), reflecting
28 marked reduction during N2 and N3 compared to W and REM (all $p's <.001$; Figure 2f). LF/HF was
29 also higher in N2 compared to N3 ($p=.019$). Nominally, LF/HF ratio was higher in HA compared to
30 LA, but this comparison did not reach a significant level ($F(1, 42.92)=2.689, p=.112$).

31
32 As we observed an increased RR interval in all sleep stages in the HA group (possibly related
33 to the slightly older age of this group), and it has previously been shown that there is a strong
34 dependency of frequency-domain parameters on heart rate³⁷⁻³⁹, we repeated the analysis described
35 above including the mean RR interval for each individual and each sleep period as covariates in the
36 models, using raw values for each HRV parameters. We also repeated the analysis on RR intervals
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3 including age as a covariate. The direction of the results was similar even with this correction (see full
4 analysis in the supplemental material). The previous trend level for total power, Group \times Stage
5 interaction was lost ($F(3, 100.88)=.553, p=.647$), while conversely the Group effect of LF/HF,
6 became significant ($F(1,42.73)=5.05, p=.030$), showing higher LF/HF ratio at HA compared to LA.
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8 Importantly, although the main Group effect for VLF was trending, but still not significant ($F(1,$
9 $38.77)=3.68, p=.062$), the greater VLF power during REM sleep in the HA group remained significant
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15 (p = .006).
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18 Discussion

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22 We aimed to investigate cardiac autonomic activity during sleep in Andean children native to
23 high altitude (HA) compared to age, gender and genetic ancestry-similar low altitude (LA) children.
24 We hypothesized that the hypoxic burden of sleep at HA could provoke increased sympathetic
25 activity. Furthermore, altitude differences in REM sleep, with characteristic high sympatho-vagal
26 tone, could reveal subtle changes in autonomic regulation. As children have otherwise healthy
27 cardiovascular systems, such alterations could provide early markers of later cardiovascular disease.
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35 Regardless of altitude, children showed marked fluctuations in HRV parameters across sleep
36 stages consistent with previous research^{19,20,24} indicating that sleep-dependent modulation of
37 autonomic activity is reliable across altitudes. Specifically, mean RR intervals lengthened during all
38 sleep stages compared to wake, with a peak during N2 sleep and a shortening during REM sleep. On
39 the other hand, total power decreased during N3 and increased dramatically during REM sleep,
40 mainly driven by LF power, which showed a marked decrease in N3 followed by a strong increase in
41 REM. The LF fluctuations also influenced the LF/HF ratio. Indeed, LF/HF was also at its lowest
42 during N3 sleep, consistently with previous reports⁴⁰.
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51 Although we filtered our data to exclude periods of irregular breathing, it is possible that
52 respiration might have had a confounding influence⁴¹ on HRV indices, when respiratory rate
53 approaches the boundary between the LF and HF band (0.15 Hz). Respiratory rate is generally higher
54 in children than adults⁴², and our participants exhibited a respiration cycle length of 3.60s to 3.80s
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3 across sleep stages. With this range (0.26-0.28 Hz), therefore, confounding in the interpretation of HF
4 during sleep is not expected to occur⁴³. Nonetheless, we cannot completely exclude the possibility
5 that HA may have altered cardiopulmonary coupling due to hypopnea episodes, but this was
6 minimized by only including periods of regular breathing in the analysis. This could be assessed in the
7 future using coherence or cross-power analysis⁴⁴.

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12 Our principle aim was to explore altitude related differences in HRV during sleep. We report
13 for the first time a nominally higher LF/HF ratio across sleep stages in children living at HA
14 compared to LA after adjusting for R-R interval. Post-hoc comparisons showed that this was
15 significantly higher in REM sleep. This finding should be interpreted with caution. While historically
16 this would have been interpreted as representing an increase in cardiac sympathetic activation in REM
17 sleep at HA⁴⁵ this assumption has been challenged⁴⁶. Experimental studies show that LF power
18 correlates poorly with cardiac sympathetic nerve activation and that the relationship between
19 sympathetic and parasympathetic activity is non-linear⁴⁶. Some authors have proposed that LF may
20 represent the vagally-mediated function of the baroreflex⁴⁷⁻⁴⁹. HA children in this study had higher
21 age and height adjusted daytime blood pressure than LA children. It is possible that higher LF/HF
22 ratio may reflect a greater baroreflex adaptive response to the increased blood pressure that is
23 generally observed during REM sleep⁵⁰. Future study of autonomic function in sleep, using non-
24 invasive technologies such as peripheral arterial tonometry⁵¹ alongside HRV measures, could provide
25 further insights.

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40 However, intriguingly, we also found an increased VLF power in children living at HA
41 compared to those at LA during REM sleep. VLF power is rarely reported in research on sleep and
42 HRV, mostly because its interpretation is still debated. There are several possible explanations for this
43 novel finding.

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48 Firstly, VLF power may be associated with increased metabolic demand and higher energy
49 expenditure⁵² at HA as a response to decreased oxygen availability^{53,54}. This is likely to be more
50 marked in REM sleep when metabolic demands are higher compared to NREM sleep⁵⁵.

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54 VLF power is also affected by the renin-angiotensin system with an inverse relationship
55 between angiotensin-converting enzyme and VLF power². In the current study, the higher VLF may

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3 be the result of lower renin-angiotensin system activity, in response to the higher systolic blood
4 pressures observed in HA participants. This effect would be predicted to be more prominent during
5 waking states and REM sleep, when the blood pressure is higher⁵⁶.

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8 VLF may also be related to thermoregulatory processes². Specifically, core hypothermia
9 increases VLF power⁵⁷. Importantly, during REM sleep body temperature regulation mechanisms are
10 less sensitive⁵⁸ as thermoregulatory centers in the pre-optic hypothalamic nuclei are switched off. This
11 creates a poikilothermic state with absence of shivering in response to cold and lack of peripheral
12 vasodilation in warm environments⁵⁹. As the HA children were studied in a cooler climate
13 (average November temperature 9°C) than LA children (average October temperature 26°C), it is
14 possible that increased VLF activity reflected a compensatory thermoregulatory effect during the
15 recording. However, it should be noted that children were studied in a modern building and slept in
16 appropriate warm bedding so core hypothermia would have been unlikely. Future studies in this field
17 should further explore the relationship between VLF, thermoregulation, and REM sleep.

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29 Finally, it is possible that the high VLF power in REM relates to low SpO₂ during sleep at
30 HA. Several studies have shown that VLF power is higher in patients with mild and severe obstructive
31 sleep apnea syndrome (OSAS) compared to controls, and that OSAS severity is associated with
32 increased VLF power⁶⁰⁻⁶³. Montemurro et al.⁶⁴ suggested that the increased VLF activity in OSAS
33 patients may be due to increased sympathetic activity. Specifically, that during obstructive apneas, the
34 spectral peak usually observed in the LF range shifts into the VLF range. They suggested that this
35 shift is due to the entrainment of the sympathetic discharge by the longer apnea-hyperpnea cycle. HA
36 children in this study had more obstructive hypopnea and central apnea, but not obstructive apnea,
37 than LA children. This is likely to represent a scoring anomaly driven by sleep-related fluctuations in
38 ventilation at HA causing larger drops in SpO₂ due to the steep slope of the oxyhaemoglobin
39 dissociation curve at low oxygen tensions^{10,26}. These exaggerated swings in SpO₂ may also stimulate
40 the sympathetic nervous system. The trend for higher systolic blood pressure readings in HA children
41 lends further support to the possibility of early subtle sympathetic activation that could presage
42 cardiac autonomic activity during wakefulness noted in adult Andean highlanders^{65,66}. While we are
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3 not aware of any publications reporting HRV in Andean children, it has been also reported that
4 Himalayan HA resident children have lower heart rates and higher HRV than lowlanders⁶⁷ – as also
5 generally observed in this study.
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10 **Conclusions**

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12 Here we confirm sleep stage-dependent modulation of HRV in children living at different
13 altitudes, with higher vagal activity during NREM sleep and greater variability of the heart rate during
14 REM. Moreover, we observed that children living at high altitude show higher VLF power during
15 REM sleep compared to children living in lower altitude. Whether these differences are the results of
16 an adaptation to high altitude living, or an indirect effect of differences in body temperature,
17 respiration and oxyhaemoglobin saturation remain unclear, and further research is required to address
18 these questions.
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Abbreviations

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4		
5	AIM	Ancestry informative marker
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7	ANOVA	Analysis of variance
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9	BMI	Body mass index
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11	CMS	Chronic mountain sickness
12		
13	DNA	Deoxyribonucleic acid
14		
15		
16	HA	High altitude
17		
18	HF	High frequency
19		
20	HRV	Heart rate variability
21		
22		
23	LA	Low altitude
24		
25	LF	Low frequency
26		
27	LMM	Liner mixed model
28		
29	PSD	Power Spectral Density
30		
31		
32	NREM	Non rapid eye movement sleep
33		
34	REM	Rapid eye movement
35		
36	SpO ₂	Oxyhaemoglobin saturation
37		
38		
39	VLF	Very low frequency
40		

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Supplementary analysis

Cardiac Autonomic Activity across Sleep Stages adjusting for RR interval

Given concerns raised by some authors regarding the confounding effect of correlation between mean HR and HRV [1-3] we corrected our HRV analysis including both the mean RR interval of each individual and each sleep period as covariate in the models, using raw values for each HRV parameter (i.e. not normalized [3]). The results of the subsequent statistical analyses (linear mixed models) were similar to those we observed using non-corrected variables, as discussed below.

The corrected total power showed Stage effects ($F(3, 106.38)=20.167, p<.001$) consistent with our earlier analysis, with a significant total power increase during REM relative to N2 and N3 (all $p's<.001$), indicating an increase variability of the heart rate in REM. Total power was also higher in Wake compared to N2 ($p=.013$) and N3 ($p<.001$). The previous trend level Group \times Stage interaction was lost ($F(3, 100.88)=.553, p=.647$).

The VLF still showed a significant main Stage effect ($F(3, 107.16)=19.14, p<.001$), with an increased total power during REM relative to N2 and N3 (all $p's<.001$) and a marked power reduction during N3 compared to the other stages (all $p's<.002$). We also observed a trend for altitude ($F(1, 38.77)=3.68, p=.062$), with higher VLF in HA, and, importantly, we still observed the previously noted higher VLF during REM sleep in the HA group ($p=.006$).

Consistent with the non-adjusted analysis, we found a Stage effect for LF ($F(3,107.92)=18.62, p<.001$), with a significant increase of LF during REM relative to N2 and N3 (all $p's<.001$), and a reduction in N3 compared to Wake ($p<.001$). While the reduction of LF in N3 compared to N2 was previously at trend level, this was now significant ($p=.001$). Again, no group or interaction effect was observed.

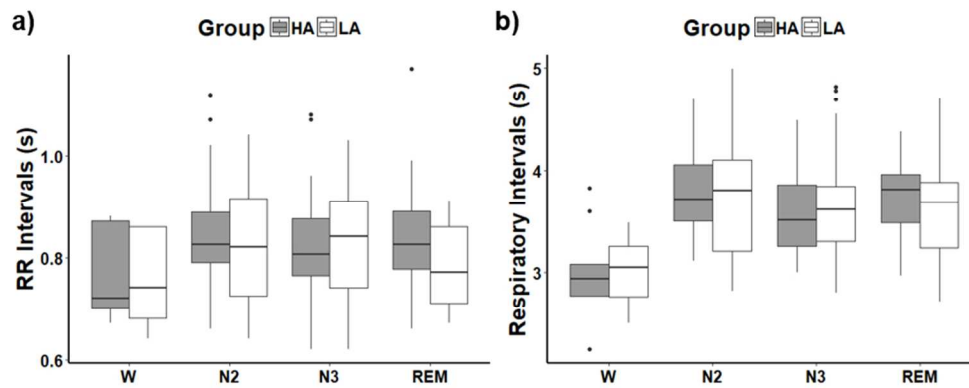
The previously observed Stage effect for HF disappeared ($F(3,101.24)=.64, p=.592$), whereas the HFnu again showed a Stage effect ($F(3, 106.82)=31.71, p<.001$) with a marked increase during N3 compared to W and REM (all $p's <.001$), indicating a strong vagal modulation of N3, and an increased

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3 HFnu during N2 compared to Wake and REM ((all p 's $<.001$). We also observed an altitude trend for
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5 HFnu ($F(1, 39.55)=3.43, p=.071$), with higher HFnu in LA participants.
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8 Consistent with the non-adjusted data, the LF/HF showed a Stage effect ($F(3,109.96)=10.36,$
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10 $p<.001$) with a marked decrease during N3 compared to Wake, and REM ($p=.006$ and $p<.001,$
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12 respectively), and a reduced ratio in N2 compared to REM ($p=.002$). However, we now observed a Group
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14 main effect, with LF/HF significantly higher in HA compared to LA ($F(1,42.73)=5.05, p=.030$).
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16 Although we did not find a significant Group \times Stage interaction ($F(3, 107.04)=1.474, p=.226$), similarly
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18 to what observed for the VLF, LF/HF was significantly higher in REM sleep at HA compared to LA
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20 ($p=.013$)
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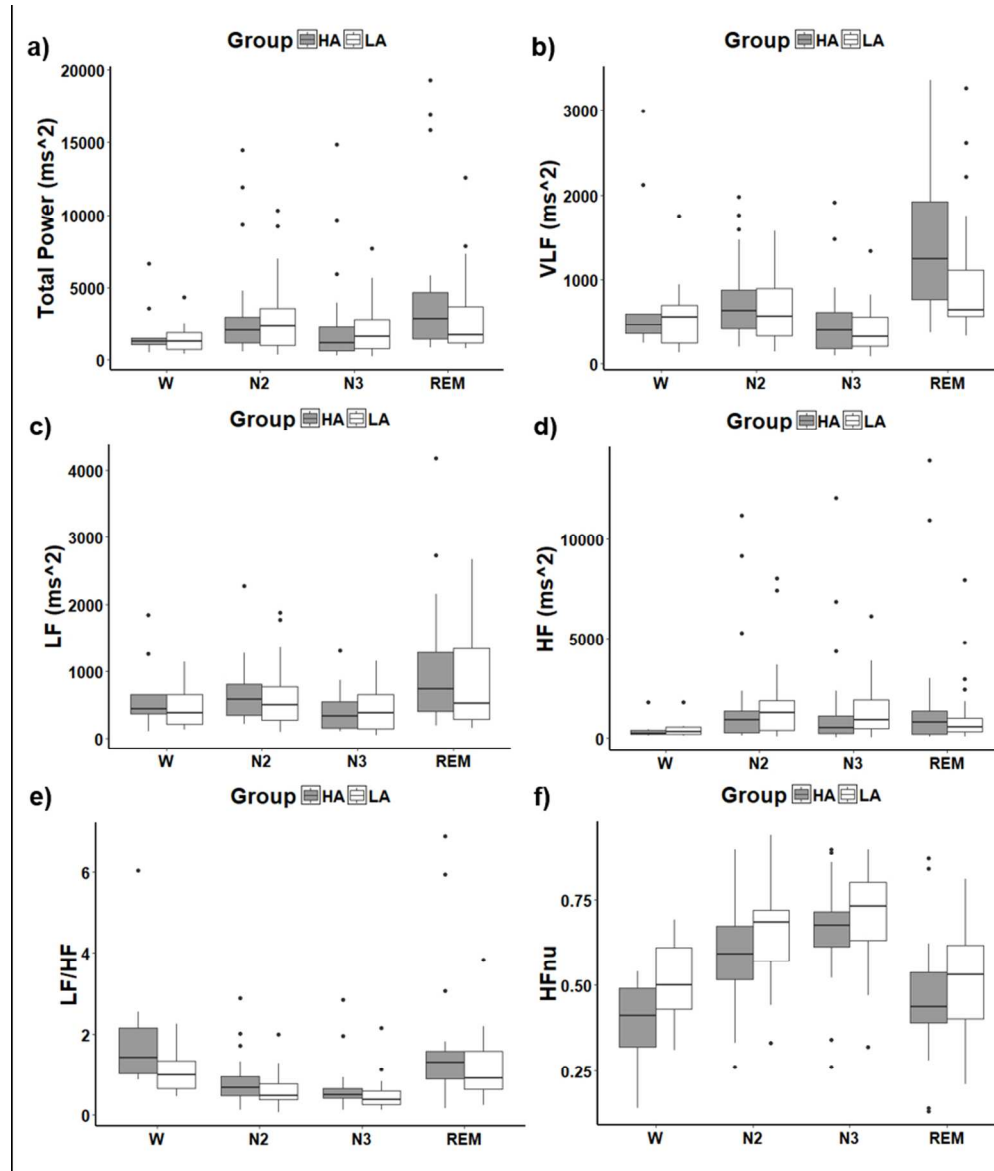
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24 Overall, the correction reduced the group differences. This was indeed expected since the two
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26 groups showed different RR intervals across the sleep stages. Nevertheless, even with this correction, we
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28 confirmed that ANS fluctuations across stages and the group differences (e.g., LF/HF, VLF during REM)
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30 were still in the same direction, suggesting that the differences were not due to changes in the heart rate
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32 per se or merely due to age differences, rather involved physiological mechanisms beyond those that
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34 control mean heart-rate.
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Box plot for inter-beat time (RR) and respiratory intervals across sleep stages in low altitude (LA) and high altitude (HA) children.

160x67mm (150 x 150 DPI)



Box plots of heart rate variability components across sleep stages in low altitude (LA) and high altitude (HA) children. VLF: very low frequency; LF: low frequency; HF: high frequency; HFnu: high frequency normalized units; LF/HF: low frequency to high frequency ratio

163x190mm (150 x 150 DPI)

Table 1. Demographic and clinical characteristics by altitude.

	Low altitude	High altitude	<i>p</i> -value*
	N = 26	N= 18	
Gender (M: F)	12:14	10:8	.76
Mean age (years)	10.8 (2.9)	12.3 (3.1)	.10
BMI (kg/m²)	19.3 (3.3)	20.7 (3.1)	.17
Ancestry informative markers			
% European	49.3 (7.6)	44.7 (10.0)	.13
% Native American	48.9 (7.6)	53.9 (10.2)	.10
% African	1.8 (1.4)	1.5 (0.5)	.78
Resting SBP (mmHg)	103 (10.6)	112.2 (12.24)	.04 [‡]

Notes. Data are presented as means and (standard deviation). BMI: body mass index; F: female; M: male; SBP: systolic blood pressure. **p*-values are reported as t-test or chi-squared comparisons and are not controlled for age. [‡]*p*-value is reported as analysis of covariance controlling for age, gender and height of the participants.

Table 2. Summary of the main polysomnographic data by altitude.

	Low altitude N = 26	High altitude N= 18	<i>p-value</i>
Total sleep time (mins)	395.0 (60.0)	365.4 (72.6)	.148
Sleep efficiency (%)	93.4 (5.9)	89.5 (11.0)	.141
% N1	3.8 (1.8)	5.2 (3.7)	.106
% N2	44.6 (5.9)	50.1 (8.0)	.013
% N3	31.5 (8.0)	26.1 (7.2)	.029
% REM	20.1 (5.2)	19.2 (5.6)	.580
Mean SpO₂	97.3 (0.8)	88.5 (2.0)	<.001
Min SpO₂	90.3 (3.6)	78.2 (5.8) ^a	<.001
3% ODI	0.9 (0.8) [#]	3.4 (2.6) ^a	<.001
OAHl index	0.9 (1.1)	2.3 (2.2)	.005
Obstructive apnea index	0.07 (0.2)	0.01 (0.1)	.213
Central apnea index	0.4 (0.4)	1.5 (2.5)	.035

Notes. Data are presented as means and (standard deviation). ODI: oxygen desaturation index; OAHl: Obstructive Apnea Hypopnea Index; REM: rapid eye movement sleep; SpO₂: blood oxygen saturation level; *p-values are reported as t-test comparisons and are not controlled for age. [#] n=24; ^a n = 17

Table 3. Autonomic parameters for the two groups for each sleep stage.

	High Altitude				Low Altitude			
	W (n=9)	N2 (n=18)	N3 (n=18)	REM (n=18)	W (n=13)	N2 (n=26)	N3 (n=25)	REM (n=23)
Segment duration (s)	209.75±710.78	6026.94± 539.67	4118.0 ± 539.64	1996.50 ± 539.64	435.62 ± 85.70	4901.08 ± 449.00	5394.92 ± 456.50	1362.61 ± 471.68
RR (s)	0.798±0.027	0.854±0.025	0.835±0.025	0.845±0.025	0.739±0.023	0.831±0.021	0.820±0.021	0.793±0.021
Resp. (s)	3.145±0.138	3.797±0.123	3.599±0.123	3.717±0.123	2.971±0.115	3.797±0.103	3.678±0.103	3.664±0.105
TP (ln ms²)	7.523±0.242	7.681±0.221	7.225±0.221	8.035±0.221	7.179±0.202	7.612±0.184	7.349±0.185	7.742±0.187
VLF (ln ms²)	6.531±0.212	6.448±0.175	5.897±0.175	7.227±0.175	6.183±0.177	6.303±0.146	5.704±0.147	6.701±0.151
LF (ln ms²)	6.283±0.225	6.318±0.192	5.737±0.192	6.283±0.192	5.875±0.188	6.125±0.192	5.737±0.192	6.651±0.192
HF (ln ms²)	6.084±0.316	6.716±0.296	6.457±0.296	6.484±0.296	5.933±0.263	6.809±0.246	6.723±0.247	6.546±0.249
HFnu	0.439±0.043	0.583±0.037	0.654±0.037	0.439±0.043	0.508±0.036	0.650±0.030	0.705±0.031	0.528±0.031
LF/HF	1.059±0.033	0.959±0.027	0.909±0.027	1.054±0.027	1.006±0.028	0.911±0.023	0.868±0.023	0.997±0.028

Notes. Data are presented as mean±standard error estimated from the linear mixed models analysis. ln denotes natural logarithm. RR: interbeat intervals; Resp: respiration intervals; TP: total power; VLF: very low frequency; LF: low frequency; HF: high frequency; HFnu: high frequency normalized units; LF/HF: low frequency to high frequency ratio; Note participants with data available from pre-sleep wakefulness are limited as some children fell asleep during the polysomnography set up.