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

Catherine Mary Hill (PhD, FRCPCH)*[1,2,3] and Romola Starr Bucks (PhD)*[3], Nicola Cellini (PhD) [4], Shayan Motamedi (PhD)[5], Annette Carroll (BSc)[6], Kate Heathcote (FRCS)[7], Rebecca Webster (PhD)[8], David Simpson (PhD)[9]

*Catherine Hill and Romola Bucks are joint first authors

Author affiliations

1. Division of Clinical Experimental Sciences, Faculty of Medicine, University of Southampton, UK
2. Southampton Children's Hospital Department of Sleep Medicine, Southampton, UK.
3. School of Psychological Science, University of Western Australia, Perth, Australia
4. Department of General Psychology, University of Padova
5. Department of Mechanical Engineering, University of Southampton
6. Sleep Disorders Unit, Canberra Hospital, Australia
7. Department of Otolaryngology, Poole General Hospital, UK
8. Laboratory for Cancer Medicine, Harry Perkins Institute of Medical Research and University of Western Australia Centre for Medical Research, Perth, Australia
9. Institute of Sound and Vibration Research, University of Southampton, UK

Address where work was conducted

Universidad Privada de Santa Cruz de la Sierra and Universidad de La Salle, La Paz, Bolivia.

Correspondence address

Dr Catherine Mary Hill. e-mail: cmh2@soton.ac.uk

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.

Conclusions: 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 in 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.

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

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 system.[1] 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 processes.[2] 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 mortality.[3] This is potentially relevant to populations living at high attitude (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 output[4] 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 ago[5] 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 altitude.[9] Furthermore, a correlation between severity of CMS and decreased baroreflex suggests that alteration in the autonomic nervous system may be a biomarker of CMS.

We have reported that Andean HA native children experience additional hypoxic challenge during sleep compared to the waking state.[10] This intermittent hypoxic 'load' during sleep could, in theory, mimic the chronic intermittent hypoxia seen in conditions such as obstructive sleep apnea, leading to increased sympathetic tone[11,12] and, in turn, increased the risk of hypertension, stroke, myocardial infarction[13,14] and, possibly, CMS.

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

Methods

Subjects

As part of a larger study (Development and Sleep at Altitude - DeSAt) investigating the effects of altitude on sleep disordered breathing[26] and neurocognitive performance,[27] 59 children and adolescents from low altitude (LA) Santa Cruz, 500m above sea-level (n_L=32) and La Paz, 3700m above sea-level (n_H=27), Bolivia, underwent a single night of attended polysomnography. Subjects were aged

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

Measures

Socio-demographic data.

Data were collected for mothers of participant children on age of completion of full-time education.

Physical examination

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

Genetics

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

Polysomnography

Attended polysomnography was carried out in an established sleep laboratory setting (Santa Cruz) and in a temporarily adapted facility (La Paz) using computerized ambulatory systems

(Compumedics PS2 system, Melbourne, Australia) according to accepted guidelines.[29] All studies were performed by an experienced polysomnographic technologist (AC). A standard ambulatory sleep montage included electroencephalography (C3/A2, C4/A1, O1/A2, O2/A1) with electrode placement according to the international 10-20 system,[30] electromyography at submentalis and anterior tibialis, bipolar electrooculography, electrocardiography and oxyhaemoglobin saturation monitoring (SpO2). Respiratory inductance plethysmography bands were used to measure abdominal and thoracic excursions and nasal thermistors provided a constant flow monitor. Polysomnographs were scored (AC) based on the established sleep staging[31] and respiratory[32] criteria for pediatrics and all studies were peer reviewed (CMH). All PSG included continuous ECG recording and standard neurophysiological sleep stage scoring based on EEG, EOG, and EMG. Only stages N2, N3, and REM sleep are reported in this report as N1 is a transition state comprising less than 5% of the night.

Data Pre-processing

All polysomnography studies were initially visually assessed to ensure presence of adequate quality ECG and the nasal airflow signals for a minimum of 4 hours of the overnight recording.

Heart-beats were automatically detected in the ECG, sampled at 256 Hz. R-waves were detected after band-pass filtering (0.9-36 Hz, 349^{th} order FIR filter, applied in the forward and reverse direction to avoid phase-shift) using a peak-detector, and then visually checked. Segments of signal where no clear R-waves could be identified were marked as bad data and removed from further analysis (marked as gaps in the data). Automatically identified R-waves were replaced by nearby peaks when deemed incorrect, based on regularity of RR intervals and the expected shape of the QRS complex. The resulting RR-interval time-series was then linearly interpolated to obtain a regularly sampled signal with a sampling rate of 5 Hz. This signal was then high-pass filtered (in the forward and reverse directions) at 0.003 Hz using a first-order Butterworth filter (applied in the forward and reverse directions) to remove its DC component and slow trends.

To account for any potential influence of respiration on HRV, all polysomnograms were automatically inspected for abnormal respiration. For regular (normal) respiratory cycle detection, the

thermistor generated nasal flow signal was first band-pass filtered between 0.08 and 0.8 Hz using a 5th order Butterworth filter to remove the DC component of signal and slow trends and avoid spectral components which are well above the average fundamental frequency of respiration in children (0.23 Hz to 0.36 Hz in children from 4 to 16.[33] Local maxima and minima of the flow signal corresponding to the start and end of expiration/inspiration were identified separately using envelope detectors. The initial sets of extrema detected were refined using amplitude thresholds. This was to avoid peaks and trough with undesirable amplitudes (e.g. a local peak with negative amplitude and troughs with positive amplitudes) and ensure normal respiratory cycle detection. Duration thresholds were then applied to the refined extrema to eliminate those which are abnormally close or distant from one another. Undergoing this procedure resulted in regular (normal) respiratory cycle detection and hence regular respiratory rate quantification. Finally, the editing on both respiratory and ECG signals was combined (logic AND operator) to produce a final edit that ensured that the HRV parameters were extracted from portions of polysomnograms with both normal cardiac patterns and regular respiration.

HRV calculation

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

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 ECG 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: HF/(LF+HF)]
- LF/HF ratio
- Respiratory interval (s)

Note that normalised LF power [LF/(LF+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/HF, [35] 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 results.[36] For each HRV

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

Results

Sample

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

Demographic and daytime systolic blood pressure

Table 1 shows the main demographic and clinical characteristics of the sample. There was no significant difference in age (t_{42}=-1.69, p=.10), gender distribution (χ^2=0.38, p=.54), or genetic ancestry differences of children between altitude locations. Daytime resting systolic blood pressure was significantly higher (Table 1) at high altitude (t_{42}=2.73, p=.009). However, blood pressure is influenced by age, gender and height centile during childhood. Given the absence of normative data for this population the contribution of altitude to blood pressure was explored through an analysis of covariance regression analysis controlling for age, height and gender, as per routine clinical practice. Controlling for age, height and gender, altitude contributed significantly to systolic blood pressure (F(1,39)= 4.757, p=.035), but BMI may be a partial mediator of the effect of altitude on BP as this altitude difference was only at trend level when BMI was controlled for (F(1,39)= 2.693, p=.109)..

Polysomnographic results

We observed some differences in sleep architecture between HA and LA children (Table 2). The ANCOVAs showed that HA children spent more time in N2 (%) than LA children (F(1,41)= 4.740, p=.035), to the detriment of N3 (F(1,41)= 4.013, p=.052). No other significant differences were

observed in sleep architecture. The ANCOVAs also highlighted the expected differences in mean nocturnal oxyhaemoglobin saturation (SpO_2) and respiratory polysomnographic variables at HA. Specifically, HA children had lower mean overnight SpO_2 levels ($F(1,41)= 383.61$, $p<.001$), higher 3% desaturation index ($F(1,38)= 19.63$, $p<.001$) and higher hypopnea index ($F(1,41)=8.455$, $p=.006$), compared to LA children. (see Table 2 and see Hill et al., 2016 for discussion of these results).

Cardiac Autonomic Activity across Sleep Stages and altitude settings

Descriptive values for all the cardiac parameters across the different stages are presented in Table 3. Note that we have a limited number of participants with data available from pre-sleep wakefulness as some children fell asleep during, or shortly after, the polysomnography set up before quality data acquisition was established. Nevertheless, our linear mixed model analysis takes into account missing data and provides a corrected estimate of the mean and standard error of the Wake period

Summary results for RR interval analysis are shown in Fig. 1. LMM on RR showed a main effect of sleep stage ($F(3,100.29)=23.504$, $p<.001$), with a significant lengthening of RR during N2, N3 and REM sleep compared to Wake (all *p's*<.001), and a shortening of RR in REM compared to N2 (p=.008). We also observed a Group × Sleep Stage interaction ($F(3, 100.29)=3.60$, $p=.016$), with a nominal lengthening of RR during Wake and REM sleep in HA compared to LA, but these contrasts did not reach significance ($p=.096$ and $p=.133$, respectively, Figure 1a).

Analysis of the respiratory intervals showed only a main effect of sleep stage ($F(3,101.25)=48.36$, $p<.0001$), with shorter respiratory intervals in Wake compared to all sleep stages (all *p's*<.0001), and a reduction of respiratory intervals from N2 to N3 (p=.011; Figure 1b). No effect of altitude was observed.

A similar result was observed for total power, with a main effect of sleep stage ($F(3, 100.41)=12.520$, $p<.001$), with a significant total power reduction during N3 relative to N2 ($p=.001$), and increased total power during REM sleep relative to W ($p<.001$), N2 ($p=.019$), and N3 ($p<.001$), indicating an increase in heart rate variability during REM sleep. We also observed increased total power during N2 relative to W ($p=.026$). We found a trend for Group × Stage interaction ($F(3,$

100.41)=2.923, p=.056), with a nominal power increase during Wake and REM in HA compared to LA, but these contrasts did not reach the significance (p=.279 and p=.317, respectively, Figure 2a).

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

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

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

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

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

As we observed an increased RR interval in all sleep stages in the HA group (possibly related to the slightly older age of this group), and it has previously been shown that there is a strong dependency of frequency-domain parameters on heart rate,[37-39] we repeated the analysis described above including the mean RR interval for each individual and each sleep period as covariates in the models, using raw values for each HRV parameter. We also repeated the analysis on RR intervals including age

as a covariate. The direction of the results was similar even with this correction (see full analysis in the supplemental material). The previous trend level for total power, Group × Stage interaction was lost (F(3, 100.88)=.553, p=.647), while conversely the Group effect of LF/HF, became significant (F(1,42.73)=5.05, p=.030), showing higher LF/HF ratio at HA compared to LA. Importantly, although the main Group effect for VLF was trending, but still not significant (F(1, 38.77)=3.68, p=.062), the greater VLF power during REM sleep in the HA group remained significant (p = .006).

Discussion

We aimed to investigate cardiac autonomic activity 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 provoke increased sympathetic activity. Furthermore, altitude differences in REM sleep, with characteristic high sympatho-vagal tone, could reveal subtle changes in autonomic regulation. As children have otherwise healthy cardiovascular systems, such alterations could provide early markers of later cardiovascular disease.

Regardless of altitude, children showed marked fluctuations in HRV parameters across sleep stages consistent with previous research[19,20,24] indicating that sleep-dependent modulation of autonomic activity is reliable across altitudes. Specifically, mean RR intervals lengthened during all sleep stages compared to wake, with a peak during N2 sleep and a shortening during REM sleep. On the other hand, total power decreased during N3 and increased dramatically during REM sleep, mainly driven by LF power, which showed a marked decrease in N3 followed by a strong increase in REM. The LF fluctuations also influenced the LF/HF ratio. Indeed, LF/HF was also at its lowest during N3 sleep, consistently with previous reports[40].

Although we filtered our data to exclude periods of irregular breathing, it is possible that respiration might have had a confounding influence[41] on HRV indices, when respiratory rate approaches the boundary between the LF and HF band (0.15 Hz). Respiratory rate is generally higher in children than adults,[42] and our participants exhibited a respiration cycle length of 3.60s to 3.80s across sleep stages. With this range (0.26-0.28 Hz), therefore, confounding in the interpretation of HF during sleep

is not expected to occur.[43] Nonetheless, we cannot completely exclude the possibility that HA may have altered cardiopulmonary coupling due to hypopnea episodes, but this was minimized by only including periods of regular breathing in the analysis. This could be assessed in the future using coherence or cross-power analysis.[44]

Our principle aim was to explore altitude related differences in HRV during sleep. We report for the first time a nominally higher LF/HF ratio across sleep stages in children living at HA compared to LA after adjusting for R-R interval. Post-hoc comparisons showed that this was significantly higher in REM sleep. This finding should be interpreted with caution. While historically this would have been interpreted as representing an increase in cardiac sympathetic activation in REM sleep at HA[45] this assumption has been challenged.[46] Experimental studies show that LF power correlates poorly with cardiac sympathetic nerve activation and that the relationship between sympathetic and parasympathetic activity is non-linear.[46] Some authors have proposed that LF may represent the vagally-mediated function of the baroreflex.[47-49] HA children in this study had higher age and height adjusted daytime blood pressure than LA children. It is possible that higher LF/HF ratio may reflect a greater baroreflex adaptive response to the increased blood pressure that is generally observed during REM sleep.[50] Future study of autonomic function in sleep, using non-invasive technologies such as peripheral arterial tonometry[51] alongside HRV measures, could provide further insights.

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

Firstly, VLF power may be associated with increased metabolic demand and higher energy expenditure[52] at HA as a response to decreased oxygen availability.[53,54] This is likely to be more marked in REM sleep when metabolic demands are higher compared to NREM sleep.[55]

VLF power is also affected by the renin-angiotensin system with an inverse relationship between angiotensin-converting enzyme and VLF power.[2] In the current study, the higher VLF may be the result of lower renin-angiotensin system activity, in response to the higher systolic blood pressures

observed in HA participants. This effect would be predicted to be more prominent during waking states and REM sleep, when the blood pressure is higher.[56]

VLF may also be related to thermoregulatory processes.[2] Specifically, core hypothermia increases VLF power.[57] Importantly, during REM sleep body temperature regulation mechanisms are less sensitive[58] as thermoregulatory centers in the pre-optic hypothalamic nuclei are switched off. This creates a poikilothermic state with absence of shivering in response to cold and lack of peripheral vasodilation in warm environments.[59] As the HA children were studied in a cooler climate (average November temperature 9°C) than LA children (average October temperature 26°C), it is possible that increased VLF activity reflected a compensatory thermoregulatory effect during the recording. However, it should be noted that children were studied in a modern building and slept in appropriate warm bedding so core hypothermia would have been unlikely. Future studies in this field should further explore the relationship between VLF, thermoregulation, and REM sleep.

Finally, it is possible that the high VLF power in REM relates to low SpO_2 during sleep at HA. Several studies have shown that VLF power is higher in patients with mild and severe obstructive sleep apnea syndrome (OSAS) compared to controls, and that OSAS severity is associated with increased VLF power.[60-63] Montemurro et al.[64] suggested that the increased VLF activity in OSAS patients may be due to increased sympathetic activity. Specifically, that during obstructive apneas, the spectral peak usually observed in the LF range shifts into the VLF range. They suggested that this shift is due to the entrainment of the sympathetic discharge by the longer apnea-hyperpnea cycle. HA children in this study had more obstructive hypopnea and central apnea, but not obstructive apnea, than LA children. This is likely to represent a scoring anomaly driven by sleep-related fluctuations in ventilation at HA causing larger drops in SpO_2 due to the steep slope of the oxyhemoglobin dissociation curve at low oxygen tensions.[10,26] These exaggerated swings in SpO_2 may also stimulate the sympathetic nervous system. The trend for higher systolic blood pressure readings in HA children lends further support to the possibility of early subtle sympathetic activation that could presage cardiac autonomic activity during wakefulness noted in adult Andean highlanders.[65,66] While we are not aware of any publications reporting HRV in Andean children, it has been also reported that Himalayan HA resident children have lower heart rates and higher HRV than lowlanders[67] – as also generally observed in this study.

Conclusions

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

Abbreviations

AIM	Ancestry informative marker
ANOVA	Analysis of variance
BMI	Body mass index
CMS	Chronic mountain sickness
DNA	Deoxyribonucleic acid
FIR	Finite impulse response
HA	High altitude
HF	High frequency
HRV	Heart rate variability
LA	Low altitude
LF	Low frequency
LMM	Liner mixed model
PSD	Power Spectral Density
NREM	Non rapid eye movement sleep
REM	Rapid eye movement
SpO_2	Oxyhaemoglobin saturation
VLF	Very low frequency

Acknowledgements:

We are indebted to Dr Ana Baya of the Universidad Privada de Santa Cruz de la Sierra (Santa Cruz); colleagues at the Universidad de La Salle (La Paz) and Dr Mario Camargo Villarreal and team at the Santa Cruz NeuroCenter who provided facilities for the conduct of these studies. The authors gratefully acknowledge the assistance of the Australian Medical Bioinformatics Resource, a National Health and Medical Research Council of Australia Medical Bioinformatics Genomics Proteomics Program. Compumedics, Australia, generously provided a no obligation loan of polysomnography

equipment for the study. We also thank Mr. Fabio Casagrande Hirono and Dr. Dragana Nikolic for their help in data preparation.

Conflict of interest statement: Dr Hill received a no-obligation loan of equipment from Masimo Inc and Compumedics to conduct this study. Co-authors have no conflicts of interest to declare with respect to this publication.

Funding statement: This study was funded by grants from the Gerald Kerkut Trust and the London Law Trust.

References

1. Billman GE, Huikuri HV, Sacha J, Trimmel K. An introduction to heart rate variability: methodological considerations and clinical applications. *Front. Physiol.* 2015;6:55.

2. Kleiger RE, Stein PK, Bigger JT. Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 2005;10(1):88-101.

3. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 2010;141(2):122-131.

4. Tassorelli CS, Nappi RE, Blandini F, Micieli G, Nappi G. Stress and the autonomic nervous system. In: Appenzeller O, ed. *Handbook of clinical neurology.* Vol 741999:307-350.

5. Moore LG. Comparative human ventilatory adaptation to high altitude. *Respir. Physiol.* 2000;121(2-3):257-276.

6. León-Velarde F, Arregui A, Monge C, y Ruiz HR. Aging at high altitudes and the risk of chronic mountain sickness. *Journal of Wilderness Medicine.* 1993;4(2):183-188.

7. León-Velarde F, Maggiorini M, Reeves JT, et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt. Med. Biol.* 2005;6(2):147-157.

8. Virues-Ortega J, Hogan AM, Baya-Botti A, et al. Survival and mortality in older adults living at high altitude in Bolivia: a preliminary report. *J. Am. Geriatr. Soc.* 2009;57(10):1955-1956.

9. Keyl C, Schneider A, Gamboa A, et al. Autonomic cardiovascular function in high-altitude Andean natives with chronic mountain sickness. *J. Appl. Physiol.* 2003;94(1):213-219.

10. Hill CM, Baya A, Gavlak J, et al. Adaptation to life in the high Andes: nocturnal oxyhemoglobin saturation in early development. *Sleep.* 2016;39(5):1001-1008.

11. Bhattacharjee R, Kheirandish-Gozal L, Pillar G, Gozal D. Cardiovascular complications of obstructive sleep apnea syndrome: evidence from children. *Prog. Cardiovasc. Dis.* 2009;51(5):416-433.

12. O'Driscoll DM, Horne RS, Davey MJ, et al. Increased sympathetic activity in children with obstructive sleep apnea: cardiovascular implications. *Sleep Medicine.* 2011;12(5):483-488.

13. McNicholas W, Bonsignore M, B26 MCoECA. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. *Eur. Respir. J.* 2007;29(1):156-178.

14. Zhang W, Si L-Y. Obstructive sleep apnea syndrome (OSAS) and hypertension: pathogenic mechanisms and possible therapeutic approaches. *Ups. J. Med. Sci.* 2012;117(4):370-382.

15. Bilan A, Witczak A, Palusiński R, Myśliński W, Hanzlik J. Circadian rhythm of spectral indices of heart rate variability in healthy subjects. *J. Electrocardiol.* 2005;38(3):239-243.

16. Bonnemeier H, Wiegand UK, Brandes A, et al. Circadian profile of cardiac autonomic nervous modulation in healthy subjects. *J. Cardiovasc. Electrophysiol.* 2003;14(8):791-799.

17. Massin MM, Maeyns K, Withofs N, Ravet F, Gérard P. Circadian rhythm of heart rate and heart rate variability. *Arch. Dis. Child.* 2000;83(2):179-182.

18. Boudreau P, Yeh W-H, Dumont GA, Boivin DB. Circadian variation of heart rate variability across sleep stages. *Sleep.* 2013;36(12):1919.

19. Busek P, Vankova J, Opavský J, Salinger J, Nevsimalova S. Spectral analysis of heart rate variability in sleep. *Physiol. Res.* 2005;54(4):369.

20. de Zambotti M, Cellini N, Baker FC, Colrain IM, Sarlo M, Stegagno L. Nocturnal cardiac autonomic profile in young primary insomniacs and good sleepers. *Int. J. Psychophysiol.* 2014;93(3):332-339.

21. de Zambotti M, Covassin N, Cellini N, Sarlo M, Torre J, Stegagno L. Hemodynamic and autonomic modifications during sleep stages in young hypotensive women. *Biol. Psychol.* 2012;91(1):22-27.

22. Villa MP, Pagani J, Paggi B, Massa F, Ronchetti R, Calcagnini G. Effects of sleep stage and age on short-term heart rate variability during sleep in healthy infants and children. *Chest.* 2000;117(2):460-466.

23. Tobaldini E, Nobili L, Strada S, Casali KR, Braghiroli A, Montano N. Heart rate variability in normal and pathological sleep. *Front. Physiol.* 2013;4:294.

24. Cellini N, Whitehurst LN, McDevitt EA, Mednick SC. Heart rate variability during daytime naps in healthy adults: Autonomic profile and short-term reliability. *Psychophysiology.* 2016;53(4):473-481.

25. Cellini N, Torre J, Stegagno L, Sarlo M. Cardiac autonomic activity during daytime nap in young adults. *J. Sleep Res.* 2017.

26. Hill CM, Carroll A, Dimitriou D, et al. Polysomnography in Bolivian children native to high altitude compared to children native to low altitude. *Sleep.* 2016;39(12):2149-2155.

27. Hill CM, Dimitriou D, Baya A, et al. Cognitive performance in high-altitude Andean residents compared with low-altitude populations: from childhood to older age. *Neuropsychology.* 2014;28(5):752.

28. Hoggart CJ, Shriver MD, Kittles RA, Clayton DG, McKeigue PM. Design and analysis of admixture mapping studies. *Am. J. Hum. Genet.* 2004;74(5):965-978.

29. Loughlin G, Brouillette R, Brooke L, et al. Standards and indications for cardiopulmonary sleep studies in children. *Am. J. Respir. Crit. Care Med.* 1996;153(2):866-878.

30. Klem GH, Luders HO, Jasper HH, Elger C. The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. *Electroencephalogr. Clin. Neurophysiol. Suppl.* 1999;52:3-6.

31. Rechtschaffen A, Kales A. *A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects.* . Bethesda, Md.: U. S. National Institute of Neurological Diseases and Blindness, Neurological Information Network; 1968.

32. Iber C. *The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications*: American Academy of Sleep Medicine; 2007.

33. Wallis LA, Healy M, Undy MB, Maconochie I. Age related reference ranges for respiration rate and heart rate from 4 to 16 years. *Arch. Dis. Child.* November 1, 2005 2005;90(11):1117-1121.

34. Camm AJ, Malik M, Bigger J, et al. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 1996;17(3):354-381.

35. Burr RL. Interpretation of normalized spectral heart rate variability indices in sleep research: a critical review. *Sleep.* 2007;30(7):913-919.

36. Baayen RH, Davidson DJ, Bates DM. Mixed-effects modeling with crossed random effects for subjects and items. *J. Mem. Lang.* 2008;59(4):390-412.

37. Kazmi SZH, Zhang H, Aziz W, et al. Inverse correlation between heart rate variability and heart rate demonstrated by linear and nonlinear analysis. *PLoS ONE.* 2016;11(6):e0157557.

38. Monfredi O, Lyashkov AE, Johnsen A-B, et al. Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. *Hypertension.* 2014:HYPERTENSIONAHA. 114.03782.

39. Sacha J, Pluta W. Alterations of an average heart rate change heart rate variability due to mathematical reasons. *Int. J. Cardiol.* 2008;128(3):444-447.

40. Burgess HJ, Penev PD, Schneider R, Van Cauter E, Estimating cardiac autonomic activity during sleep: impedance cardiography, spectral analysis, and Poincaré plots. *Clin Neurophysiol.* 2004;115(1):19-28.

41. Beda A, Simpson DM, Carvalho NC, Carvalho ARS. Low-frequency heart rate variability is related to the breath-to-breath variability in the respiratory pattern. *Psychophysiology.* 2014;51(2):197-205.

42. Fleming S, Thompson M, Stevens R, et al. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. *The Lancet.* 2011;377(9770):1011-1018.

43. Trinder J, Kleiman J, Carrington M, et al. Autonomic activity during human sleep as a function of time and sleep stage. *J. Sleep Res.* 2001;10(4):253-264.

44. Thomas RJ, Mietus JE, Peng C-K, Goldberger AL. An electrocardiogram-based technique to assess cardiopulmonary coupling during sleep. *Sleep.* 2005;28(9):1151-1161.

45. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability:standards of measurement, physiological interpretation, and clinical use. *Circulation*. 1996; 93, 1043–1065.

46. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Frontiers in Physiology*. 2013;4:26.
47. Nisbet LC, Yiallourou SR, Walter LM, Horne RS. Blood pressure regulation, autonomic control and sleep disordered breathing in children. *Sleep Med Rev*. 2014;18(2):179-89.
48. Goldstein DS, Bentho O, Park MY, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 2011;96(12):1255-1261.
49. Reyes del Paso GA, Langewitz W, Mulder LJ, Roon A, Duschek S. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology*. 2013;50(5):477-487.
50. Rahman F, Pechnik S, Gross D, Sewell L, Goldstein DS. Low frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Clin. Auton. Res.* 2011;21(3):133-141.
51. Trinder J, Waloszek J, Woods MJ, Jordan AS. Sleep and cardiovascular regulation. *Pflugers Arch.* 2012;463(1):161-168.
52. Millis RM, Austin RE, Hatcher MD, Bond V, Goring KL. Metabolic energy correlates of heart rate variability spectral power associated with a 900-calorie challenge. *Journal of Nutrition and Metabolism.* 2011;2011.
53. Palmer BF, Clegg DJ. Ascent to altitude as a weight loss method: the good and bad of hypoxia inducible factor activation. *Obesity.* 2014;22(2):311-317.
54. Hill N, Stacey M, Woods D. Energy at high altitude. *J. R. Army Med. Corps.* 2011;157(1):43-48.
55. Sharma S, Kavuru M. Sleep and metabolism: an overview. *International journal of endocrinology.* 2010;2010.
56. Trinder J, Waloszek J, Woods MJ, Jordan AS. Sleep and cardiovascular regulation. *Pflugers Arch.* 2012;463(1):161-168.
57. Fleisher LA, Frank SM, Sessler DI, Cheng C, Matsukawa T, Vannier CA. Thermoregulation and heart rate variability. *Clin. Sci.* 1996;90(2):97-103.
58. Bach V, Telliez F, Libert J-P. The interaction between sleep and thermoregulation in adults and neonates. *Sleep Med. Rev.* 2002;6(6):481-492.
59. Okamoto-Mizuno K, Mizuno K. Effects of thermal environment on sleep and circadian rhythm. *J. Physiol. Anthropol.* 2012;31(1):14.
60. Shiomi T, Guilleminault C, Sasanabe R, Hirota I, Maekawa M, Kobayashi T. Augmented very low frequency component of heart rate variability during obstructive sleep apnea. *Sleep.* 1996;19(5):370-377.
61. Aydin M, Altin R, Ozeren A, Kart L, Bilge M, Unalacak M. Cardiac autonomic activity in obstructive sleep apnea: time-dependent and spectral analysis of heart rate variability using 24-hour Holter electrocardiograms. *Tex. Heart Inst. J.* 2004;31(2):132.
62. Wakai M, Samejima Y, Goshima K, Yamamoto J. Altered heart rate variability in severe sleep apnea syndrome. *Sleep Biol. Rhythms.* 2004;2(1):87-88.
63. Song M-K, Ha JH, Ryu S-H, Yu J, Park D-H. The effect of aging and severity of sleep apnea on heart rate variability indices in obstructive sleep apnea syndrome. *Psychiatry investigation.* 2012;9(1):65-72.
64. Montemurro LT, Floras JS, Picton P, et al. Relationship of heart rate variability to sleepiness in patients with obstructive sleep apnea with and without heart failure. *J. Clin. Sleep Med.* 2014;10(3):271.
65. Passino C, Bernardi L, Spadacini G, et al. Autonomic regulation of heart rate and peripheral circulation: comparison of high altitude and sea level residents: Portland Press Limited; 1996.
66. Bernardi L, Passino C, Spadacini G, et al. Cardiovascular autonomic modulation and activity of carotid baroreceptors at altitude. *Clin. Sci.* 1998;95(5):565-573.
67. Zhuang J, Zhu H, Zhou Z. Reserved higher vagal tone under acute hypoxia in Tibetan adolescents with long-term migration to sea level. *Jpn. J.Physiol.* 2002;52(1):51-56.

Figure Captions

Figure 1: Box plot for inter-beat time (RR) and respiratory intervals across sleep stages in low altitude (LA) and high altitude (HA) children.

Figure 2: 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

Table 1. Demographic and clinical characteristics by altitude.

	Low altitude **N = 26**	**High altitude** **N= 18**	***p-value****
Gender (M: F)	12:14	10:8	.76
Mean age (years)	10.8 (2.9)	12.3 (3.1)	.10
BMI (kg/m^2)	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 altidude.

	Low altitude N = 26	**High altitude N= 18**	***p-value***
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 SpO_2	97.3 (0.8)	88.5 (2.0)	<.001
Min SpO_2	90.3 (3.6)	78.2 (5.8) $^{\alpha}$	<.001
3% ODI	0.9 (0.8) $^{\neq}$	3.4 (2.6) $^{\alpha}$	<.001
OAHI 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; OAHI: Obstructive Apnea Hypopnea Index; REM: rapid eye movement sleep; SpO_2: blood oxygen saturation level; *p-values are reported as t-test comparisons and are not controlled for age. $^{\neq}$ n=24; $^{\alpha}$ 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 ms^2)	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 ms^2)	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 ms^2)	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 ms^2)	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. In 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.

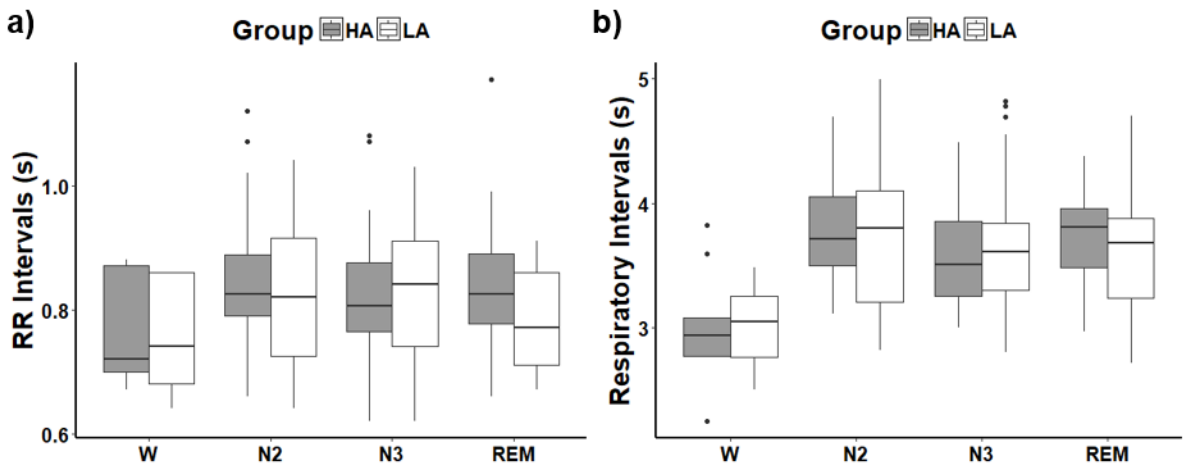

Figure 1. Box plot for interbeat time (RR) and respiratory intervals across sleep stages in low altitude (LA) and high altitude (HA) children.

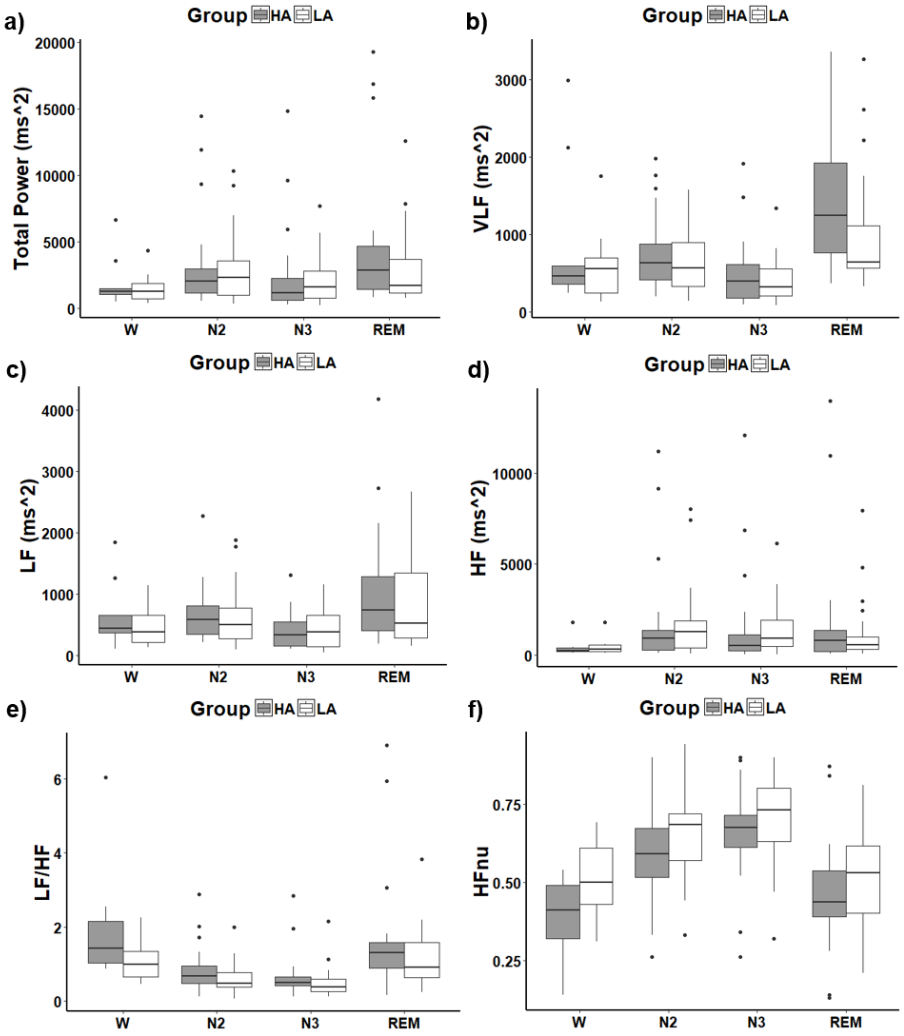

Figure 2. Box plots of heart rate variability components across sleep stages in low altitude (LA) and high altitude (HA) children. VLF: very low frequency; LF: low frequency; HF: high frequency; HFnu: high frequency normalized units; LF/HF: low frequency to high frequency ratio.