DISSERTATION

Autonome und endokrine Folgen eines Mittagsschlafs in simulierter Höhe bei gesunden Flachlandbewohnern

Autonomic and endocrine consequences of a daytime napping at simulated altitude on healthy lowlanders

zur Erlangung des akademischen Grades

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Table of Contents

List of tables

List of figures

List of abbreviations

AHI Apnea-Hypopnea Index

BIA Bioelectrical Impedance Analysis

BMI Body Mass Index

BW Body Water

DBPr Diastolic Blood Pressure right arm

EBW Extracellular Body Water

ECG Electrocardiogram

EEG Electroencephalogram

EMG Electromyography

EPO Erythropoietin

FFM Fat Free Mass

FiO_2 Fraction of inspired Oxygen

FT Fat Mass

GGT Gamma-glutamyl transferase

GOT Glutamic Oxaloacetic Transaminase

GPT Glutamic Pyruvic Transaminase

HbA1c Glycated hemoglobin

HDL High-Density Lipoprotein

HF High Frequency

HR Heart Rate

HRV Heart Rate Variability

LDL Low-Density Lipoprotein

LF Low Frequency

m asl Meters above sea level

MCHC Mean Corpuscular Hemoglobin Concentration

MCV Mean Corpuscular Volume

N1 NREM stage 1

N2 NREM stage 2

N3 NREM stage 3

NH13 Normobaric Hypoxia FiO_2 12.5%

NH15 Normobaric Hypoxia FiO_2 14.7%

NN Normobaric Normoxia FiO_2 20.9%

NREM Non-REM sleep

PBI Periodic Breathing Index

pNN50 Proportion of successive NN intervals that exceeds more than 50 ms

PNS Parasympathetic Nervous System index

PVT Psychomotor Vigilance Task

RDW Red Blood Cell Distribution Widht

REM Rapid Eye Movement sleep

RMSSD Root Mean Squeare of Sucessive RR Interval Differences

SBPr Systolic Blood Pressure right arm.

SD1 Standard Deviation of the instantaneous beat-to-beat variability

SD2 Standard Deviation of the continuous long-term variability

SI Baevsky's Stress Index

SNS Sympathetic Nervous System index

SOL Sleep Onset Latency

SpO_2 Peripheral Oxygen Saturation

TSH Thyroid-Stimulating Hormone

TST Total Sleep Time

Ur. pH Urine pH

Ur.den. Urine density

UrA Uric Acid

VLF Very Low Frequency

WASO Wake After Sleep Onset

WHR Waist/Hip ratio

Abstract

Altitudinal hypoxia is a problem of growing interest, given the increased population that voluntarily or involuntarily is exposed to this condition. Despite the fact that a hypoxic context could have marked the dawn of humanity, most humans currently live below 2500 m asl in the so-called lowlands. Although physiological adaptive mechanisms have allowed the incursion of population groups above that level, non-acclimatized lowlanders may suffer maladaptive changes. Among these changes, sleep disturbances stand out, leading to impaired physical and mental performance. Hence, implementing countermeasures is desirable to improve the performance and well-being of these vulnerable populations.

Napping has been recognized as an effective strategy against sleep disturbances under normoxic conditions; yet, its use under altitudinal hypoxia lacks experimental support. This gap is due, among other things, to a poor understanding of the physiological nature of a daytime nap under hypoxic condition. Thus, here our goal was to analyze cognitive, endocrine, and autonomic changes produced around a daytime nap under normobaric hypoxia.

Our results show a sleep architecture alteration in individuals when napping in normobaric hypoxia, including less REM and N3 sleep and a concomitant increase in N2, relative to individuals in the normoxic condition. As expected, there was a classic dose-dependent endocrine response with an elevation of EPO and cortisol. These changes were strongly correlated with indirect markers of autonomic activity, namely, Heart Rate Variability parameters. There was an increase in cardiovagal tone while napping, suggested by larger RR intervals and RMSSD; this increase was less in hypoxia. Similarly, sympathetic activity after a 90-min nap increased. These drastic neuroendocrine changes did not have significant cognitive repercussions.

Regarding respiratory activity, a notable difference between men and women was evident. Men exhibited high peripheral oxygen desaturation and dose-dependent periodic breathing. The significant findings in men (and one woman with polycystic ovary) point to a potential role of testosterone in modifying the ventilatory threshold and the CO_2 reserve.

Considering our results, the prescription of a nap as a countermeasure against night sleep disturbances at altitude should be limited, given the little evidence favoring its implementation. Additional strategies, such as oxygen administration, may be necessary.

Zusammenfassung

Hypoxie in Höhenlagen ist ein Problem von wachsendem Interesse, da immer mehr Menschen freiwillig oder unfreiwillig diesem Zustand ausgesetzt sind. Trotz der Tatsache, dass ein hypoxischer Kontext die Anfänge der Menschheit markiert haben könnte, leben die meisten Menschen heute in den so genannten Tieflandgebieten unterhalb von 2500 m ü. NN. Obwohl physiologische Anpassungsmechanismen das Vordringen von Bevölkerungsgruppen oberhalb dieses Niveaus ermöglicht haben, können nicht akklimatisierte Tieflandbewohner unter maladaptiven Veränderungen leiden. Zu diesen Veränderungen gehören vor allem Schlafstörungen, die zu einer Beeinträchtigung der körperlichen und geistigen Leistungsfähigkeit führen. Aus diesem Grund ist es wünschenswert, Gegenmaßnahmen zu ergreifen, um die Leistungsfähigkeit und das Wohlbefinden dieser anfälligen Bevölkerungsgruppen zu verbessern.

Obwohl das Mittagschlaff unter normoxischen Bedingungen als wirksame Strategie gegen Schlafstörungen anerkannt ist, fehlt für seine Anwendung unter Höhenhypoxie die experimentelle Unterstützung. Dieser Mangel ist unter anderem auf die mangelnde Kenntnis der physiologischen Natur eines Tagesschlafs unter hypoxischen Bedingungen zurückzuführen. Aus diesem Grund war es das Ziel der vorliegenden Untersuchung, die kognitiven, endokrinen und autonomen Veränderungen zu analysieren, die bei einem Tagesschlaf unter normobarer Hypoxie auftreten.

Die Ergebnisse der vorliegenden Studie zeigen eine Veränderung der Schlafarchitektur beim Mittagsschlaf unter normobarer Hypoxie mit einer Abnahme des REM- und N3-Schlafs und einer gleichzeitigen Zunahme des N2-Schlafs im Vergleich zur normoxischen Kontrolle. Wie erwartet, kam es zu einer klassischen dosisabhängigen endokrinen Reaktion mit einem Anstieg von EPO und Cortisol. Diese Veränderungen korrelierten stark mit indirekten Markern der autonomen Aktivität, d. h. mit den Parametern der Herzfrequenzvariabilität. Obwohl der kardiovagale Tonus während des Mittagsschlaf anstieg, was durch den Anstieg des RR-Intervalls und der RMSSD belegt wird, war dieser Anstieg bei Hypoxie geringer. In ähnlicher Weise stieg die sympathische Aktivität nach einem 90-minütigen Mittagsschlaf an. Diese neuroendokrinen Veränderungen hatten keine signifikanten kognitiven Auswirkungen.

In Bezug auf die Atmungsaktivität war ein bemerkenswerter Unterschied zwischen Männern und Frauen festzustellen, da die Männer eine hohe periphere

Sauerstoffentsättigung aufwiesen und auch eine dosisabhängige periodische Atmung entwickelten. Die signifikanten Befunde bei Männern (und einer Frau mit polyzystischem Ovar) deuten auf eine grundlegende Rolle des Testosterons bei der Veränderung der ventilatorischen Schwelle und der CO_2-Reserve hin.

In Anbetracht unserer Ergebnisse sollte die Verschreibung eines Mittagsschlafs als Gegenmaßnahme gegen nächtliche Schlafstörungen in der Höhe begrenzt werden, da es nur wenige Belege für ihre Anwendung gibt. Möglicherweise sind ergänzende Strategien wie die Verabreichung von Sauerstoff erforderlich.

1 Introduction

1.1 Altitudinal hypoxia: an ancestral physiological challenge

Altitudinal hypoxia has been a major environmental challenge shaping the adaptive mechanisms of human physiology since its dawn [1]. Evolutionary theorists point to significant geological changes in the Rift Valley approximately 4 million years ago that led to our ancestors being exposed to altitudes exceeding 2500 m asl [1]. As higher elevation is intrinsically associated with lower availability of oxygen, early human evolution must have been linked to improving endurance performance and tolerance to hypoxia. Nevertheless, the dispersion of our species around the planet led it to settle in less challenging regions, including coasts and other lowland areas where oxygen availability is more favorable. As a result, and along other cultural evolutionary processes, such as sailing and trading, major urban centers are currently at sea level, and 98.93% of humanity lives in regions considered low altitude (below 2500 m asl) [2]. Remarkably, several environmental and sociopolitical factors are forcing humankind to expose again to altitudinal stress.

One of these factors is the forced displacement that causes lowlander populations to be temporarily or permanently exposed to high altitudes. A notable example occurs in Colombia, the country with the highest internal migration on the planet [3]. Millions of lowlanders have been forced to move to high-altitude cities, primarily the capital of the country (Bogotá) settled at over 2600 m asl, which usually offer greater socio-political stability. This internal context is compounded by the mobilization of Venezuelans who, due to socio-economic instability in their country, have migrated from their lowlands (the ten most populated cities are located below 1000 m asl) to other areas, including the Colombian Andes.

From another perspective, millions of people around the globe happily and voluntarily expose themselves to high mountain conditions for pleasure or work. This includes the growing interest in climbing and high-altitude sports (e.g., snowboarding and hiking), as well as populations exposed to hypoxia in aircraft cabins. For example, over 12 million travelers are in the air daily which exceeds the population of many countries, and has earned it the name of Flyland, the country in the air (2020 IATA report). Importantly, the

conditions in aircrafts emulate those of mountains at approximately 2400 m asl, barely below what is considered high altitude. Yet, many physiological processes are known to be affected during flight [4].

Finally, and of growing concern, is population displacement because of climate change [5]. Due to rising sea levels, increased climatic phenomena such as storms, hurricanes, earthquakes, and rising temperatures or droughts, millions of people are forced to move from their usual places of residence every year [5]. This has led the Institute of Economics and Peace to predict that by 2050, 1.3 billion people will have been forced to move due to climate change. Although human migration is a complex phenomenon rooted in the very history of our evolution [1], making it difficult to predict, it is not surprising that migrating to the mountains avoiding coastal phenomena and high temperatures is an option.

1.2 Neuroendocrine response to hypoxic stress and sleep

At the heart of the hypoxic challenge is the need to meet the demands of metabolic processes. Given its central role in the physiology of the system, the metabolism is regulated by neuroendocrine components that balance energy expenditure and storage for example during exposition to extreme temperatures [1]. As for the neural component, the autonomic nervous system exerts control via a sympathetic branch (in charge of ergotropic or energy-releasing contexts) and a parasympathetic branch (in charge of trophotropic or energy-storing contexts). Moreover, the endocrine component that responds to hypoxic stress involves renal (erythropoietin) and adrenal (cortisol and catecholamines) hormones that ensure energy mobilization in the face of the stressor [1,6].

One of the most intriguing metabolic scenarios in which this neuroendocrine control is exerted is during sleep, a state with trophotropic and restorative properties [7]. These properties have been supported by some findings [8], including:

- The neural orexinergic system that promotes the search for energy resources (i.e., appetite) is attenuated during sleep.
- A metabolite of the ATP molecule, namely the adenosine, is one of the main somnogens.

- A metabolic clearance layout of the central nervous system (glymphatic system) is activated mainly during sleep.

The trophotropic and restorative function of sleep has been directly linked to the phase known as NREM (Non-Rapid Eye Movement), especially the third stage (N3), which is characterized by the presence of electrical and vascular waves (delta rhythm) that clears the brain interstitium [7]. As expected, a predominance of the parasympathetic component accompanies the changes described during NREM sleep. In contrast to N3, the subsequent sleep phase, namely rapid eye movement sleep (hereafter REM), is characterized by an autonomic shift towards ergotropism and, thus, sympathetic dominance [8]. This tropho- and ergo-trophic sleep oscillation explains that NREM predominates at the beginning of sleep, where homeostatic pressure is high [8]. Then, as the restorative process advances and the homeostatic pressure decreases, REM sleep prevails [8].

As for the endocrine components that respond to hypoxic stress, a morning acrophase for cortisol and an evening acrophase for erythropoietin cause nighttime sleep to be accompanied by the lowest levels of these two hormones [6]. This would be in line with the parasympathetic predominance of NREM sleep, whose metabolic demands are lower than those of wakefulness. The disruption of this endocrine components, added to the autonomic previously mentioned, explains the sleep disturbances inherent to conditions such as shift work, jetlag, or exposure to high altitude.

The connection between altitudinal hypoxia and nocturnal sleep is well documented following by the profound impact of hypobaric hypoxia on physiological parameters during sleep [9–11]. Exposure to high altitude characterized by rise of cortisol and EPO may, for example, decrease the amount of REM and N3 sleep [6,12]. Consistent with this decrease in N3, there is a withdrawal of cardiovagal influx in high-altitude sleep [9]

Changes in respiratory regulation also accompany autonomic changes in cardiovascular regulation [9]. Drastic modifications in ventilatory control lead to the appearance of periodic breathing and central apnea [9,13]. These sleep disturbances could explain the appearance of the characteristic symptoms of mountain sickness, ranging from decreased physical or mental performance to alterations that compromise the general state, such as the brain edema [14].

In this context, developing counterregulatory measures against the impairments induced by disrupted sleep is critical to improve the well-being of the population voluntarily or involuntarily exposed to altitudinal hypoxia. Pharmacological and behavioral approaches to improve sleep quality are certainly available; yet a slightly neglected strategy of physiological nature emerges as a strong candidate to protect against sleep disturbances, the daytime nap.

1.3 Daytime Napping as a Countermeasure for Sleep Disturbances

Implementing a daytime nap to improve physical and mental well-being is of interest in public health given its potential positive impact for a society increasingly burdened by the lack of quality sleep [15]. Napping has been associated with cardiovascular, or metabolic dysfunctions [16], yeti t clearly has a physiological nature. For instance, a propensity to sleep two hours after the noon can be predicted by the classic two-process model, which combines homeostatic pressure with the circadian component of sleep [17]. Moreover, its implementation may improve physical (aerobic endurance and muscle power) and mental (attention and memory) performance [18,19].

Despite the evidence about the potential benefit of daytime napping, physiological description is limited, and an unrestricted extrapolation of nighttime sleep traits are typically implied [8]. As previously discussed, a differential hormonal and autonomic activity between day and night suggests that daytime napping may exhibit contrasting changes with respect to nighttime [6,20]. Moreover, the evaluation of the daytime nap in conditions of altitudinal hypoxia is limited. This lack of studies in a process with high potential and impact highlights the need for further experimental support. For this reason, the objective of the present work was to carry out a comprehensive evaluation of the neural (autonomic tone, sleep architecture, and cognition), physiological (oxygen saturation, ventilatory pattern, and temperature), and endocrine (release of EPO, cortisol, and catecholamines) changes of volunteers taking a nap at a simulated altitude.

Two factors could interact significantly with the characterization of daytime nap. First, we would like to determine whether there is a dose-dependent relationship, so we evaluated napping at simulated altitudes of 2600 m and 4000 m asl. Second, we evaluated individuals of both sexes given potential differences [11].

2 Methods

2.1 Participants: screening and ethical considerations

A total of 20 volunteers were evaluated for eligibility following the inclusion criteria: men and women, 18 to 45 years old, with a normal nutritional balance evident by a Body Mass Index between 20 to 28 kg/m^2 and a standard body composition. Exclusion criteria included: native highlanders, postoperative phases, acute and chronic infections, hematologic disorders, diabetes, thyroid disorders, dyslipidemia, cardiac arrhythmias, hepatic or renal dysfunction, sleep apnea, insomnia, somnolence, exposition to high altitude (more than 2500 m asl) within the last six months before inclusion in the study, plane travel two weeks before enrolment, cardiac or pulmonary diseases, migraine, smoking habit, high-performance athlete, participation in a weight reduction program, change in body weight of more than 2 kg in the previous month of the study, incapacity or other circumstances that do not allow the subject to fully understand the nature, importance, and scope of this study, and drug or alcohol abuse. An open call through Charité outlets was launched to recruit volunteers. A medical doctor screened those interested in participating during a visit to the Charité Experimental and Clinical Research Center under fasting conditions. This visit included anamneses, physical examination, administration of questionnaires to evaluate insomnia (>15 points in the Insomnia Sleep Index) and sleep apnea (>2 points in the STOP-band score), a 12-lead standard ECG, Bioelectrical Impedance Analysis, blood sample draw, and urine specimen collection. After evaluation, two volunteers were excluded (one nutritional disbalance and one acute infection with COVID-19), and three declined to participate, leaving a total of 15 volunteers to be enrolled in the experimental phase. Before participation, each volunteer signed the informed consent previous explanation of the procedures and their implications. After participation, volunteers received monetary compensation for their time. All experimental methods and data management protocols were reviewed and approved by the Ethics Committee of Charité Universitätsmedizin Berlin (N°. EA1/226/19). The total sample size of participants followed the rule of thumb for pilot studies [21]. The protocol was registered in Clinicaltrials.gov under the identifier NCT04146857.

2.2 Experimental design

A cross-over design was chosen because the primary outcomes are more stable within volunteers than between volunteers. Each volunteer was exposed to three distinct levels of FiO_2 in a random sequence assigned by Randomizer ® v. 2.1.0 (Factor = sex; method = minimization) and remained blinded about that condition. On the first day, the volunteer was evaluated under one of these FiO_2, repeating the procedures with other levels seven and fourteen days later until completing all the levels. One week between experiments was established to avoid residual effects in the subsequent evaluation. Likewise, the same day of the week was intended to minimize the impact of a circaseptan rhythm. The night before the experiment, all volunteers followed a sleep restriction protocol by going to bed two hours later than usual but waking up at the regular time. The goal was to increase the chances of inducing napping at noon. On the day of the experiment, all volunteers arrived at the laboratory at 10:00 am for an initial and general evaluation, entering the bedroom around 10:30 am to set and start the polygraphy recording and take a cognitive test (**Figure 1**). 10 minutes before turning off the bedroom lights the volunteer was instructed to lie supine, quiet, and awake. Then, the light bedroom was turned off from 12:00 m until 1:30 pm, constituting a 90-minutes nap opportunity period followed by 5 minutes for full awakening. Then, the volunteer was instructed again to lie supine, quiet, and awake for 10 minutes. A blood sample was collected at the end of this 10 minutes phase. Finally, the volunteer repeated the cognitive test, followed by a final and general evaluation, including a Lake Louise AMS score, and the experiment was concluded.

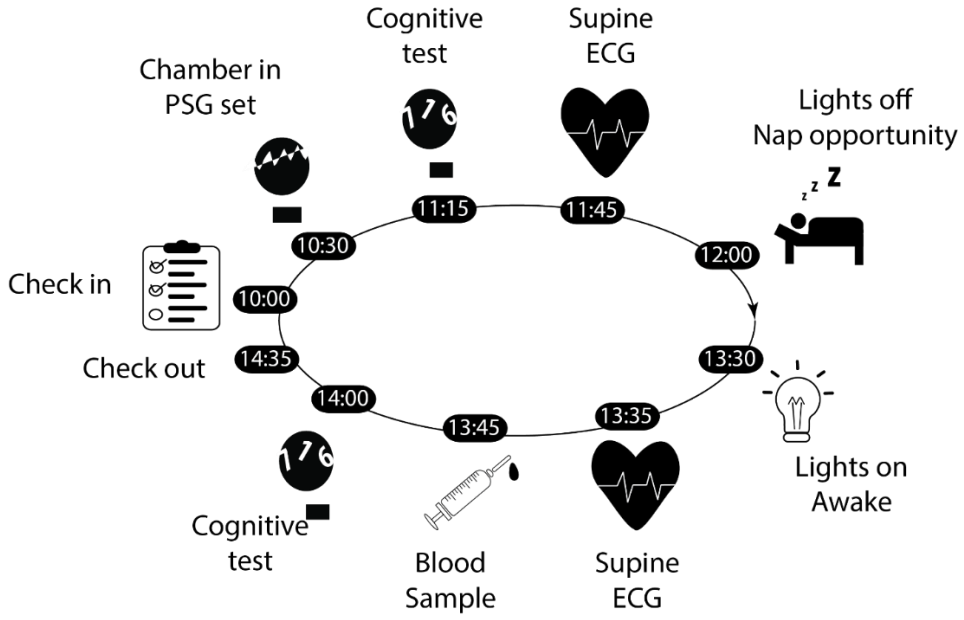

Figure 1. Experimental protocol

2.3 Normobaric hypoxia

A bedroom in the Experimental Clinical Research Center laboratory at the Buch Campus of Charité was set to induce hypoxia. For each visit, the conditions were adjusted according to the random sequence established for every volunteer. Hence, nitrogen gas was introduced to the bedroom, changing the FiO_2 to 14.7 % (simulating 2660 m asl = NH15 condition) or to 12.5% (simulating 4000 m asl = NH13 condition). The control set corresponded to the natural conditions in Berlin (35 m asl, FiO_2 20.9% = NN condition). Additionally, to ensure that the temperature was appropriate for sleeping, the bedroom temperature was set to 22°C. Continuous measurement of O_2 and CO_2 percentages, temperature, barometric pressure, and humidity in the room was monitored. A more detailed description of the Berlin-Buch Low Oxygen System has been previously published [22].

2.4 Body composition analysis

A Bioelectrical Impedance Analysis (BIA) was conducted to determine the body composition of the volunteers using the BIACorpus RX Spectral (MEDICAL Healthcare GmbH, Karlsruhe, Germany). This multi-frequency BIA device applied 50 kHz AC by electrodes placed on the wrists and ankles and measured electrical currents 5 cm apart. The resistance measurements were exported to the proprietary software BodyComp v. 8.3 to calculate the percentual body composition and generate a report.

2.5 Sleep monitoring and staging

The sleep evaluation was done in two ways. First, to evaluate sleep duration and quality on the days around the experiment and compliance with the restriction protocol the night before, an actigraphy recording was collected three days before and after each visit. The data was obtained by an ActiGraph GT3X and processed on 60 s epochs by the Sadeh algorithm in the proprietary software Actilife v. 6.13.3 (ActiGraph, Pensacola, FL, USA). Second, to stage the nap, a polysomnographic recording was obtained with a BWIII PSG Plus Sleep System™ and processed using the proprietary software BWAnalysis v 1.98.0.98 (Neurovirtual, Fort Lauderdale, FL, USA) according to the Manual for the

Scoring of Sleep and Associated Events by the American Academy of Sleep Medicine, v. 2.6. All signals were sampled at 2000 Hz and stored at 500 Hz. EEG signals were filtered with a 0.03 high-pass filter and a 35 Hz low-pass filter. EMG signals were filtered with a 10 Hz high-pass filter and a 70 Hz low-pass filter.

2.6 Heart Rate Variability (HRV) Analysis

To evaluate the HRV, an ECG-modified chest lead (MCL-DII) was set and recorded by the BWIII PSG Plus Sleep System™ with a 0.03-100 Hz pass-band filter. Data were exported in European Data Format (edf) to LabChart Pro software v. 8.1.16 (ADInstruments, Castle Hill, NSW, Australia) for detecting RR intervals using the HRV Module v. 2.0.3. After the algorithmic detection, a visual inspection was executed, and manual correction if needed was applied according to the HRV Task force guidelines[23]. 5-min intervals were extracted from the supine phases before and after the nap, and from representative sleep stages during the nap. These intervals were processed in Kubios HRV Standard v. 3.4.2 software (University of Eastern Finland, Kuopio, Finland) [24] with a 4 Hz interpolation rate to obtain mean RR interval (RR), mean Heart Rate (HR), Root mean square of successive RR interval differences (RMSSD), Poincaré plot index of the Standard Deviation of the instantaneous beat-to-beat variability (SD1) and the continuous long-term variability (SD2), and the Baevsky's stress index (SI). A proprietary computing of RR, RMSSD, and SD1 compared each parameter with the normal population values to yield a Parasympathetic nervous system index (PNS). This PNS index represents how many standard deviations the evaluated volunteer has above or below the mean of the population in resting conditions. A similar computation with HR, SI, and SD2 is applied to obtain the Sympathetic nervous system index (SNS).

2.7 Blood Analysis

Fifteen minutes after napping, three 5-mL blood samples were collected for biochemical analysis. A complete sample was sent to the laboratory for hemogram analysis. The remaining samples were centrifuged immediately to obtain plasma (EDTA-sample) and serum. Samples were adequately cooled and sent for chromatographic analysis in the

laboratory. Serum levels of erythropoietin (EPO), and cortisol, and plasmatic levels of epinephrine, norepinephrine, and dopamine were reported. Because dopamine and epinephrine were reported as categorical parameters, an ordinal categorization was applied following a threshold for high values of dopamine (60 ng/L) and epinephrine (30 ng/L).

2.8 Cognitive evaluation

Cognitive evaluation of memory, attention, vigilance, and other executive functions was done with the following sequence: forward digit span test, backward digit span test, Color-word Stroop test, and 5-min version of the Psychomotor Vigilance Task (PVT) [25]. To collect and compute the performance of each volunteer, the Psychology Experiment Building Language software v. 2.1 was implemented [26]. The forward digit span test consisted of an initial presentation of three digits which disappeared after 5 seconds. The volunteer was asked to remember and type the same sequence and was granted two opportunities to get it right. In that case, a new series of four digits was presented. The procedure was repeated sequentially to offer a maximum of nine digits when the test was stopped. The maximum number of digits remembered for the volunteer was computed. The backward digit span test was like the forward test, but the volunteer was instructed to type the sequence in the opposite direction. The Stroop test administered was a simplified version of the classical test adapted to a computer where the volunteer must select the color of a list of words that sometimes include names of colors. The conflict between the color of the word and the word itself origins a latency in the decision. This latency and accuracy were computed. Finally, the PVT consisted of the sudden presentation of a red dot at the center of a black screen. The volunteer had to activate a key as soon as this dot appeared. The latency dot presentation-key activation and the lapses (responses after 10 s) were computed.

2.9 Data management: protection and presentation

For compliance with the data management regulation, all data collected were captured and cryptically stored at the Charité Universtitätsmedizin Berlin Hosting using the

Research Electronic Data Capture tool (REDCap [27]), to which only the researchers have access. The identifiable volunteer information was pseudonymized, replacing it with a numerical code and keeping it separate from the experimental data. Additionally, data always will be presented with statistical descriptors of the sample as a group or subgroup. When data are individually shown, a numerical code replaces any natural identifier. The graphical presentation was done using Adobe Illustrator software v. 16.0.0 (Adobe Systems Incorporated, San Jose, CA, USA) for figures and Origin Pro v. 9.3.226 software (OriginLab Corporation, Northampton, MA, USA), and ggplot and corrplot R packages for graphs.

2.10 Statistical analysis

The statistical design was reviewed and approved for the Charité Institute of Biometry and Clinical Epidemiology. Shapiro-Wilk test and QQ plots evaluated the normal distribution of the data with the detection of outliers using Humber M-estimate (winsorized = 1.5 SD, converge tolerance = 1e-06). According to Rubin's classification, missingness data considerations include MACR (blood samples damaged) and MAR (PSG data missed when the volunteer cannot sleep). Mean and Standard Deviation were used to describe the sample, and correlation between parameters was conducted using Pearson's r analysis. Due to the nature of the pilot study, an exploratory analysis was implemented. The demographic, hematologic, endocrine, and HRV differences between the hypoxic conditions were analyzed by a linear mixed model using FiO_2, sex, and sleep phase as fixed factors, intercept-volunteer as a random factor, and a post hoc analysis with multiple comparisons with Holm's correction. The normal distribution of residuals was verified. Ordinal parameters (epinephrine and dopamine) were described as percentages, and the changes between hypoxic conditions were analyzed with McNemar's test. A p-value less than 0.05 was considered the statistical significance level for all the inferential tests. All the statistical computing was carried out with Jamovi v. 1.6.16 (The Jamovi project 2021) and complemented with Rstudio v. 1.4.1103 based on R v. 4.0.4 (R Project for Statistical Computing).

3 Results

3.1 Anthropometric, biochemical, and hematological description

Table 1 presents the anthropometric parameters classified by sex. Although men tended to be older than women, these differences were not statistically significant. Differences in body mass index and distribution of fat and muscle tissues corresponding with the expected human sexual dimorphism were found. The biochemical analysis of blood and urine samples during the screening phase is presented in **Table 2**. The blood count found no differences in leukocyte and platelet values between the sexes but only in the erythrocyte values; no differences were found when compared by condition (**Table 3**). RDW, Reticulocytes (number, %, Hb, production index), and platelets were not significantly different.

Table 1. Anthropometric comparison of men vs. women

	Units	Men	Women	p
Age	years	32.6±8.1	26.5±3.21	0.072
Weight	kg	81.9±8.44	61.1±4.68	< .001**
Height	cm	182±9.8	167±5.04	0.002*
BMI	kg/cm^2	24.8±2.79	22±1.71	0.034*
SBPr	mmHg	126±6.59	107±5.99	< .001**
DBPr	mmHg	74.7±9.14	66.5±8.37	0.092
SBPl	mmHg	120±6.21	110±7.35	0.014*
DBPl	mmHg	72.9±7.1	68.6±11.6	0.418
HR	b.p.m.	56.1±6.12	62.6±6.05	0.06
Waist	cm	89.1±7.25	74.3±6.71	0.001*
Hip	cm	101±5.46	97.3±2.22	0.115
WHR		0.88±0.04	0.76±0.06	< .001**
FM	kg	18.3±5.9	17.3±3.31	0.691
FM%	%	22.1±5.02	28.3±4.34	0.023*
FFM	kg	63.6±5.24	43.8±3.62	< .001**
FFM%	%	77.9±5.02	71.7±4.34	0.023*
BW	kg	46.7±4.81	30.7±2.6	< .001**
BW%	%	57.1±4.01	50.4±3.52	0.004*
EBW	kg	19.2±3.09	14.2±1.16	< .001**
EBW%	%	41.1±3.43	46.4±2.84	0.006*
Cel.Mass	kg	37.1±5.52	22.1±2.75	< .001**

BMI: Body Mass Index. SBPr: Systolic Blood Pressure right arm. DBPr: Diastolic Blood Pressure right arm. HR: Heart Rate. WHR: Waist/Hip ratio. FT: Fat Mass. FFM: Fat Free Mass. BW: Body Water. EBW: Extracellular Body Water. Data presented as mean±SD. t-test *p<0.05 **p<0.001. Modified of Riveros-Rivera et al. 2022 [28].

Table 2. Biochemical comparison of men vs women

	Units	Men	Women	p
Na	mEq/L	140±2.8	139±1.7	0.476
K	mEq/L	4.17±0.4	3.83±0.3	0.089
Cl	mEq/L	101±2.5	102±2.2	0.557
Ca	mEq/L	2.33±0.05	2.3±0.06	0.428
Urea	mg/dL	29.1±8	21.4±4.8	0.037*
Creatinine	mg/dL	0.889±0.1	0.752±0.1	0.036*
GOT	mg/dL	27.3±7.8	19.4±2.4	0.017*
GPT	mg/dL	25.1±9	14.5±3.9	0.01*
GGT	mg/dL	19.7±7.1	11.6±2.6	0.01*
UrA	mg/dL	5.51±1.8	4.25±1.2	0.124
Bilirubin	mg/dL	0.696±0.3	0.431±0.2	0.051
Cholesterol	mg/dL	157±44	155±22.3	0.917
HDL	mg/dL	47.6±13.5	65.4±16.2	0.039*
non-HDL	mg/dL	104±40	90±15.9	0.382
LDL	mg/dL	97±33	86.6±14.4	0.433
Triglycerides	mg/dL	90±46.4	45.4±10.5	0.02*
Glucose	mg/dL	82.1±5.2	78.9±4.9	0.231
HbA1c	%	5.29±0.3	5.06±0.5	0.283
HbA1c	mmol/molHb	34.1±2.5	31.8±5.2	0.292
TSH	mU/L	2.14±1.3	1.6±1.3	0.442
Ur. den.		1.02±0.003	1.01±0.007	0.302
Ur. pH		6.29±1.3	5.63±1	0.295

GOT: Glutamic Oxaloacetic Transaminase. GPT: Glutamic Pyruvic Transaminase. GGT: Gamma-glutamyl transferase. UrA: Uric Acid. HDL: High-Density Lipoprotein. LDL: Low-Density Lipoprotein. HbA1c: Glycated hemoglobin. TSH: Thyroid-Stimulating Hormone. Ur.den.: Urine density. Ur. pH: Urine pH. Data presented as mean±SD. t-test *p<0.05. Modified of Riveros-Rivera et al. 2022 [28].

Table 3. Hemogram comparison of men vs women by condition

		Men				Women			
	Units	Screen	NN	NH15	NH13	Screen	NN	NH15	NH13
Erythrocytes	10^6cel/µL	5.13±0.38	4.89±0.42	5±0.44	5.07±0.35	4.31±0.21*	4.24±0.23*	4.27±0.24*	4.31±0.32*
Hemoglobin	g/dL	15.2±0.8	14.7±1	14.9±1	15.2±0.9	12.8±0.7**	12.7±0.8*	12.7±0.8*	12.9±1**
Hematocrit	%	45±2.8	43.2±2.9	43.5±3.1	44.8±2.7	38.5±1.9*	37.7±1.8*	37.6±2.2*	37.7±2.4**
Leukocytes	10^3cel/µL	5.29±1.62	4.92±1.52	4.67±1.26	5.37±1.45	5.45±1.85	6.36±1.93	6.58±1.83	7.41±3.48
Platelets	10^3cel/µL	226±54.6	209±37.3	200±37.9	216±32.6	221±27.6	225±36.2	227±36.8	225±26.5

NN: Normobaric Normoxia. NH15: Normobaric Hypoxia FiO_2 14.7%. NH13: Normobaric Hypoxia FiO_2 12.5%. Data presented as mean±SD. *p<0.05 and **p<0.001 in comparison with Men at the same condition (Linear Mixed Model). Modified of Riveros-Rivera et al. 2022 [28].

3.2 Environmental monitoring

Oxygen levels corresponded to the control condition in Berlin (FiO_2 20.9 ± 0.3 %; altitude 39 ± 10 m asl) and the simulated altitudes for NH15 (FiO_2 14.7 ± 2.5 %; altitude 2660 ± 40 m asl), and NH13 (FiO_2 12.5 ± 0.3 %; altitude 4000 ± 30 m asl) conditions. In agreement with Berlin altitude and the normobaric protocol, the barometric pressure slightly oscillated around 760 mmHg. CO_2 and humidity were lower during NH13 and NH15, probably due to the infusion of fresh air during the hypoxia protocol (**Figure 2**).

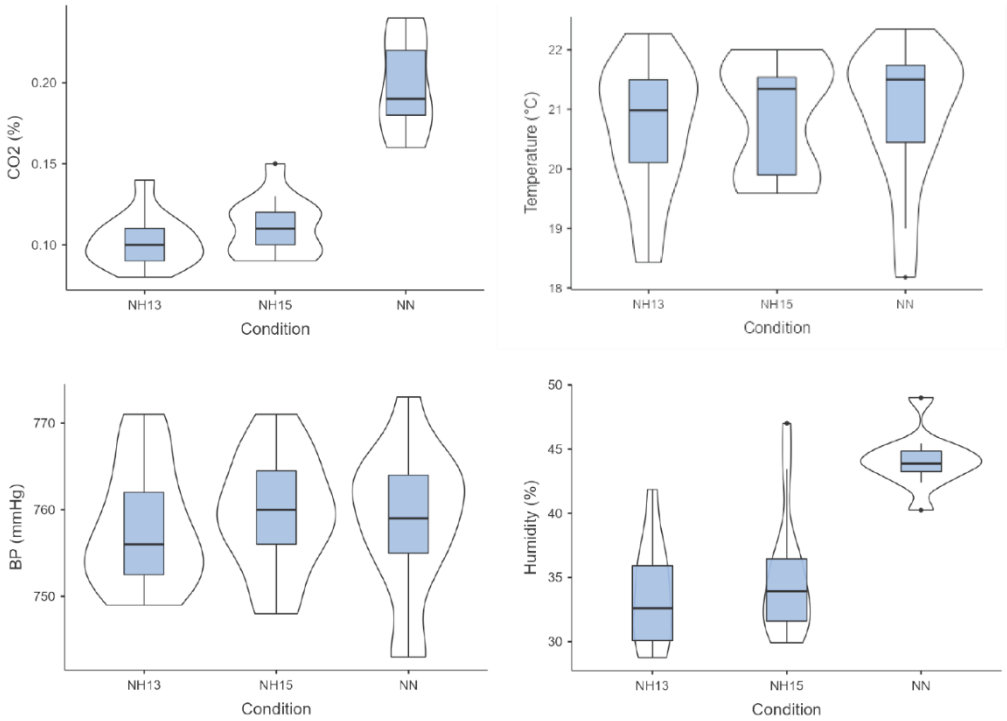

Figure 2. Environmental parameters in the Hypoxia Chamber.
Violin plot representing the probability around the median. BP: Barometric Pressure.

3.3 Sleep monitoring around the experiment (actimetry)

Sleep quality and efficiency after the acute hypoxia exposition were not affected. The summary of the actimetry parameters three days before and after each visit is presented in **Table 4**. No statistical significance was found comparing days and conditions except for Total Minutes in Bed and Total Sleep Time the night before the experiment. Those changes were expected in compliance with the 2-h sleep restriction instruction. However, it is striking that the only protocol with statistically significant differences for all days was for the week around the normobaric normoxia experiment (**Figure 3**).

Table 4. Sleep quality by actimetry

Day	Week	Efficiency	WASO	Fragmentation Index	Sleep Fragmentation Index	Movement Index
-3	WEEK13	86.6±5.39	68.6±33.9	8.59±5.82	20.9±7.37	12.3±3.88
	WEEK15	88±6.62	54.7±33.4	8.62±7.08	19.8±9.43	11.2±3.52
	WEEK21	90.3±4.88	48.9±33	5.15±4.29	14.4±5.99	9.22±2.78
-2	WEEK13	87±5.74	63.4±32.8	10±9.4	21.9±11.8	11.8±3.88
	WEEK15	90.9±5.38	44.2±28.1	7.81±6.15	17.3±8.61	9.46±4.39
	WEEK21	86±5.28	66.8±29.9	12.1±11.8	24.7±12.3	12.6±3.02
-1	WEEK13	88.4±4	39.3±17.3	11.7±7.26	22.5±8.65	10.7±2.64
	WEEK15	91.2±2.69	31.8±13.2	6.42±8.07	15.1±8.59	8.69±1.39
	WEEK21	85.4±6.81	52.4±30.4	10.2±10.2	22.6±14.4	12.4±4.71
+1	WEEK13	85.3±5.55	71±32.2	9.66±5.73	22.7±7.58	13.1±3.67
	WEEK15	87.8±3.6	57±24.5	9.77±8.92	21.4±10.1	11.6±2.83
	WEEK21	85.8±8.36	67.5±33.3	11.7±11.3	24.5±14.7	12.8±5.26
+2	WEEK13	88.4±5.32	51.4±26.7	10.7±9.48	21.9±11	11.1±2.68
	WEEK15	89.9±4.97	45.5±24	9.52±7.66	19.5±9.25	9.99±2.86
	WEEK21	85.8±6.99	64.4±32.6	9.72±9.83	22.5±11.7	12.8±3.92
+3	WEEK13	89.1±5.65	57±37.6	10.1±7.41	20.9±8.37	10.8±3.17
	WEEK15	86.6±5.53	64.8±33.2	10±7.3	21.3±7.21	11.3±3.68
	WEEK21	88.1±4.46	56.1±27	9.49±6.61	20.3±7.77	10.9±2.61

Three days before (negative) and three days after (positive) the experiment. WEEK21: Week around NN. WEEK15: Week around NH15. WEEK13: Week around NH13. WASO: Wake After Sleep Onset. Data presented as mean±SD. No significant differences (Linear Mixed Model). Modified of Riveros-Rivera et al. 2022 [28].

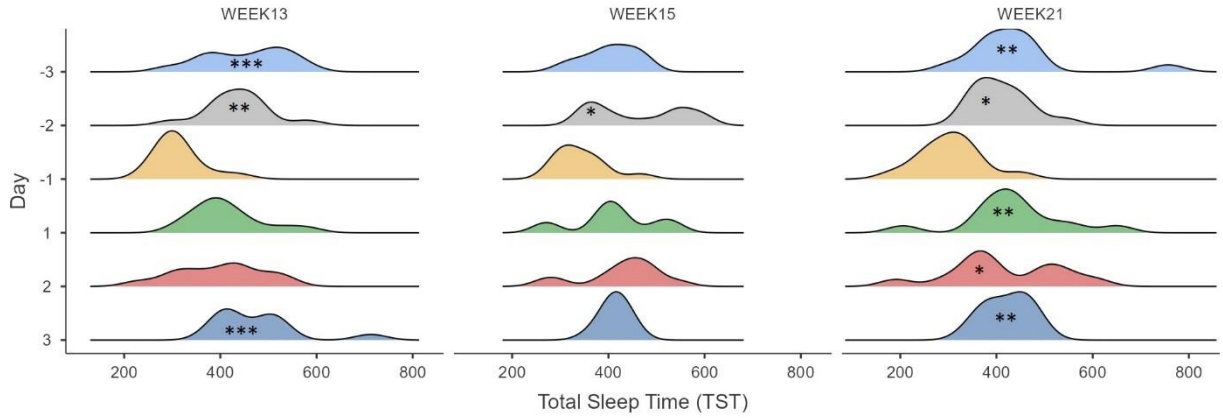

Figure 3. Total Sleep Time by actimetry.
Three days before (negative) and three days after (positive) the experiment. WEEK13: Week around NH13. WEEK15: Week around NH15. WEEK21: Week around NN. TST in minutes. * p<0.05 ** p<0.01 *** p<0.001 with respect to -1 day. (Linear Mixed Model).

3.4 Sleep monitoring during the nap opportunity (polysomnography)

Table 5. Oxygen saturation and respiratory events by polysomnography

	Units	Condition	Phase	All	Men	Women
SpO_2	%	NN	Pre	97.8±1.4	97.1±1.6	98.4±0.9
			Sleep	96±1.3	95.9±1.1	96.1±1.6
			Pos	96.8±1.4	96.5±1.6	97.1±1.1
SpO_2	%	NH15	Pre	90.3±3.4**	88.7±3.5	91.7±2.9
			Sleep	86.8±2.7**	85.8±2.9	87.7±2.5
			Pos	89±2.5**	88.5±1.5	89.4±3.2
SpO_2	%	NH13	Pre	77.5±3.6**~	75.3±3.9	79.5±1.8
			Sleep	75.9±5.4**~	73.1±5.8	78.4±3.8^
			Pos	79.4±3.1**~	77.4±2.6	81.1±2.4
AHI	Events/h	NN	Sleep	0.2±0.7	0.4±1.1	0.1±0.4
		NH15		13.1±29.3	28±39.6	0.3±0.6
		NH13		32.9±45.2*	68.3±44.6**	1.8±5.2^^
PBI	Events/h	NN	Sleep	0±0	0±0	0±0
		NH15		21.3±40.5	46±50.9	0.2±0.5
		NH13		39.7±58.4*	80.8±64.4**	3.8±10^^

SpO_2: peripheral oxygen saturation. AHI: Apnea-Hypopnea Index. PBI: Periodic Breathing Index. *p<0.01 in comparison with NN. **p<0.001 in comparison with NN same phase. ~p<0.001 in comparison with NH15 same phase. ^p<0.05 ^^p<0.001 compared with Men at the same phase and condition. Data presented as mean±SD. (Linear Mixed Model). Modified of Riveros-Rivera et al. 2022 [28].

Figure 4 presents parameters related to sleep quality by conditions. The mean SE and TST were lower during NH13. Similarly, WASO and SOL of this condition were higher. However, there was no statistical significance compared to the NH15 and NN conditions. Analysis of the distribution of sleep phases by condition (**Figure 5**) shows an evident increase at N2 %TST in hypoxic conditions in comparison with NN (p<0.001). In the sex analysis, this difference is only presented in women NN vs. NH13 (p<0.001). No differences between sexes were found under the same condition. As for N3, a reciprocal change was seen in the hypoxic conditions, with a significant decrease in NH13 concerning NN (p<0.001). Similarly to N2, women had a significant difference between NN vs. NH13 (p=0.029). In men, the difference was more marked in NH15 (p=0.006) than in NH13 (p=0.05) compared to NN. However, comparing sexes under the same condition did not show any differences. Finally, no changes in REM sleep between conditions or sexes were evident. Nevertheless, the total absence of this phase in men is striking during the NH15 condition.

Regarding respiratory parameters, **Table 5** shows peripheral oxygen saturation (SpO_2), the Apnea-Hypopnea Index (AHI), and the Periodic Breathing Index (PBI) by sex and condition. As expected, there was a decremental change as normobaric hypoxia increased, with significant differences from normoxia. An analysis between sexes showed differences during the nap in the NH13 condition but no in NN or NH15.

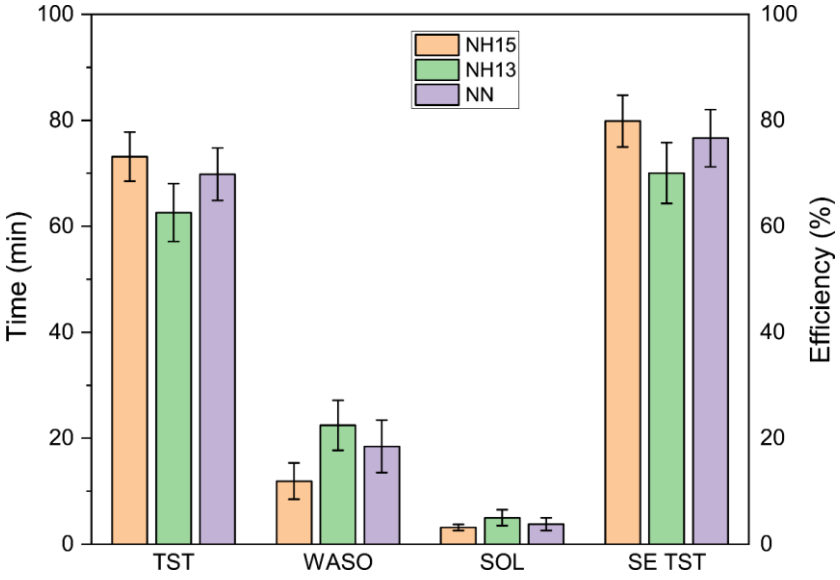

Figure 4. Sleep quality by polysomnography during napping.
Bar chart representing the mean and SD. TST: Total Sleep Time. WASO: Wake After Sleep Onset. SOL: Sleep Onset Latency. SE TST: Sleep Efficiency in TST. Data presented as mean±SEM. No statistical significance (Lineal Mixed Model).

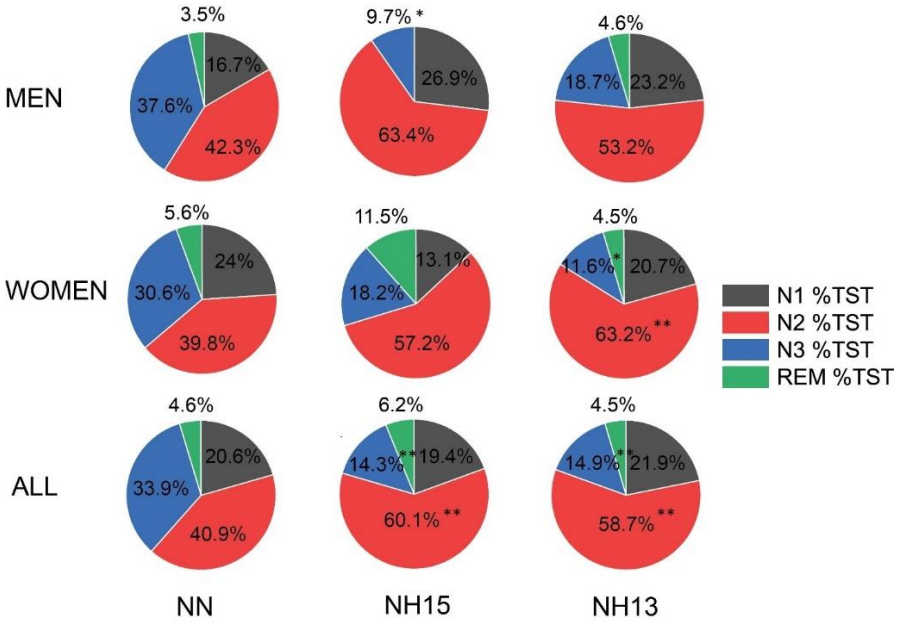

Figure 5. Sleep phases distribution by polysomnography.
N1: NREM stage 1. N2: NREM stage 2. N3: NREM stage 3. REM: Rapid Eye Movement sleep. *p<0.05 **p<0.001 compared with NN (Linear Mixed Model).

3.5 Heart Rate Variability Analysis

Due to the nap's timing, most volunteers had short sleep stages. Given that at least 5 minutes of recording is required for HRV assessment, the results presented during sleep correspond exclusively to stage N2. A decrease in parasympathetic tone in men during N2 sleep was evident at 4000 m (RMSSD=52.3±32 ms; HR=57.7±5 bpm; SD2=93±45.6 ms) vs. control (RMSSD=48.7±24 ms; HR=49.4±5.5 bpm; SD2=49.1±19.6 ms; $p<0.001$). The same change was evident during N2 in women at 4000 m (SD1=26±23 ms; SI=16.6±12.5) vs. control (SD1=53±30 ms; SI=7.8±3.3; $p<0.001$).

The parasympathetic (PNS) and sympathetic (SNS) indexes synthetize the results for all group. PNS decreased from control values (1.53 ± 1.54) to NH13 (-0.03 ± 1.64; $p<0.001$). In contrast, a reciprocal increase in SNS was observed from control (-0.98 ± 1) to NH13 (0.67 ± 2.59; $p<0.001$). In the pre-pos napping analysis, no significant changes occurred during NN. However, both hypoxic conditions showed a statistically significant decrease in PNS (preNH15: 0.69 ± 1.81; posNH15: -0.36 ± 1.19; preNH13: -0.3 ± 1.26; posNH13: -1.2 ± 0.92; $p<0.05$) and an increase in SNS (preNH15: -0.36 ± 1.13; posNH15: 0.73 ± 1.6; preNH13: 0.49 ± 1.39; posNH13: 1.73 ± 1.83; $p<0.05$).

3.6 Hypoxic stress hormone analysis

The endocrine response to hypoxic stress was evaluated through catecholamines, cortisol, and EPO. The endocrine response to hypoxic stress was evident in the EPO and cortisol levels in NH13 (**Table 6**). No differences between women and men were found.
This endocrine stress response had a high correlation with the nervous response. As expected, high levels of EPO were positively correlated with sympathetic activity and negatively with parasympathetic activity (**Figure 6**). However, this change was evident only in the HRV before the nap. Cortisol reactivity was like EPO; however, correlations occurred with HRV both before and after sleep and, crucially, with HRV during the nap. Norepinephrine had no significant correlations with HRV parameters. Although there is a negative association between EPO levels and peripheral oxygen saturation, this relationship was not significant.

Table 6. Endocrine response after napping in hypoxic condition

Hormone	Condition	Units	Value
Norepinephrine	NN	ng/L	186.2±83.7
Norepinephrine	NH15	ng/L	170.4±110.4
Norepinephrine	NH13	ng/L	211.1±121.8
EPO	NN	IU/L	11.9±7.1
EPO	NH15	IU/L	12.1±4
EPO	NH13	IU/L	15±4.6*~
Cortisol	NN	nmol/L	227±51.7
Cortisol	NH15	nmol/L	264.8±86.2
Cortisol	NH13	nmol/L	421.3±265*~
Epinephrine	NN	%High	14.3
Epinephrine	NH15	%High	23.1^
Epinephrine	NH13	%High	42.9
Dopamine	NN	%High	23.1
Dopamine	NH15	%High	38.5
Dopamine	NH13	%High	42.9

*p<0.001 comparing with NN. ~p<0.05 compared with NH15. Data presented as mean±SD. (Linear Mixed Model). ^p=0.021 (McNemar test). Modified of Riveros-Rivera et al. 2022 [28].

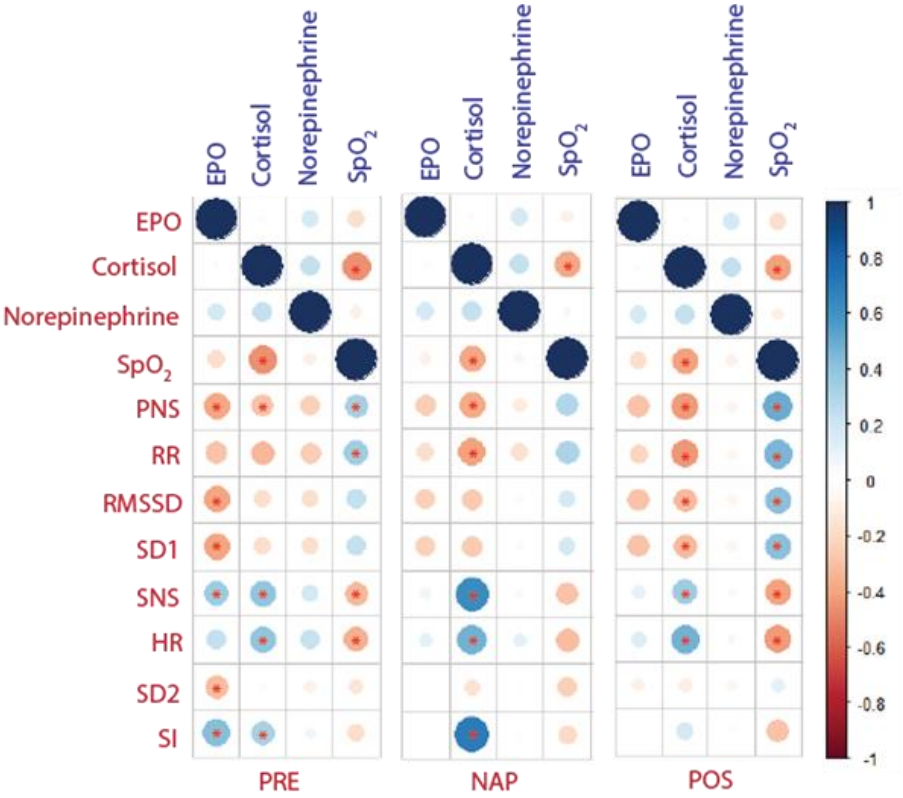

Figure 6. HRV-Hormones correlogram.
Measured before (PRE), during (NAP), and after (POS) the nap. Positive correlations are displayed in dark blue, and negative correlations in dark red. Circle size and intensity are proportional to the correlation coefficients. SpO_2: peripheral oxygen saturation. PNS: Parasympathetic index. SNS: Sympathetic index. RR: RR interval. RMSSD: Root Mean Square of Successive Differences between normal heartbeats. SD1: Standard Deviation of the instantaneous beat-to-beat variability. SD2: Standard Deviation of continuous long-term variability. HR: Heart rate. SI: Baevsky's stress index. *p<0.05(Pearson's r). Modified of Riveros-Rivera et al. 2022 [28].

3.7 Cognitive evaluation

Although attention and memory tended to increase after napping (as seen through the digit span tests), no significant differences were found (**Table 7**). In contrast, there were changes in performance during the Stroop test in normoxic conditions, evident by decreased errors and latency. This improvement after the nap was not significant in the two hypoxic conditions. On the other hand, latencies during the incongruent Stroop test were significantly longer in the hypoxic conditions before sleep compared to normoxia performance. Finally, PVT showed improvements after napping, but its difference was significant only under normoxic conditions at the 9-second latency.

Table 7. Cognitive performance before and after napping in hypoxic condition

Condition	Phase	Fds	Bds	STerrors	STlatencyCon (ms)	STlatencyInc (ms)	STaccuCon (%)	STaccuInc (%)	PVTlatency9 (ms)	PVTlapse
NN	pre	7.3±1.6	7.4±1.5	7.7±3.7	737±129	813±164	94.2±4.1	94.6±3.1	372±128	4.1±5.6
NN	pos	7.4±1.5	7.2±1.6	4.8±3.8*	654±118*	731±127	96.5±3.7	96±3.9	312±42.7*	1.6±1.5
NH15	pre	6.7±1.6	6.7±1.1	7.1±4.2	721±159	848±193~	96.4±2.8	93.6±4.8	345±58.9	3.6±5.3
NH15	pos	7.5±1.5	7.3±1.8	5.1±4.3	678±104	795±164	97.1±3.2	95.4±3.6	313±57	1.1±1.3
NH13	pre	7.1±2	7.5±1.5	7.3±3.3	726±143	840±176~	95.8±2.6	94±2.8	343±52.5	3.9±4.3
NH13	pos	7.7±1.2	7.1±1.7	6.6±3.6	677±96.8	793±102	96.4±2.3	94.2±4	321±41.7	2±2.8

Fds: Forward digit span test (maximum number of digits recalled). Bds: Backward digit span test (maximum number of digits recalled). STerrors: errors in Stroop test. STlatencyCon: latency in congruent Stroop test. STlatencyInc: latency in incongruent Stroop test. STaccuCon: accuracy in congruent Stroop test. STaccuInc: accuracy in incongruent Stroop test. PVTlatency9: latency in PVT test when the red dot appeared 9 seconds after the key. Pre: before napping. Pos: after napping. Data presented as mean±SD. * $p<0.05$ with respect to pre. ~$p<0.05$ with respect to NNpos. Modified of Riveros-Rivera et al. 2022 [28].

4 Discussion

4.1 Short summary of results

Significant endocrine and autonomic regulation changes accompany the physiological challenge of exposure to hypoxia. Here we evaluated the consequences of exposure to hypoxia by simulating altitude conditions to healthy lowlanders around a daytime nap of approximately 90 min. Our results confirm a drastic shift to an ergotropic state before, during and after napping (**Figure 6**). As hypoxia increased, the percentage of stage N2 sleep increased while N3 decreased. However, these changes were not accompanied by decreased sleep efficiency or total sleep time. Along with the expected changes in peripheral oxygen saturation due to normobaric hypoxia, there was also a marked increase in apnea episodes and periodic breathing. The last effect was more dependent on changes in the male group. As for the autonomic changes during the nap, when hypoxic conditions were compared with normoxia, an increase in sympathetic activity and a decrease in the parasympathetic were evidenced. In contrast, after the nap, the sympathetic tone increased, and the parasympathetic tone decreased for each condition relative to the pre-sleep stage. However, this change was significant in hypoxic but not in normoxic conditions. A concomitant change in autonomic adaptations was also found in the endocrine response, with a significant increase in cortisol and EPO levels in NH13 and epinephrine in NH15. Finally, there were no general cognitive changes when comparing performance between the different conditions, yet the before-after nap assessment offered positive results. Overall, subjects improved their performance after napping, with significant changes only under the normoxic condition in both the Stroop test (errors and congruent latency) and the psychovigilance task (9 s latency).

4.2 Interpretation of results

We found that the changes in the architecture typical of nocturnal sleep at high altitude [6,8,29] also appear in a daytime nap of less than 90 minutes. Although there was a decrease in REM sleep that was inversely proportional to the degree of hypoxia, the representation of this phase was small even under normoxic conditions. Since the ultradian NREM-REM cycle lasts approximately 90 minutes, it is to be expected that there would be a low proportion of REM sleep in a short nap that lasts less than that period.

Intriguingly, the proportion of N3 sleep also fell as hypoxia increased, probably because N3 sleep emerges as a trophotropic component involved in the restorative function of sleep [30]. Thus, activation of the parasympathetic component is necessary to reach this stage, requiring the resetting of the baroreflex among other factors [13]. In this process, the participation of the parabrachial nucleus and the nucleus of the solitary tract, which function as controller and outlet to the peripheral autonomic system, is essential. However, given the constant activation of the carotid glomus by normobaric hypoxia, the influx of information towards these nuclei could depress or block the initiation of processes that activate N3 sleep and its parasympathetic predominance.

The increase in light sleep (i.e., N2 sleep), as opposed to deep sleep, may trigger the manifestations of high-altitude sleep. Sleep activates brain interstitial clearance mechanisms whose purpose is to sweep away products of daily metabolic activity [30]. Such clearance is evident by the coupling between electrical and vascular phenomena typical of N3 sleep (delta waves). However, the depression of this state could, among other things, explain the tendency towards developing brain edema characteristic of maladaptation to altitude [14].

Another important finding was that a daytime nap under normobaric hypoxia reveal the same respiratory adaptations documented in nocturnal sleep, particularly periodic breathing. We show that napping at simulated altitude has sex-dependent consequences on ventilatory pattern, just as occur during night-time sleep in natural high altitude [11]. There is a tendency to exhibit episodes of periodic breathing during night sleep once humans 2400 m asl likely due to the role of the carotid glomus, a detector of arterial oxygen pressure [13]. Under hypoxic conditions, low levels of plasmatic oxygen increase the firing rate of nerves in the glomus and activate compensatory responses, including hyperventilation. This is accompanied by hypocapnia, triggering a ventilatory depression and initiating a cycle of inhibitions and activations characteristic of periodic breathing.

The essential parameter in this periodic breathing is the CO_2 reserve (the change in blood CO_2 pressure the system can withstand before triggering a reflex ventilatory response [13]). The CO_2 reserve is modified, among others, by sex steroids, which have a rich expression of receptors in fundamental components of respiratory regulation [31]. Although there is evidence that estrogens and progestogens could explain this

modulation, our results provide more support for the role of testosterone in this process. For example, the periodic breathing presentation was almost exclusively in the men group. Also, the only exception in the women group was a volunteer diagnosed with polycystic ovary, a condition characterized by significant increases in testosterone [32]. In fact, the administration of testosterone to premenopausal women can modify the apneic threshold and consequently disrupts the respiratory control [33]. Hence, the effect of testosterone would be an increase in the ventilatory response to hypocapnia, being the role of central or peripheral chemoreceptors remains unknown.

Napping under normoxic conditions leads to an increased parasympathetic tone with a reciprocal change in sympathetic activity while the volunteers slept. Then, waking up is accompanied by a withdrawal of the parasympathetic tone and an increase of the sympathetic that reaches higher values than before the nap [34]. Our results corroborate this last change, but the pre-post difference was only significant in the highest state of hypoxia (NH13). As noted above, the influence of carotid sensors (both baro- and chemo-receptors) is critical in sleep onset and subsequent autonomic oscillation [13]. Its activation, exacerbated by hypoxia, could target the ventrolateral preoptic area (VLPO), which is responsible for inhibiting the wakefulness nuclei. Also, norepinephrine can hyperpolarize these neurons, thereby blocking their sleep-inducing action and explaining an increased LF/HF ratio with depression of delta waves [35].

We found paradoxical results regarding oxygen saturation and its relationship with autonomic activation. On the one hand, men presented the lowest values of SpO_2, but women had the most increase in the sympathetic index. This could be partly explained by the ventilatory modulation produced by periodic breathing in men, which can increase vagal bulbar feedback that tend to increase parasympathetic tone differentially. Indeed, the depth of ventilation characteristic of periodic respiration followed by central apnea is accompanied by changes in autonomic modulation [9]. Alternatively, the differential response between men and women could be the effect of EPO on carotid chemoreceptors. We previously showed that the activity of this hormone is different between men and women [6]. Moreover, the receptors for EPO in type I glomus cells conditionate differential responses according to testosterone concentration [36].

Finally, although previous studies have documented significant changes in cognitive evaluation by altitudinal hypoxia [19], we did not replicate that outcome around a daytime nap probably due to the existence of a threshold from which these cognitive changes appear [37]. These point out to buffering mechanisms to explain the scant cognitive affectation [37].

4.3 Embedding the results into the current state of research

Previous evaluations of the relationship between altitudinal hypoxia and sleep indicated changes in linear parameters of heart rate variability. Here we showed that non-linear parameters, such as those obtained through Poincaré plots, i.e., SD1 and SD2, can also serve as markers of hypoxia. We also corroborated results from previous studies that showed that taking a nap under normoxic conditions is accompanied by an elevation in HRV markers associated with cardiovagal regulation. We have complemented this information for simulated altitudes of 2,600 and 4,000 m asl, revealing a depression of parasympathetic tone and the release of factors typical of the response to hypoxic stress, namely cortisol and catecholamines.

Taking a daytime nap is a practice that has been recommended for populations that have an altered sleep pattern (such as crew members or shift workers) or who want to enhance their physical or mental performance (elite athletes or workers with high cognitive demand). Our results contradict this recommendation for those at high altitude since dramatic changes in the ventilatory pattern, peripheral oxygen saturation, and autonomic imbalance could worsen their general condition. Although to a lesser extent, previous studies evaluating nocturnal sleep under altitudinal hypoxia suggest that women also develop periodic breathing. Our results show that as far as daytime naps are concerned, such a ventilatory pattern is a phenomenon almost exclusively of male nature. The fact that other studies under altitudinal hypoxia have been able to see ventilatory disruption in women during their night-time sleep, but we did not replicate these outcomes in a short daytime, nap leads us to think about the importance of the duration of exposure.

Contrary to previous studies that have demonstrated the usefulness of cognitive tests such as the Stroop test or the PVT when evaluating executive functions under hypoxic conditions, our results do not demonstrate an evident sensitivity of these tests, at least in

terms of short exposure. Although the cognitive reserve of our young volunteers may have worked in their favor, there is evidence that cognitive modifications should accompany autonomic changes such as parasympathetic withdrawal. Previous studies in normoxia have shown that, for example, memory and attention have a notable increase after napping. Our results indicate an improvement after napping in all conditions, but changes were only marked in the condition with the greatest hypoxia.

Previous studies showed significant correlations between HRV parameters and hormones responding to hypoxic stress, including cortisol and catecholamines; however, such correlation have been not stablished for EPO. Our study is the first to document a strong correlation between EPO and HRV parameters, including RMSSD, SD1, SD2, SI, PNS, and SNS indexes. In conclusion, a daytime nap under normobaric hypoxia significantly modifies cardiorespiratory and neuroendocrine responses similarly to night sleep. We have additionally shown that sex is a fundamental determinant of these responses and that it should always be considered when evaluating the effects of hypoxic exposure.

4.4 Strengths and weaknesses of the study

To our knowledge, this study is the first to document physiological changes in lowlanders taking a daytime nap under normobaric hypoxic conditions. An integrative approach was made to analyze the association between endocrine and autonomic responses, and the gold standard for sleep monitoring, polysomnography, was implemented. However, scrutiny must be considered in the future to carry out studies that corroborate and strengthen the conclusions we have reached. As a pilot study, inferential analyses are more exploratory than definitive, so the conclusions must be analyzed carefully. However, although the sample was small, we used a cross-over model to resemble a parallel study's power and effect size that would have included more volunteers [38].

From an environmental point of view, some limitations of the present study must be recognized for its implementation in field conditions. While it is true that the simulated altitude allows for reaching hypoxic conditions like those found in the natural context, there is still controversy about the accurate extrapolation of its conclusions [39]. Factors associated with high altitude, such as radiation, temperature, or humidity, directly change

the autonomic and endocrine components described in this work [1]. On the other hand, although most of the experimental sessions were conducted in late summer and autumn, seasonal changes could substantially impact the volunteers' responses. For example, exposure to longer summer days could have changed circadian oscillation and, consequently, contrasting with short autumn days [1].

In addition to the environmental limitations mentioned above, factors linked to our experimental design must be stated. On the one hand, there is the effect that the restriction of two hours of sleep could have had on the volunteers. Whether due to an additive or collaborative effect, sleep deprivation has been pointed out as one of the primary triggers for sleep disturbances. However, this effect is dose-dependent, and longer exposures than ours have not generated changes in parameters such as cortisol [20]. On the other hand, although we have characterized a differential response for women, an effect related to the menstrual cycle phase was impossible since the sample size was small. However, the volunteers were exposed to a random sequence of conditions to avoid bias related to the menstrual pattern. In theory, this should lead to a balanced relationship between the menstrual phase and the hypoxic condition.

Finally, genetic considerations may limit the external validity of our conclusions. Since the volunteers were not randomly selected but were recruited from the local community characterized by a central European phenotype, extrapolation to other populations requires further studies. Of particular interest for our future projects is the impact that the present work could have on South American populations. However, it is necessary to consider that different American ethnicities have inherited a genetic repertoire that could give them advantages in tolerating high altitudes [40].

4.5 Implications for practice and/or future research

Determining the degree of hypoxic stress an individual is experiencing is critical for medical assessment in field conditions. Hormonal measurements of factors such as cortisol, EPO, or catecholamines, account for such a response but are challenging to implement given technical limitations, including sample storage and processing times. We have shown a strong correlation between changes in these hormones and some parameters of heart rate variability. For example, Baevsky's index while sleeping

presented a robust significant correlation with cortisol levels measured after napping. Even early assessment (i.e., before napping) of variables such as RMSSD, SD1, or SD2 was associated with later increments in EPO concentrations. Since these HRV parameters can be evaluated with portable, low-cost, and highly accurate equipment, our results ratify their implementation in field medicine to evaluate the hypoxic response to altitudinal stress.

Contrary to the benefits of napping under normoxic conditions, it has become clear that sleeping under hypoxic conditions is diverted to negative consequences. The changes could be seen even from the simulated altitude of 2600 m asl, which is close to the conditions maintained in the aircraft cabin. For this reason, we conclude that a short nap while traveling by plane or climbing in the mountains could negatively impact susceptible groups, presumably targeting men or those with elevated testosterone levels. Therefore, and as a tentative working hypothesis, the recommendation of napping as a countermeasure to sleep disturbances at altitude should be limited or accompanied by complementary strategies. Among them, oxygen supplementation, as has recently been recommended for air crews, stands out [4].

The study of the relationships between sleep and hypoxia is crucial not only for mountain or aerospace medicine but also for understanding general clinical conditions. Failures in perfusion or oxygenation, ventilation disorders, and neurological or endocrine disturbances benefit from studies that evaluate this connection. Although considering the specific properties of daytime nap that we have discussed above, we have shown here that a short daytime nap can generate a valid model to manifest the typical changes in sleep during hypoxia. The decrease in operational, financial, and time costs should encourage its implementation as a model to study these conditions.

Bearing in mind our goal of understanding the implications of mountain migration by forced displacement, our future research will evaluate the changes described here under natural conditions, particularly at 2600 m. Given that we have identified the primary HRV markers related to this response, we hope to apply this approach to Andean populations. With these results, we might demonstrate that normobaric hypoxia is a model whose conclusions can be extrapolated to hypobaric hypoxia and, from another point of view,

analyze whether, despite a different genetic repertoire, the physiological responses are similar.

5 Conclusions

A lowlander taking a daytime nap in hypoxic conditions exhibits a classical stress reaction. With increased sympathetic tone and adrenal activity, the fight-or-flight response coordinates a sleep-disturbing response. This disturbance is dose and sex-dependent, with a marked ventilatory reaction in men and a notable depression of cardiovagal modulation in women. The implementation of the nap as a counterregulatory measure for sleep disorders at altitude should be limited or at least supported by oxygen supplementation.

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7 Statutory Declaration

“I, Alain Riveros Rivera, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic Autonomic and endocrine consequences of a daytime napping at simulated altitude on healthy lowlanders (Autonome und endokrine Folgen eines Mittagsschlafs in simulierter Höhe bei gesunden Flachlandbewohnern), independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

8 Declaration of my own contribution to the publication

Alain Riveros-Rivera contributed the following to the below publication:

Riveros-Rivera A, Penzel T, Gunga HC, Opatz O, Paul F, Klug L, Boschmann M, Mähler A. **Hypoxia Differentially Affects Healthy Men and Women During a Daytime Nap with a Dose-Response Relationship: A Randomized, Cross-Over Pilot Study.** Front Physiol. 2022 May 24;13.

Contributions:
I developed and substantiated the research hypothesis based on an in-depth literature review (**Section 1**). I conceptualized the methods necessary to evaluate the research hypothesis and, thanks to the feedback from the co-authors, submitted the protocol to the Charité research committee (**Section 2.1**). In collaboration with Dr. Mähler, I registered the protocol in Clinicaltrials.gov and obtained the approval of the Charité Ethics Committee (**Section 2.1**). I completed the CONSORT 2010 Checklist and designed the flow diagram (**Figure 1a**).
Together with Drs. Penzel and Mähler, I adapted the hypobaric chamber into a sleep laboratory (**Section 2.7**). Actively participate, together with Dr. Boschmann, in the recruitment and screening of volunteers, performing the baseline cognitive tests (**Section 2.2 and 2.5**). Under Dr. Mähler supervision, I designed the forms and questionnaires on the RedCap platform (**Section 2.5 and 2.13**). I randomized the volunteers for the hypoxia protocols and was in charge of setting up the hypoxic chamber in each session (**Sections 2.1 and 2.3**).
I designed the protocol for the actimetry evaluation of the volunteers and set them up in each session. I was also in charge of downloading, processing, and analyzing the information stored by the actimeters (**Section 2.6**).
I set and conditioned the electrophysiological recordings and their subsequent processing and extraction of the variables (**Sections 2.7 and 2.13**). I administered all assessment questionnaires and computer cognitive tests at each session (**Section 2.9**).
Based on the blood samples obtained by Mrs. Gabriele Rahn and analyzed by Labor Berlin, I registered the biochemical analysis and integrated them with the electrophysiological data (**Section 2.13**).

I analyzed and staged the sleep record and generated polysomnographic reports for each volunteer (**Section 2.7**). I ran the heart rate variability analysis of each electrocardiographic recording (**Section 2.8**).

I carried out all the statistical analysis presented and based on them, created graphical support (**Tables 1-3**, **Figures 3-4**).

I synthesized the methods and results (**Sections 2-3**) and designed the respective figures (**Figures 1b and 2**).

I analyzed and interpreted the results creating reports to discuss with my coauthors (**Section 4**). I compiled the references to support our hypothesis and analysis, creating the introduction and discussion draft (**Sections 1 and 4**). Based on the contributions of my coauthors, I wrote and submitted the final version of the paper.

_____

Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

9 Printing copy of the publication

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ORIGINAL RESEARCH
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Hypoxia Differentially Affects Healthy Men and Women During a Daytime Nap With a Dose-Response Relationship: a Randomized, Cross-Over Pilot Study

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Context: The use of daytime napping as a countermeasure in sleep disturbances has been recommended but its physiological evaluation at high altitude is limited.

Objective: To evaluate the neuroendocrine response to hypoxic stress during a daytime nap and its cognitive impact.

Design, Subject, and Setting: Randomized, single-blind, three period cross-over pilot study conducted with 15 healthy lowlander subjects (8 women) with a mean (SD) age of 29(6) years (Clinicaltrials identifier: NCT04146857, https://clinicaltrials.gov/ct2/show/NCT04146857?cond=napping&draw=3&rank=12).

Interventions: Volunteers underwent a polysomnography, hematological and cognitive evaluation around a 90 min midday nap, being allocated to a randomized sequence of three conditions: normobaric normoxia (NN), normobaric hypoxia at FiO_2 14.7% (NH15) and 12.5% (NH13), with a washout period of 1 week between conditions.

Results: Primary outcome was the interbeat period measured by the RR interval with electrocardiogram. Compared to normobaric normoxia, RR during napping was shortened by 57 and 206 ms under NH15 and NH13 conditions, respectively ($p < 0.001$). Sympathetic predominance was evident by heart rate variability analysis and increased epinephrine levels. Concomitantly, there were significant changes in endocrine parameters such as erythropoietin (~6 UI/L) and cortisol (~100 nmol/L) (NH13 vs. NN, $p < 0.001$). Cognitive evaluation revealed changes in the color-word Stroop test. Additionally, although sleep efficiency was preserved, polysomnography showed lesser deep sleep and REM sleep, and periodic breathing, predominantly in men.

Conclusion: Although napping in simulated altitude does not appear to significantly affect cognitive performance, sex-dependent changes in cardiac autonomic modulation and respiratory pattern should be considered before napping is prescribed as a countermeasure.

Keywords: napping, sleep, hypoxia, high altitude (low air pressure), autonomic nervous system, physiological stress

1 INTRODUCTION

Sleep is a cornerstone for maintaining physiological homeostasis. Modifications of sleep due to changes in schedule, duration, dark-light cycle, and oxygen availability may result in decreased physical and cognitive performance (Wu et al., 2010; Bloch et al., 2015; Souissi et al., 2020). Among these factors, oxygen availability, particularly in the form of hypobaric hypoxia, calls for more attention given the increasing exposure of humans to such environments. According to the International Air Transport Association, 4,543 million people air-traveled worldwide in 2019 (IATA, 2020), with 12 million people daily exposed to cabin atmosphere corresponding to 2,438 m above sea level, a condition that could significantly decrease oxygen saturation (Humphreys et al., 2005; Muhm et al., 2009). Indeed, it has recently been shown that nighttime sleep in these circumstances induces hypoxia (Elmenhorst et al., 2022). Other causes for the increase in exposure to hypoxic environments come from two distant phenomena, namely global warming, and sociopolitical instability, resulting in migration to high-land cities that may continue in the years to come (Camargo et al., 2020; Hauer et al., 2020). Thus, fully understanding the connection between exposure to hypoxic environments, sleep quality and physical and cognitive performance, are key to developing strategies to face this growing challenge.

Latterly, the impact of daytime napping on sleep health and performance has stood relative to other strategies implemented to complement daily sleep hygiene (Faraut et al., 2017). Daytime napping benefits physiological and mental parameters such as endurance, strength, memory, and attention (Dutheil et al., 2021; Lastella et al., 2021). For instance, healthy adults increased maximal voluntary isometric contraction after 40 or 90 min of a daytime nap vs. no nap, with a concomitant increase in attention evaluated through the digital cancelation test (Boukhris et al., 2020). Also, it has been shown that factual knowledge task is boosted after a 30 min nap with improvement in learning and consolidation processes (Cousins et al., 2019). From the above, an interest arises that associates the implementation of napping as a countermeasure for sleep disturbances characteristic of altitudinal hypoxia. Might daytime napping support cognitive and physical performance during exposure to hypoxic environments?

Over the years, most studies evaluating the effects of hypoxia on sleep have focused on nocturnal sleep (Bloch et al., 2015; Tseng et al., 2015; Horiuchi et al., 2017; Hughes et al., 2017; Steier et al., 2017; Hill et al., 2018; Bird et al., 2021), leaving the impact on daytime sleep under hypoxic environments unclear. Furthermore, direct extrapolations may be inaccurate because of fundamental differences between daytime and nighttime sleep due to circadian changes of essential physiological components such as the endocrine and autonomic systems. For example, the secretion of erythropoietin or cortisol, two key hormones released during hypoxic stress, present higher levels during the day in comparison with the night (Cristancho et al., 2016; Mohd Azmi et al., 2021). Also, the autonomic balance evaluated by LF/HF ratio in a Heart Rate Variability (HRV) analysis has significant differences in a day-night comparison (Boudreau et al., 2012). Nevertheless, important information derived from overnight sleep studies indicates that sex, and divergent lengths and intensity of exposures to hypoxia are critical factors for the degree of hypoxemia reached by the individuals, conditioning physiological and clinical impact (Lombardi et al., 2013). Therefore, prior to its clinical implementation as a countermeasure to nocturnal sleep disturbances, it is necessary to characterize physiologically daytime sleep during hypoxia exposure. Does acute hypoxia alter daytime napping in a dose-and sex-dependent manner? Does acute hypoxia impair cognitive performance after a daytime nap?

Here, our goal was threefold. First, we aimed to characterize the quality and architecture of a daytime nap under two incremental degrees of hypoxia. We monitored napping in a normobaric hypoxia chamber by polysomnographic recording, a standardized and clinically validated methodology, which allows to reliably assess the quality and architecture of sleep (Silber et al., 2007). Although sleep architecture assessment using actimetry or cardiorespiratory monitoring has undergone a remarkable development in recent years (Fekedulegn et al., 2020; Gaiduk et al., 2022), polysomnography remains the gold standard. Despite some limitations, its synchronous and integrated approach of cardiovascular, respiratory, and neural parameters makes it the most objective, sensitive, and specific assessment of sleep duration and its phases (Lee et al., 2022).

Second, we aimed to evaluate the neuroendocrine response to hypoxic stress before, during and after a daytime nap. We implemented HRV analysis using the ECG recordings obtained from the polysomnography (PSG). HRV is a well described proxy of cardiac autonomic balance due to the dynamic and complex interactions between the sympathetic and parasympathetic loops with the sinoatrial node (Heart rate variability, 1996; MacDonald et al., 2020). We further corroborated and correlated the autonomic parameters with blood markers of the hypoxic stress response including catecholamines, cortisol, and erythropoietin (Dimai et al., 2000; Mackenzie et al., 2008). Integrating both neuroendocrine components is ideal for highlighting the coherence between neural and humoral factors triggered during the allostatic response to the hypoxic stress.

Third, we aimed to test the cognitive effect of napping under normobaric hypoxia. We focused on attention, vigilance, and

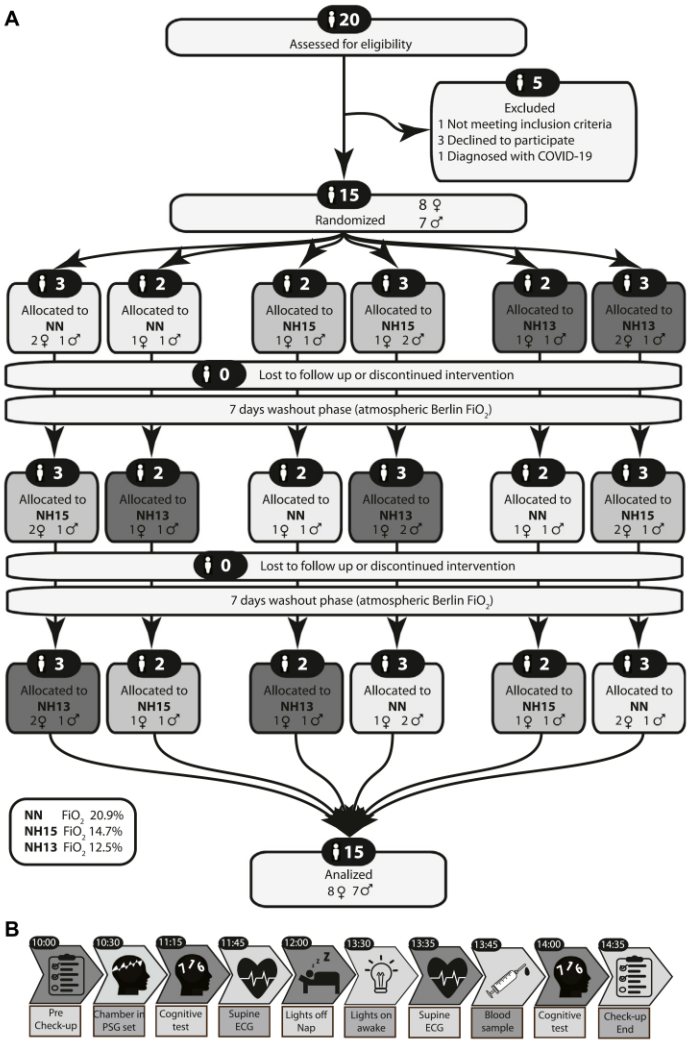

FIGURE 1 | CONSORT 2010 flow diagram **(A)** and study protocol **(B)**.

memory since previous studies have shown that these cognitive parameters are affected after sleeping under hypoxic conditions. (De Aquino Lemos et al., 2012). Thus, we conducted psychological evaluations including Digit Span and Stroop Color word tests and the Psychomotor Vigilance Task. To our knowledge, this is the first time that an integrated approach is provided for characterizing daytime napping under normobaric hypoxia conditions.

2 MATERIALS AND METHODS

2.1 Study Design

This was a single-blind, randomized, cross-over pilot study conducted at the Experimental and Clinical Research Center of Charité Universitätsmedizin Berlin from October 2019 to December 2020 (ClinicalTrials.gov 31/10/2019, NAPOXIA

study, identifier: NCT04146857). Since the main outcomes are more stable within-volunteers than between volunteers, a cross-over design was chosen. Because hypoxia-related symptoms due to three exposures over 3.5 h are mild and usually do not extend beyond 24 h, a significant dropout rate and a long carryover effect were not expected. Consequently, we chose a washout period of 1 week. Randomization of the treatment sequence was done with Randomizer® v. 2.1.0 (Research Randomizer, RRID:SCR_008563), setting sex as factor and minimization as method. Volunteers were blinded to the treatment allocation until the end of the study. They signed informed consent before their participation. The study followed the regulations of the currently applicable version of the Declaration of Helsinki and respective German legislation. The institutional ethics board of Charité Universitätsmedizin Berlin approved the study protocol (EA1/226/19).

2.2 Subjects

Healthy volunteers were recruited and screened (**Figure 1A**) by physical examination, blood tests, bioelectrical impedance analysis (BIA), and electrocardiogram. Key inclusion criteria were: women and men, age 20–45 years and body mass index 20–28 kg/m^2. Key exclusion criteria were: native highlanders, altitude exposure >2,500 m above sea level (asl) 6 months and plane travel 2 weeks before enrollment, smoking, cardiac or pulmonary diseases, insomnia, and obstructive sleep apnea.

2.3 Hypoxia Chamber

Normobaric hypoxia was produced by the nitrogen dilution technique according to methods described previously (Klug et al., 2018; Mähler et al., 2018). In brief, a 38 m^3 chamber with the Berlin baseline (35 m asl, FiO_2 = 20.9%) was insufflated with nitrogen gas to decrease the oxygen percentage and simulate 2,660 m asl (FiO_2 = 14.7%) or 4,000 m asl (FiO_2 = 12.5%). Environmental parameters such as percentage oxygen and carbon dioxide content, temperature, barometric pressure, and humidity were continuously monitored.

2.4 Bioelectrical Impedance Analysis

Body composition was obtained from bioelectrical multifrequency segmental impedance analysis using the BIACorpus RX Spectral (MEDICAL Healthcare GmbH, Karlsruhe, Germany).

2.5 Sleep Questionnaires

A validated German version of the Insomnia Severity Index (ISI) was used for insomnia screening (Gerber et al., 2016). Volunteers with clinical insomnia (>15 points) were excluded. Risk of Obstructive Sleep Apnea was evaluated by the German version of the STOP-bang questionnaire downloaded from the official STOP-bang website. Scoring more than 2 points was considered as an exclusion criterion.

2.6 Actimetry

We instructed volunteers to follow a 2 h-sleep restriction protocol the night before each experiment, meaning they had to go to bed 2 h later than usual but get up at their usual time. Previous reports using this approach have demonstrated an increase in the sleep drive in a dose-response relationship (Van Dongen et al., 2003). We chose delayed sleep rather than early awakening to ensure volunteers adherence to the protocol. To verify compliance with these instructions and detect sleep modifications around the experiments, volunteers wore an actimeter on their non-dominant wrist for 3 days before and after each experiment (ActiGraph GT3X, Pensacola, FL, United States; ActiGraph Activity Monitor Devices, RRID:SCR_008399). Collected data were processed using ActiLife v. 6.13.3 (ActiGraph, Pensacola, FL, United States) and sleep parameters obtained using the Sadeh algorithm on 60-s epochs.

2.7 Polysomnography and Sleep Staging

All volunteers underwent diurnal polysomnographic monitoring (Neurovirtual BWIII PSG Plus Sleep System™, Fort Lauderdale, FL, United States) in accordance with the Manual for the Scoring of Sleep and Associated Events by the American Academy of Sleep Medicine, v. 2.6. Scoring and sleep staging was manually conducted using the BWAnalysis software v 1.98.0.98 (Fort Lauderdale, FL, United States). Electrophysiological signals were sampled at 2,000 Hz, stored at 500 Hz and pass-band filtered at 0.03–35 Hz (electroencephalogram), 0.03–100 Hz (electrocardiogram) and 10–70 Hz (electromyogram).

2.8 Electrocardiography and Heart Rate Variability Analysis

Electrocardiographic signals were exported to LabChart Pro software v. 8.1.16 (ADInstruments, Castle Hill, NSW, Australia; Data Acquisition Systems for Life Science, RRID: SCR_001620). Following the HRV Task force guidelines, only sleep stages longer than 5-min were considered for HRV analysis. The HRV Module v. 2.0.3 algorithm automatically detected RR intervals in 5-min Electrocardiography (ECG) segments. Visual inspection and manual correction were applied in accordance with the HRV Guidelines (Malik et al., 1996). The series of RR intervals were exported to Kubios HRV Standard v. 3.4.2 software (University of Eastern Finland, Kuopio, Finland) (Tarvainen et al., 2014), and 4 Hz interpolation rate was applied to obtain HRV linear and non-linear parameters.

2.9 Cognitive Tests

Volunteers took a battery of three computer-based cognitive tests before and after the nap using the PEBL v. 2.1 software [Psychology Experiment Building Language (PEBL), RRID: SCR_014794] (Mueller and Piper, 2014). In the first test, a sequence of three digits was presented and the volunteer had to repeat it in the same order (forward digit span test). In a second test, the sequence presented had to be repeated by the volunteer in reverse order (backward digit span test). Both sequences increased the number of digits up to a maximum of nine. In a third test, words naming colors were presented in different colors. The colors of these words may or may not match the name. The volunteer was asked to inhibit the naming of the words and select the color in which they were presented (Color-word Stroop test). In a fourth and final test, a red dot appeared suddenly at irregular intervals in the center of a black screen. The volunteer was asked

to be attentive for 5 min to the black screen and activate a key when the red dot appeared with the shortest possible reaction time [5-min version of the Psychomotor Vigilance Task (PVT) (Loh et al., 2004)]. This tests sequence did not change within and between volunteers.

2.10 Hormone Analysis

After the nap, 5 ml of EDTA-blood samples were collected from all volunteers for blood count, dopamine, epinephrine, and norepinephrine measurements. Likewise, serum erythropoietin (EPO) and cortisol levels were measured from 5 ml of centrifuged blood. Centrifugation was done after finishing each experiment session and the sample was properly cooled and immediately delivered to the laboratory for analysis. Low levels of dopamine and epinephrine were reported as categorical parameters. For this reason, we handled them as ordinal parameters with the following categories: dopamine low (<60 ng/L), dopamine high (>60 ng/L), epinephrine low (≤30 ng/L), epinephrine high (>30 ng/L).

2.11 Experimental Protocol

Volunteers were instructed to abstain from caffeine and alcohol on the preceding day and from vigorous physical activity on the day of the experiment. Before entering the hypoxic chamber, the general condition, vital signs, quality of sleep, and compliance with the 2-h sleep restriction were evaluated through a general check-up (**Figure 1B**). Inside the chamber, polysomnography electrodes were positioned. Then sitting on the bed, the volunteers took the pre-sleep cognitive tests. Afterward, a 10-min baseline ECG was recorded while volunteers lay supine and awake. After this, the lights were switched off and volunteers were asked to sleep for 90 min. After a 5-min awakening period, another 10-min ECG was recorded, followed by blood sample collection. Finally, volunteers repeated the cognitive tests. All volunteers repeated this experiment at three conditions in a randomized sequence: normobaric normoxia at FiO_2 20.9% (NN), normobaric hypoxia at FiO_2 14.7% (NH15), and normobaric hypoxia at FiO_2 12.5% (NH13) (**Figure 1A**).

2.12 Outcome Measures

2.12.1 Primary Outcome Measure

Primary outcome measure was the change in the cardiac autonomic balance during or after napping, assessed by RR intervals as a component of HRV under hypoxia vs. normoxia.

2.12.2 Secondary Outcome Measures: Polysomnography

1) Parasympathetic Nervous System Index (PNS) resulting from the computation of mean RR interval, Root mean square of successive RR interval differences (RMSSD) and Poincaré plot index of the Standard Deviation of the instantaneous beat-to-beat variability (SD1) in normalized units
2) Sympathetic Nervous System Index (SNS) resulting from the computation of mean Heart Rate (HR), Baevsky's stress index (SI) and Poincaré index of the Standard Deviation of the continuous long-term variability (SD2) in normalized units
3) Peripheral Oxygen Saturation (SpO_2).
4) Sleep Efficiency (SE).
5) Wake After Sleep Onset (WASO).
6) Sleep Onset Latency (SOL).
7) Apnea-Hypopnea Index (AHI).
8) Periodic Breathing Index (PBI).
9) Percentage of REM sleep and stage 1 (N1), stage 2 (N2) and stage 3 (N3) of non-REM sleep.

2.12.3 Secondary Outcome Measures: Hormone Levels

1) Erythropoietin (EPO).
2) Cortisol
3) Norepinephrine
4) Epinephrine
5) Dopamine.

2.12.4 Secondary Outcome Measures: Cognition

1) Length of digit span tests
2) Lapses and reaction time in PVT
3) Accuracy and reaction time in color-word Stroop test

2.13 Data Management, Analysis, and Statistical Analysis

Study data were pseudonymized, collected, and managed using REDCap (REDCap, RRID:SCR_003445) electronic data capture tools (Harris et al., 2009) hosted at Charité Universitätsmedizin Berlin. The sample size was determined following the rule of thumb for pilot studies proposed by Julious SA (Julious, 2005). Data graphing were carried out by Origin Pro v. 9.3.226 software (OriginLab Corporation, Northampton, MA, United States; RRID: SCR_014212). Rstudio v. 1.4.1103 (RStudio, RRID:SCR_000432) based on R v. 4.0.4 software (R Project for Statistical Computing, RRID:SCR_001905) and Jamovi v. 1.6.16 (The jamovi project 2021; RRID:SCR_016142) were used for statistical analysis. R packages included ggplot, corrplot and MASS. According to Rubin's classification, missingness was considered MAR (PSG data) or MACR (blood analysis). Normal distribution was evaluated by Shapiro-Wilk test and QQ plots. Robust statistical processing using Huber M-estimate with winsorized at 1.5 standard deviations and converge tolerance 1e-06 was applied to detect outliers. Descriptive statistics are presented by mean ± SD unless stated otherwise. Ordinal parameters are presented as percentage. Since this was a pilot study, inferential statistics should be understood as an exploratory approach to the data analysis. Multiple comparison test with Holm's correction is presented only to describe the main patterns, but it is limited for the reasons previously stated. Since within-subject correlations arose from the cross-over design, a linear mixed model approach was chosen. To estimate differences between normoxia and hypoxia, modeling was applied using FiO_2, sex, or sleep phase as fixed factors and intercept-volunteer as random factor. Normal distribution of residuals was verified. Sex differences in demographic and hematologic parameters at baseline were analyzed by a *t*-test. McNemar's test of paired contingency tables was performed to analyze epinephrine and dopamine. Correlations between parameters were calculated by Pearson's r. The overall significance level was set at two-tailed $p = 0.05$.

TABLE 1 | Anthropometric and hematologic parameters of 15 healthy men and women. Data as mean ± SD.

	Women (8)	Men (7)	All (15)	Sex difference p-value (*t*-test)
Age (years)	26.5 ± 3.2	32.6 ± 8.1	29.3 ± 6.6	0.07
Mass (kg)	61.1 ± 4.7	81.9 ± 8.4	70.8 ± 12.5	<0.001
Height (cm)	167 ± 5.0	182 ± 9.8	174 ± 10.8	0.002
Body Mass Index (kg/m^2)	22.0 ± 1.7	24.8 ± 2.8	23.3 ± 2.6	0.034
Fat Mass (%)	28.3 ± 4.3	22.1 ± 5.0	25.4 ± 5.5	0.023
Fat Free Mass (%)	71.7 ± 4.3	77.9 ± 5.0	74.6 ± 5.5	0.023
Body Water%	50.4 ± 3.5	57.1 ± 4.0	53.5 ± 5.0	0.004
Erythrocytes/pL	4.31 ± 0.2	5.13 ± 0.4	4.69 ± 0.5	<0.001
Hemoglobin (g/dl)	12.8 ± 0.7	15.2 ± 0.8	13.9 ± 1.4	<0.001
Hematocrit (%)	38.5 ± 1.9	45.0 ± 2.8	41.5 ± 4.0	<0.001

TABLE 2 | Heart rate variability parameters during a daytime nap in normobaric normoxia (NN) and normobaric hypoxia (NH15: FiO_2 14.7, NH13: FiO_2 12.5).

		Phase	N	RR (ms)	RMSSD (ms)	SD1 (ms)	PNS	HR (b.p.m.)	SD2 (ms)	SI	SNS
NN	Women	Pre	8	985 ± 115	66.3 ± 48	47 ± 34	1 ± 1.84	61.6 ± 7.1	64.3 ± 25.7	8.2 ± 2.7	–0.52 ± 0.92
		N2	8	1,024 ± 146	74.9 ± 43	53 ± 30	1.44 ± 1.79	59.8 ± 9.4	64.4 ± 14.8	7.8 ± 3.3	–0.73 ± 1.16
		Pos	8	938 ± 125	56.7 ± 46	40 ± 33	0.55 ± 1.81	65 ± 8.9	50.2 ± 22.1	10.8 ± 4	0.08 ± 1.21
	Men	Pre	7	1,172 ± 170	55 ± 34	39 ± 24	1.54 ± 1.76	52.1 ± 6.9	52.6 ± 15.7	8.7 ± 3.9	–1.05 ± 1.13
		N2	7	1,230 ± 157	48.7 ± 24	35 ± 17	1.64 ± 1.32	49.4 ± 5.5	49.1 ± 19.6	8.5 ± 2.7	–1.26 ± 0.75
		Pos	7	1,083 ± 95	42.8 ± 21	30 ± 15	0.77 ± 0.97	55.8 ± 5	54 ± 23.3	9.5 ± 3.7	–0.65 ± 0.74
	All	Pre	15	1,072 ± 168	61 ± 41.2	43.2 ± 29.2	1.25 ± 1.76	57.2 ± 8.4	58.8 ± 21.8	8.4 ± 3.2	–0.77 ± 1.02
		N2	15	1,120 ± 180	62.7 ± 36.6	44.4 ± 26	1.53 ± 1.54	54.9 ± 9.3	57.3 ± 18.4	8.1 ± 2.9	–0.98 ± 1
		Pos	15	1,006 ± 132	50.2 ± 36.2	35.6 ± 25.6	0.65 ± 1.43	60.7 ± 8.5	52 ± 21.9	10.2 ± 3.8	–0.26 ± 1.05
NH15	Women	Pre	8	876 ± 122	42.1 ± 21	30 ± 15	0.25 ± 1.75	69.6 ± 9.1*	61.8 ± 28.9	9 ± 3.4	0.15 ± 1.02
		N2	7[m]	940 ± 165	64.7 ± 43	46 ± 30	0.75 ± 1.91	65.6 ± 11.4	62.3 ± 23.4	9.4 ± 4.5	–0.07 ± 1.5
		Pos	7[m]	795 ± 109*	26.6 ± 17	19 ± 12	–1 ± 0.94*	76.6 ± 9.6**	37.8 ± 18.9	15.2 ± 6.5	1.61 ± 1.6*[A]
	Men	Pre	6[m]	1,135 ± 185	53.8 ± 37	38 ± 26	1.28 ± 1.88	53.9 ± 8.2	63.6 ± 19.2	7.7 ± 2.5	–1.02 ± 0.94
		N2	6[m]	1207 ± 123	51.7 ± 28	37 ± 20	1.54 ± 1.37	50.1 ± 4.7	67.9 ± 26.9	7.6 ± 2.7	–1.28 ± 0.68
		Pos	6[m]	1,036 ± 86	38.3 ± 25	27 ± 18	0.4 ± 1.03	58.3 ± 5.3	55.7 ± 26.6	10.4 ± 4.7	–0.31 ± 0.85
	All	Pre	13	987 ± 197*	47.5 ± 28.9	33.7 ± 20.5	0.69 ± 1.81	62.9 ± 11.6*	62.6 ± 24.3	8.4 ± 3	–0.36 ± 1.13
		N2	13	1,063 ± 198	58.7 ± 35.7	41.6 ± 25.3	1.12 ± 1.67	58.4 ± 11.8	64.9 ± 24.2	8.6 ± 3.7	–0.63 ± 1.31
		Pos	13	906 ± 157**[A]	32 ± 20.7	22.7 ± 14.6	–0.36 ± 1.19*[A]	68.2 ± 12.2*[A]	46.1 ± 23.7	13 ± 6	0.73 ± 1.6[A]
NH13	Women	Pre	8	781 ± 85**	30.5 ± 17	22 ± 12*	–0.96 ± 0.81**	77.5 ± 7.9**	43.9 ± 15.8	13.4 ± 4.5	1.4 ± 1.14**
		N2	8	797 ± 168**	36.8 ± 32	26 ± 23*	–0.73 ± 1.64**	78.2 ± 16**	48.4 ± 31.4	16.6 ± 12.5*	1.97 ± 2.96**
		Pos	8	708 ± 93**	15.7 ± 11	11 ± 8*	–1.77 ± 0.77**	86 ± 10.7**[A]	31.3 ± 17.5	19.3 ± 7.1*	2.95 ± 1.61**[A]
	Men	Pre	7	1,010 ± 126**	46.8 ± 28*	33 ± 20	0.45 ± 1.29	60.2 ± 7.1	81 ± 41.8	7.7 ± 2.6	–0.55 ± 0.82
		N2	7	1,048 ± 103**	52.3 ± 32*	37 ± 22	0.77 ± 1.31	57.7 ± 5.2*	93 ± 45.6*	7 ± 3.5	–0.81 ± 0.78
		Pos	7	909 ± 50**	27.3 ± 14*	19 ± 10	–0.55 ± 0.59	66.2 ± 3.7*	54.3 ± 18.8	10.8 ± 4	0.34 ± 0.74
	All	Pre	15	888 ± 156**	38.1 ± 23.3*	27 ± 16.5*	–0.3 ± 1.26**	69.4 ± 11.6**	61.2 ± 35.2	10.8 ± 4.7	0.49 ± 1.39*
		N2	15	914 ± 188**	44 ± 31.7	31.2 ± 22.5	–0.03 ± 1.64**	68.6 ± 15.9**	69.2 ± 43.8	12.1 ± 10.4	0.67 ± 2.59**
		Pos	15	802 ± 127**[A]	21.1 ± 13.5*	15 ± 9.6*	–1.2 ± 0.92*[A]	76.7 ± 12.9**[B]	42 ± 21.1	15.4 ± 7.2*	1.73 ± 1.83**[A]

*[m]Missing data due to lack of sleep or ECG artifacts. Data as mean ± SD. *p <0.05, **p <0.001 comparing the same phase with NN. [A]p <0.05, [B]p <0.001 comparing with pre in the same condition (Linear Mixed Model).*

3 RESULTS

3.1 Demographic, Haematological, and Environmental Evaluation

Total recruitment, screening, attrition, and allocation are presented in **Figure 1**. Baseline anthropometric and hematologic characteristics of 15 men and women who completed the study are shown in **Table 1**. Men were slightly, but not significantly, older than women.

In line with our instructions, actimetry showed a 2-h sleep restriction the night before tests. Along with this, there were no changes in sleep efficiency (SE, 87 ± 2%) and wake after sleep onset (WASO, 59 ± 8 min) in the nights before vs. after experiments. This apparent undisturbed sleep could be because the dose of sleep restriction was small and the exposure to hypoxia was short. In addition, similar protocols (such as the splitting sleep protocol) have even found positive effects from their implementation (Cousins et al., 2021).

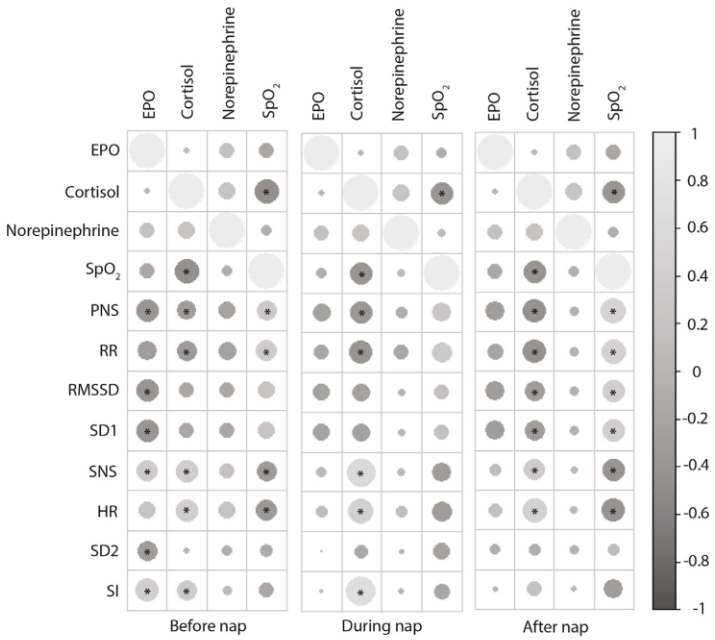

FIGURE 2 | Correlations of after nap stress hormone levels (EPO, cortisol, norepinephrine) with HRV parameters measured after, during and after the nap. Positive correlations are displayed in light gray and negative correlations in dark gray. Circle size and intensity are proportional to the correlation coefficients. *$p < 0.05$ (Pearson's r).

Environmental parameters within the chamber did not show differences throughout the study (barometric pressure: 759 ± 7 mmHg; humidity: 37.6 ± 6%; temperature: 20.8 ± 1°C).

3.2 Primary Outcome Measure

Since REM and N3 were less than 5 min long or absent in more than 30% of experiments, these phases were not included in the HRV analysis. The RR interval before, during, and after nap was shorter under hypoxia vs. normoxia (**Table 2**). Regarding the analysis by sex, men showed a longer RR interval in all conditions and phases compared to women, being pronounced for N2 in NH15 ($p = 0.05$) but not in NN ($p = 0.32$). This sex difference was reciprocally evident for HR in NH13 N2 ($p = 0.01$), NH13 pos ($p = 0.02$) and NH15 pos ($p = 0.04$) (**Table 2**). In comparison with N2, a significant shortening in RR was observed after napping in all conditions ($p < 0.001$). A comparison between pre- and post-nap RR interval revealed difference in NH15 ($p = 0.006$) and NH13 ($p = 0.007$), but not in NN ($p = 0.07$).

3.3 Effects of Hypoxic Stress on Cardiac Autonomic Balance

The SNS index changed in NH13 vs. NN, particularly in women (**Table 2**). SNS index was different in women during nap vs. pre-nap, both at NH13 ($p = 0.019$) and NH15 ($p = 0.03$). PNS index showed a similar pattern, but only at NH13 ($p = 0.04$). Finally, peripheral oxygen saturation (SpO_2) correlated positively with PNS index and RR, both before and after the nap (**Figure 2**). In line with this, SpO_2 was negatively correlated with SNS index and HR. Mean and SD of further factors determining PNS index (RMSSD and SD1) and SNS index (HR, SI and SD2) are shown in **Table 2**.

3.4 Effects of Hypoxic Stress on Sleep

A summary of polysomnography (PSG) data is presented in **Table 3**. Total sleep time (TST), sleep period time (SPT), sleep onset latency (SOL), WASO, and SE were not affected by both hypoxic conditions and sex. Interestingly, N2 percentage of TST increased in NH15 and NH13 vs. NN ($p < 0.001$). Accordingly, N3 percentage of TST decreased in NH15 and NH13 ($p < 0.001$), both vs. NN. All men showed REM sleep in NN (17 ± 4% of TST) but only 4 of 8 women. Those percentages fell in NH13, where women did not get any REM sleep, and only 3 of 7 men did.

During the nap, SpO_2 was different between all three conditions ($p < 0.001$) (**Figure 3A**). At NH13, SpO_2 values were higher in women vs. men ($p = 0.012$), but not at NH15 ($p = 0.629$) or NN ($p = 0.87$) (**Table 3** and **Figure 3A**).

Finally, the incidence of respiratory events under hypoxic conditions was higher in men vs. women, including central

TABLE 3 | Main polysomnography parameters during a daytime nap in normobaric normoxia (NN) and normobaric hypoxia (NH15: FiO_2 14.7, NH13: FiO_2 12.5).

		N	TST (min)	WASO (min)	SPT (min)	SOL (min)	N1 %TST	N2 %TST	N3 %TST	SE TST	SE SPT	% SpO_2 TST
NH	Women	8	68.3 ± 21	21 ± 24.6	87.4 ± 7.4	3.9 ± 5.8	24 ± 22.1	39.8 ± 11.5	30.6 ± 20.4	74.7 ± 25.7	94 ± 7.6	96.1 ± 1.6
	Men	7	71.5 ± 18.3	15.6 ± 11.7	84.3 ± 14.2	3.6 ± 3.2	16.7 ± 7.1	42.3 ± 14.8	37.6 ± 21.3	78.9 ± 15.5	93.3 ± 7.5	95.9 ± 1.1
	All	15	69.8 ± 19.2	18.5 ± 19.2	85.9 ± 10.8	3.8 ± 4.6	20.6 ± 16.7	40.9 ± 12.7	33.9 ± 20.4	76.6 ± 20.9	93.6 ± 7.3	96 ± 1.3
NH15	Women	7[m]	83.6 ± 4.9	6.1 ± 3.2	89.4 ± 3.9	2.4 ± 1.9	13.1 ± 4.9	57.2 ± 11.2	18.2 ± 12.6	90.8 ± 4.3	97 ± 2.2	87.7 ± 2.5**
	Men	6[m]	60.8 ± 17.4	18.6 ± 15.9	82.5 ± 3.9	4.1 ± 1.8	26.9 ± 12.2	63.4 ± 11.1	9.7 ± 10.6*	67.1 ± 19.2	91 ± 4.7	85.8 ± 2.9**
	All	13	73.1 ± 16.7	11.9 ± 12.4	86.2 ± 5.2	3.2 ± 2	19.4 ± 11.2	60 ± 11.1**	14.3 ± 12.1**	79.8 ± 17.7	94.2 ± 4.6	86.8 ± 2.7**
NH13	Women	8	62.6 ± 24.4	21.2 ± 20.7	79.1 ± 12.8	5 ± 7.2	20.7 ± 13.5	63.2 ± 13.2**	11.6 ± 15.8*	70.2 ± 26.5	88.8 ± 12.6	78.4 ± 3.8**, ^A
	Men	7	62.6 ± 18.7	23.9 ± 16.5	77.6 ± 22.9	5 ± 4.3	23.2 ± 18.6	53.4 ± 16.3	18.7 ± 18.1	69.9 ± 18.3	85.3 ± 17.2	73.1 ± 5.8 ^A
	All	15	62.6 ± 21.1	22.4 ± 18.3	78.4 ± 17.5	5 ± 5.8	21.9 ± 15.5	58.6 ± 15**	14.9 ± 16.7**	70 ± 22.2	87.1 ± 14.5	75.9 ± 5.4**

[m]*Missing data due to lack of sleep. TST, Total Sleep Time; WASO, Wake after Sleep Onset; SPT, Sleep Period Time; SOL, Sleep Onset Latency; SE, Sleep Efficiency; SpO_2, Peripheral oxygen saturation. Data as mean ± SD. *p < 0.05, **p < 0.001 comparing with NN. ^A p = 0.012 comparing with women in the same condition (Linear Mixed Model).*

sleep apneas and periodic breathing (**Figure 4**). Indeed, only one woman presented a periodic breathing pattern in NH13. The reticulocyte production index in NH13 correlated strongly with the number of apneas [r (14) = 0.57, p = 0.03] and PB [r (14) = 0.66, p = 0.008].

3.5 Effects of Hypoxic Stress on Endocrine Response

Erythropoietin (EPO) concentrations raised with increasing hypoxia with no sex-specific differences (**Figure 2B**). This EPO increase was accompanied by an increment in erythrocytes in NH13 (4.67 ± 0.13/pL; p = 0.008), but not in NH15 (4.61 ± 0.11/pL, p = 0.074), both vs. NN (4.56 ± 0.12/pL). EPO showed positive correlations with SI and SNS index and negative correlations with RMSSD, SD1, and PNS index, particularly before the nap (**Figure 2**).

Cortisol concentrations also increased with increasing hypoxia (**Figure 3C**). In contrast to EPO, cortisol was correlated with HRV parameters during nap, particularly with SI and SpO_2 (**Figure 2**).

We found an increased frequency of high concentrations of epinephrine (NN = 14.3%, NH15 = 23.1%, NN13 = 42.9%) and dopamine (NN = 23.1%, NH15 = 38.5%, NH13 = 42.9%), which were, however, not significant (**Figure 3D**). Norepinephrine concentrations did not change (data not shown).

3.6 Effects of Hypoxic Stress on Cognition

Performance in the Forward and Backward Digit Span test before the nap did not change due to hypoxia (Forward, NN: 7.3 ± 1.6, NH15: 6.7 ± 1.6, NH13 = 7.1 ± 2; Backward, NN = 7.4 ± 1.5, NH15 = 6.7 ± 1.1, NH13 = 7.5 ± 1.5). However, there was a strong correlation between cortisol concentrations and the Forward Digit Span test after the nap [r (14) = −0.61, p = 0.02] at NH13, but not NH15 or NN.

Incongruent-Congruent response times (I-C) in the Stroop test did not change markedly. Strikingly, I-C were higher under hypoxia (NN: 75.9 ± 85.5 ms, NH15: 128 ± 71.5 ms, NH13: 113 ± 93.6 ms). At NH13, congruence errors were correlated with HRV, including SNS index [r (14) = 0.54, p = 0.04], HR [r (14) = 0.53, p = 0.04] and SI [r (14) = 0.52, p = 0.04]. Response time to the congruent stimulus improved after nap at NN (pre: 737 ± 129 ms vs. pos: 654 ± 118 ms, p = 0.035). Importantly, this improvement was not observed under both hypoxic conditions.

Lapses in PVT did not change (NN: 4.1 ± 5.6, NH15: 3.6 ± 5.3, NH13: 3.9 ± 4.3). At NH13, the PVT response time was correlated with EPO [r (14) = −0.59, p = 0.02], SpO_2 during the nap [r (14) = −0.52, p = 0.05] and SI after the nap [r (14) = 0.59, p = 0.03].

4 DISCUSSION

Napping is recommended as an energetic and cognitive booster (Milner and Cote, 2009; McDevitt et al., 2018). Here, we describe the main physiological changes during a daytime nap under simulated altitude conditions, including cardiac autonomic

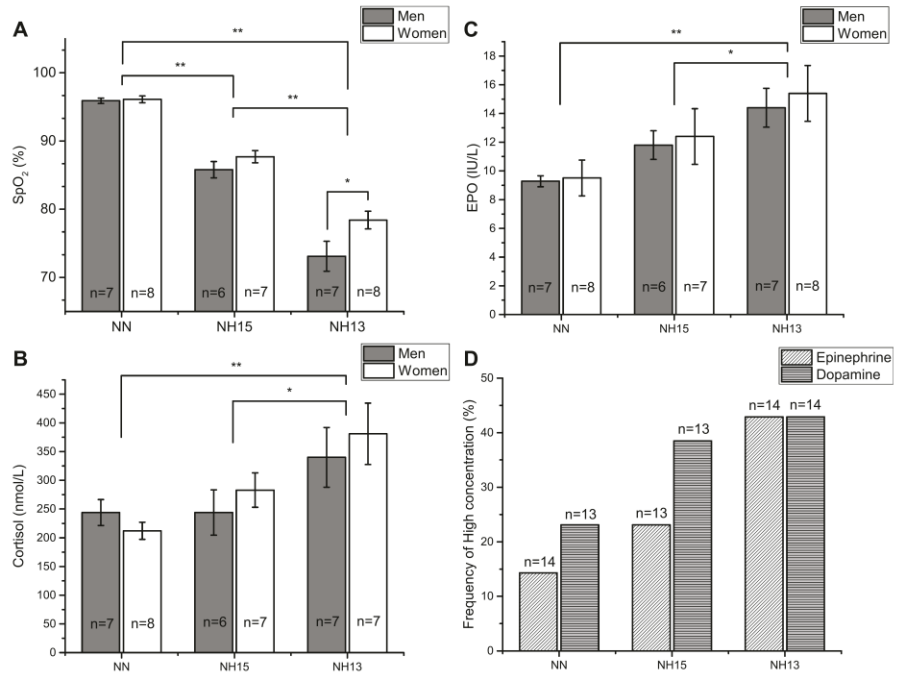

FIGURE 3 | Peripheral oxygen saturation (SpO_2) **(A)** and cortisol **(B)** and erythropoietin (EPO) **(C)** concentrations after a 90 min nap under normoxic (NN) and hypoxic conditions (NH15: FiO_2 14.7, NH13: FiO_2 12.5) in healthy men (n = 7) and women (n = 8). Data as mean ± SEM. *p < 0.05, **p < 0.001 (Linear Mixed Model). Frequency of high concentration of epinephrine and dopamine **(D)**. Missing data due to blood specimen damage during processing.

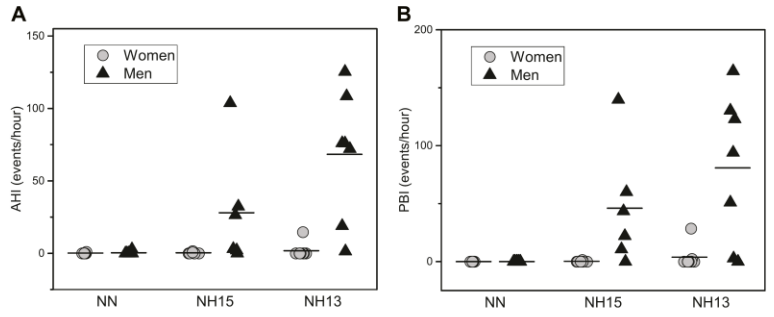

FIGURE 4 | Apnea-Hypopnea Index (AHI) **(A)** and Periodic Breathing Index (PBI) **(B)** during a 90 min nap under normoxic (NN) and hypoxic conditions (NH15: FiO_2 14.7, NH13: FiO_2 12.5) in healthy men (n = 7) and women (n = 8).

balance, stress hormone secretion, and respiratory pattern. We assessed whether the presented neuroendocrine and oxygenation changes affect cognitive performance in attention, memory, and vigilance functions. We investigated 15 healthy adult volunteers who took a short daytime nap under two hypoxic conditions.

Primary outcome measure was the change in the RR Interval under hypoxic conditions. This component of the electrocardiogram is directly correlated with cardiovagal activity (Chapleau and Sabharwal, 2011). Under normoxia we found values similar to the general population (926 ± 90 ms,

resting, normoxic) (Nunan et al., 2010). Sleep was accompanied by an RR elongation that has been reported both in nighttime (Herzig et al., 2018) and daytime sleep (Cellini et al., 2018). The evident decrease in SpO_2 under both hypoxic conditions was accompanied by a RR shortening, which affected both awake and sleep measurements. This effect aims to improve oxygen delivery and is mediated by the carotid bodies in hypoxic conditions and could be explained by a depression of cardiovagal activity *via* nucleus ambiguous or a sympathetic stimulation via the rostral ventrolateral medulla (Zera et al., 2019). During hypoxic stress, the RR interval after the nap was shorter than before, showing inertia after awakening in the increased sympathetic activity. This contrasts with the normoxic condition in which the values did not change. Although the RR interval was consistently longer in men than in women, this difference was accentuated during sleep under hypoxia. This sex-dependent difference could be due to discrepancies in breathing control, particularly in chemosensitivity to hypoxia (Gargaglioni et al., 2019; Littlejohn et al., 2020).

The cardiac autonomic change evident by RR shortening was consistent with changes of cardiovagal markers in HRV, such as the RMSSD, SD1, and the PNS index. These findings coincide with Boos et al. (Boos et al., 2017), who also found changes in parasympathetic markers dependent on sex and altitude in awake subjects early at the morning. Evaluation in field conditions after acute exposure to hypoxia at 3,150 m also showed similar changes before and after sleep (Yih et al., 2017). This decrease in cardiovagal tone could be accompanied by either a relative (i.e., no actual increase in activity) or an absolute increase in sympathetic activity. Since the increments in HR, SI, and SNS index were accompanied by an increase in the frequency of high levels of epinephrine during hypoxia, the results of this study rather point to the second possibility. Contrary to Panjwani et al. (2006), who found an increase in plasma norepinephrine after a simulated ascent to 3,500 m, our study did not show such changes. The explanation for this dissimilarity of responses could be that in our study subjects were evaluated at rest or that noradrenergic mediation is more neural than endocrine, as Rowel et al. (1989) have shown. It has also been found that although an increased sympathetic influx accompanies acute hypoxia, norepinephrine clearance is equally increased (Leuenberger et al., 1991).

In any case, this absolute or relative increase in sympathetic activity was more pronounced in women than in men, with the aforementioned post-nap inertia. This is paradoxical considering that men exhibited the lowest SpO_2 values. However, there is evidence that women have a different sensitivity to hypoxia (Gargaglioni et al., 2019), probably due to the effects of female hormones on the neural circuits controlling cardio-ventilatory responses (Littlejohn et al., 2020). Additionally, men presented periodic breathing, a phenomenon that has been linked to a RR lengthening as a consequence of increment in tidal volume and its effect on vagal tone (Insalaco et al., 2016).

Periodic breathing due to altitudinal hypoxia is a respiratory event that has been previously described both under field (Ju et al., 2021) and laboratory conditions (Pramsohler et al., 2019) during nighttime sleep. Here, we confirm this during a daytime nap and a short hypoxia exposition. As during its nocturnal presentation, periodic breathing has an accentuated sex-dependent incidence, being predominantly a male phenomenon. This could be explained either by an inhibitory effect of female hormones or a trigger effect of testosterone. Although the two possibilities are not mutually exclusive, some results of this study favor the second possibility. On the one hand, the only woman who presented periodic breathing has been diagnosed with polycystic ovary syndrome (POS), a condition in which testosterone levels are increased substantially (Lerchbaum et al., 2014). Likewise, the man who had fewer episodes of periodic breathing was the oldest one and, therefore, might have lower testosterone levels (Ferrini and Barrett-Connor, 1998). Studies evaluating nocturnal sleep have shown that although in a lower proportion than men, women also develop periodic breathing, especially at higher altitudes (Lombardi et al., 2013; Caravita et al., 2015). A notable difference in our daytime sleep findings is that no women presented periodic breathing (excluding the one with POS). These differences could be either due to infradian phenomena (the phase of the menstrual cycle in which women are evaluated), circadian phenomena (the contrast between daytime and nighttime sleep) or to a time-dependent mechanism in which only prolonged exposure triggers periodic breathing in women. Studies discriminating whether one or all these mechanisms are involved are needed.

Although there are differences between nighttime and daytime sleep, in normoxic experiments a similar distribution of N2 has been described previously (Jiang et al., 2018). Our study corroborates that, like nighttime sleep, a daytime nap in normoxia is accompanied by a higher proportion of N2 sleep followed by N3 and REM sleep. Similar to men evaluated during nighttime sleep in a simulated altitude of 2,000 m (Hoshikawa et al., 2007), we found an increased proportion of N2 and decreased proportions of N3 and REM sleep under hypoxic conditions. Considering that cerebral clearance has been associated with sleep (Xie et al., 2013) and particularly its slow waves (Fultz et al., 2019), a decrease in the N3 proportion may affect brain function. Indeed, this has been pointed out as the link between high-altitude hypoxia and cerebral edema (Simka et al., 2018). Besides hypoxia, we cannot rule out a small additive effect of factors such as sleep deprivation or caffeine withdrawal (Wu et al., 2010; Weibel et al., 2020).

As previously described (Coste et al., 2005; Keenan et al., 2020), the endocrine response to hypoxic stress was led by cortisol, which showed a positive correlation with sympathetic HRV markers, particularly during the nap. Indeed, a mutually enhancing interaction between cortisol secretion and sympathetic tone has been described *via* peripheral (Wurtman, 2002) and central mechanisms (Schulz et al., 2020). On the other hand, and as has been demonstrated during acute exposure to normobaric hypoxia in awake subjects (Mackenzie et al., 2008), taking a daytime nap of fewer than 90 min is accompanied by significant changes in EPO secretion. Unfortunately, our study design cannot answer if this response was due to hypoxia, sleep disturbances or both.

Unlike Philips et al. (2015) who found changes in cognitive performance with evaluations such as the Digit Span and Stroop tests after 30 min of exposure to hypoxia our results before the nap (60 min of hypoxia) did not reveal changes between

normoxic and hypoxic conditions. Our results are more in agreement with Latshang et al. (2013) who also failed to evoke changes. Additionally, although it has been shown that after naps there is an enhancement of mental activity (Milner and Cote, 2009; Qian et al., 2020), our results only showed a slight improvement in cognitive performance. It is possible that, as has been proposed (McMorris et al., 2017), changes become apparent only when arterial oxygen delivery is below a certain threshold. Hence, although our volunteers had low oxygen saturation levels, other oxygen delivery determinants might have compensated for that. Nevertheless, the correlation between cognitive performance and hormone levels, particularly cortisol and EPO, stands out. We corroborated that tests including reaction times were correlated with the neuroendocrine response to hypoxic stress (Phillips et al., 2015; Pramsohler et al., 2019).

Our study has a comprehensive approach to daytime sleep and hypoxic stress phenomena, including simultaneous neural and endocrine biomarker measurements. We evaluated activity and sleep both within the laboratory and on the days surrounding the experiment. However, as a pilot study several constraints need to be recognized to be improved in more extended assessments. For example, although our sample size exceeds the minimum recognized for a pilot study (Julious, 2005), larger groups under each condition may render stronger conclusions. Nevertheless, as a cross-over study, a smaller sample size has similar power and effect size than a parallel-group study with a larger one (Berry et al., 2006). Also, and linked to the constraints of the sample size is the scope of our conclusions, given that volunteers were not randomly selected and rather recruited from the local community. For this reason, the sample was cloistered to a relatively young age group of lowlanders with similar ethnic characteristics. Extrapolation to other age groups, highlanders or ethnically distant inhabitants does not apply. As a more functional aspect, there are differences between daytime and nighttime sleep (see above), hence, the interpretation of our data is limited to patterns observed during the day. Future research could address these differences by extending the study sites and populations analyzed as well as the times of day when the experiments are conducted. Moreover, the fact that the volunteers had a sleep deprivation the night before the hypoxic experiment could have an additive effect (Tobaldini et al., 2014). However, previous studies documented the preservation of cortisol release and cognitive variables, even with more extended restrictions than ours, as long as it is acute (Voderholzer et al., 2012; Schaedler et al., 2018). It is also true that in real scenarios proposed in our altitude simulations, this association between hypoxia and sleep restriction is not uncommon. However, a more detailed dissection of this relationship could be examined in future works. Adding to the clustered sample, the specific and controlled conditions of the laboratory decrease the scope of our inferences. Hence, extrapolation to natural altitudes is somewhat limited, and a differential response in hypobaric hypoxia is still debated (Millet et al., 2012; Mounier and Brugniaux, 2012). Additionally, it is well known that humidity and temperature could be different from those presented here, and both factors have important effects on sleep (Manzar et al., 2012; Okamoto-Mizuno and Mizuno, 2012) and hypoxic stress (Mugele et al., 2021). On the other hand, although most of the measurements were made during late summer and autumn, seasonal modifications (light-dark ratio, barometric pressure, humidity, and temperature) could affect sleep and the neuroendocrine responses to stress (Suzuki et al., 2019). Because we analyzed an acute exposure, long-term compensatory mechanisms to hypoxic stress are out of the scope of our conclusions. Finally, although infradian mechanisms could partly explain the respiratory response of our volunteers contrasting to previous nocturnal sleep studies (Lombardi et al., 2013; Caravita et al., 2015), given the small sample size, it was not possible to categorize responses concerning the menstrual cycle phase.

5 PERSPECTIVES AND SIGNIFICANCE

This study demonstrates that taking a daytime nap, even in mild hypoxia and during a short time, triggers a classic neuroendocrine response to hypoxic stress in lowlanders. Those changes are accompanied by modifications in sleep architecture, cardiac autonomic modulation, and respiratory pattern with marked differences between men and women, ratifying the importance of considering sex as a central determinant in all adaptive physiological responses. However, such changes do not impact cognitive performance or sleep efficiency.

Given the above, we hope that this research will lead us to corroborate these findings under natural conditions. In that case, the benefits of oxygen supplementation during napping at altitude should be evaluated. Finally, the elucidation of the specific effects that ethnicity, and sex hormones and their oscillations have on daytime napping should be analyzed in detail.

DATA AVAILABILITY STATEMENT

The data set of this study is available upon reasonable request by accredited researchers or institutions with a methodologically sound proposal.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional ethics board of Charité Universitätsmedizin Berlin (No. EA1/226/19). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors conceived and designed the study. AR-R, LK and MB screened the volunteers. AR-R, LK, AM, and MB conducted the experiments. AR-R and AM analyzed and interpreted data. Funding acquisition by FP and AM. AR-R and AM wrote the first draft of the paper. AR-R designed the figures. All authors contributed to manuscript revision, and read and approved the submitted version.

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10 Curriculum Vitae

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11 Publication list

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