



Sex Differences in Autonomic Cardiac Control and Oxygen Saturation Response to Short-Term Normobaric Hypoxia and Following Recovery: Effect of Aerobic Fitness

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Introduction: The main aims of this study were to investigate autonomic nervous system (ANS) and arterial oxygen saturation (SpO₂) responses to simulated altitude in males and females, and to determine the association between maximal oxygen uptake (VO₂max) and these responses.

Materials and Methods: Heart rate variability (HRV) and SpO₂ were monitored in a resting supine position during Preliminary (6 min normoxia), Hypoxia (10 min, fraction of inspired oxygen (FIO₂) of 9.6%, simulated altitude ~6,200 m) and Recovery (6 min normoxia) phases in 28 males (age 23.7 ± 1.7 years, normoxic VO₂max 59.0 ± 7.8 ml.kg⁻¹.min⁻¹, body mass index (BMI) 24.2 ± 2.1 kg.m⁻²) and 30 females (age 23.8 ± 1.8 years, VO₂max 45.1 ± 8.7 ml.kg⁻¹.min⁻¹, BMI 21.8 ± 3.0 kg.m⁻²). Spectral analysis of HRV quantified the ANS activity by means of low frequency (LF, 0.05–0.15 Hz) and high frequency (HF, 0.15–0.50 Hz) power, transformed by natural logarithm (Ln). Time domain analysis incorporated the square root of the mean of the squares of the successive differences (rMSSD).

Results: There were no significant differences in SpO₂ level during hypoxia between the males (71.9 ± 7.5%) and females (70.8 ± 7.1%). Vagally-related HRV variables (Ln HF and Ln rMSSD) exhibited no significant differences between sexes across each phase. However, while the sexes demonstrated similar Ln LF/HF values during the Preliminary phase, the males (0.5 ± 1.3) had a relatively higher ($p = 0.001$) sympathetic activity compared to females (−0.6 ± 1.4) during the Hypoxia phase. Oxygen desaturation during resting hypoxia was significantly correlated with VO₂max in males ($r = -0.45$, $p = 0.017$) but not in females ($r = 0.01$, $p = 0.952$) and difference between regression lines were significant ($p = 0.024$).

Conclusions: Despite similar oxygen desaturation levels, males exhibited a relatively higher sympathetic responses to hypoxia exposure compared with females. In addition, the SpO₂ response to resting hypoxia exposure was related to maximal aerobic capacity in males but not females.

Keywords: gender, autonomic nervous system, vagal activity, sympathovagal balance, simulated altitude, heart rate variability, maximal oxygen uptake

INTRODUCTION

Sojourning at high altitude is a popular pursuit for many people around the world, either as part of a vacation (mountain climbing, skiing, trekking) or as a popular strategy for improving aerobic performance, using various altitude training approaches, in elite and/or amateur endurance athletes (1–3). Due to the improvement in transportation, an increasing number of people can travel passively to high altitude for short visits, for instance by lift, car, and/or helicopter without previous training and/or acclimatization, and this may be a risk for acute mountain sickness (AMS) development (4). During the sojourn to high altitude, it is important to consider that a low oxygen environment represents an added stress to the body (5), and tolerability to high altitude exposure in human beings seems to be highly variable (6–10). Both lower atmospheric pressure or low fraction of inspired oxygen (FiO₂) induces a progressive decline in arterial oxygen saturation (SpO₂), causing immediate compensatory responses in the pulmonary and cardiorespiratory systems to ensure the adequate supply of oxygen to vital tissues (11). An acute hypoxia ventilatory response (AHVR) is thought to be a vital body response for homeostatic SpO₂ adjustment during hypoxia (12). It was demonstrated that AHVR is augmented by hypercapnia (13), and in the population, AHVR is characterized with great inter-individual variability in hypoxic chemosensitivity (14). Some authors have associated a progressive decline in SpO₂ with AMS at high altitude (15), while others associate AMS with sympathetic dominance in autonomic cardiac control (5). Previously, a higher AMS incidence was positively associated with higher VO₂max level in mountain climbers (15). In this regard, it has been established that the SpO₂ response to hypoxia during exercise is negatively affected by higher aerobic capacity in both males (8, 16, 17) and females (18). This relationship is commonly explained as a result of the relative hypoventilation mediated by blunted chemoreceptor sensitivity in individuals with higher VO₂max (17, 19, 20). However, there is conflicting evidence as to sex differences relating to the role of aerobic capacity in moderating the SpO₂ response during resting normobaric hypoxia exposure (17, 18). The acute homeostatic adjustment to systemic hypoxia is a complex stress-regulated response, which is primarily mediated by a central command mechanism (21) and changes in autonomic nervous system (ANS function) (12). The functional changes in the ANS at altitude are considered to be an adaptive response to hypoxia (22, 23) as well as a response to hypoxia inducible factor 1 α production at the cellular level (24). These changes modulate metabolic pathways as well as immune

responses that play an important part in adaptation response to hypoxia (25).

Spectral analysis of R-R interval to determine heart rate variability (HRV) is commonly accepted as a non-invasive tool for autonomic cardiac control assessment (26), especially parasympathetic (vagal) cardiac outflow (27). Vagal activity is reflected in both high-frequency power (HF, 0.15–0.50 Hz) of R-R intervals and/or in time domain root mean square of the successive R-R interval differences (rMSSD), and is associated with respiratory modulated fluctuation of heart rate (HR) that causes a phenomenon known as respiratory sinus arrhythmia (RSA) (28). Low frequency power (LF, 0.05–0.15 Hz) is considered to be modulated by baroreflex activity (29) together with bilateral sympathetic and vagal traffic (30). Ratio LF/HF is traditionally thought to be an index of sympathovagal balance (31, 32). The hypoxia-induced increase in resting HR seems to be a result from a decrease in cardiac vagal activity and an increase in relative sympathetic activity (10, 33–35). From a medical standpoint, it is well-known that long-term sympathetic predominance in autonomic cardiac regulation contributes to increasing risk of cardiovascular disease such as malignant arrhythmias (36), hypertension (37), and/or sudden cardiac death (38). A recently published meta-analysis demonstrated that healthy females showed a higher resting HR accompanied with lower global autonomic activity compared with age matched males. However, at rest, females maintained a significantly greater HF and lower LF power, that was further reflected by a lower LF/HF ratio, in normoxia conditions, representing a cardio-protective effect of vagal activity (39). Regarding, hypoxia-induced gender differences in HRV, to date, published data is inconsistent. For example Wadhwa et al. (40) demonstrated a visible decrease in vagal activity, with a concomitant increase in sympathetic cardiac control, in males compared with females, in response to intermittent normobaric hypoxia exposure (FiO₂ = 8.0%) and also during the recovery period. However, more recently, Boos et al. (41) found higher overall autonomic cardiac activity during ascent to high terrestrial altitude in males compared with age, body mass index (BMI) matched females. In addition, females and males demonstrated a similar cardiopulmonary responses during 150 min of normobaric hypoxia exposure (FiO₂ = 11.5%) (42).

Therefore, our primary objective was to test the hypothesis that age-matched females and males exhibit no differences in autonomic cardiac and SpO₂ response to equal simulated altitude. In addition, our secondary objective was to test the hypothesis that there are no sex differences in SpO₂ response to resting hypoxia in relation to VO₂max level.

MATERIALS AND METHODS

Participants

The study included 28 males and 30 females. Data for males were published previously (9) and reanalysed for comparison with females for the purpose of this study. Somatic and physiological characteristics of the experimental group are presented in **Table 1**. Subjects were healthy non-smoking, sport science students, who had not been exposed to hypoxia for at least the previous 2 years and were not on any medication or dietary supplements. They underwent preliminary medical screening to identify cardiovascular, pulmonary, and metabolic conditions that would exclude them from the study. This study was carried out in accordance with the recommendations of Ethics Committee of the Faculty of Physical Culture, Palacký University Olomouc. The protocol was approved by the Ethics Committee of the Faculty of Physical Culture, Palacký University Olomouc. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Experimental Protocol

The subjects were required to avoid eating, drinking coffee, tea, and/or any substance affecting the ANS activity for at least 2 h before the experiment. In addition, they were asked to avoid vigorous physical activity and alcohol for 48 h before the experiment. The experiment was performed between 8:00 and 11:00 a.m. in a laboratory where the ambient temperature ranged from 22 to 24°C. During the experiment, each subject rested quietly in a supine position and was shielded against acoustic and visual disturbances.

From a methodological perspective, the influence of breathing patterns and tidal volume on the HRV components is well-described (43, 44) where a decrease in breathing frequency (BF) and increased tidal volume cause an increase in the HF component. However, a BF <9 breaths per minute may lead to an artificial increase in the LF with concomitant changes in the LF/HF ratio due to an RSA peak shift from HF into the LF. This could be a limitation in terms of interpreting both the vagal and the sympathetic contribution to the sinoatrial node activity (45). To avoid the potential methodological issue of BF, HRV is frequently measured under paced breathing conditions (7, 46). However, it has been reported that paced breathing may increase sympathetic activity (47). Therefore, in the present study we used spontaneous breathing throughout the experimental protocol. In respect to methodological issues mentioned above, 4 males and 3 females were excluded from the original dataset of 32 males and 33 females due to BF <9 breaths.min⁻¹ within any phase of the experiment.

In order to minimize a potential effect of the menstrual cycle to HRV results (48), all females were measured similarly at the follicular phase of the menstrual cycle based on self-report. In addition, 6 (20%) participating females were taking oral contraceptives pills.

The hypoxic experiment proceeded as follows (**Figure 1**): The subjects first breathed ambient air without a breathing mask. Each subject lay supine for 6 min to skip the transitory phase and to ensure the stabilization of the data. After this period,

SpO₂ and electrocardiogram (ECG) data were recorded for 6 min and used for calculation of the “Preliminary phase” variables. Once preliminary recording was completed, a research assistant fitted a face mask and the subject started to breathe air with the reduced O₂ concentration. The first 5 min of hypoxia served as a stabilization period and the last 5 min were recorded and used for calculation of the “Hypoxia phase” variables. After the 10 min of hypoxia had elapsed, the mask was removed, and the subject again breathed ambient air. The first minute was used as the standardization period and 6 min of data recording followed this period. This data was used for calculation of the “Recovery phase” variables.

The altitude of the laboratory was 260 m above sea level. The normobaric hypoxia condition was equal to an altitude of 6,200 m (FiO₂ = 9.6%) and has been used widely in the literature for intermittent hypoxic exposure (49). This condition was created using a MAG-10 system (Higher Peak, Boston, MA, United States), which simulated the lower O₂ pressure found at high altitudes by lowering the percentage of O₂ in the air. Subjects breathed air with a reduced O₂ concentration via the mask from a non-rebreathing circuit with a bag acting as a reservoir.

Oxygen Saturation Measurement

The SpO₂ was measured continuously using a Nonin Avant 4000 pulse oximeter (Nonin Medical, Minneapolis, MN, United States) positioned on the right index finger. The SpO₂ was measured at a sampling frequency of 1.0 Hz, and the average value over each phase was calculated for subsequent statistical analysis. The oxygen desaturation between Hypoxia phase and Preliminary phase was calculated as $\Delta\text{SpO}_2 = \text{SpO}_2_{\text{Hypoxia}} - \text{SpO}_2_{\text{Preliminary}}$.

Heart Rate Variability Analysis

To determine the HR and HRV variables, the ECG signal was measured at a sampling frequency of 1,000 Hz using a DiANS PF8 diagnostic device (DIMEA Group, Olomouc, Czech Republic). The ECG record was examined, and all premature ventricular contractions, missing beats, and any artifacts were manually filtered. A set of 300 artifact-free subsequent R-R intervals was obtained from each phase. A spectral analysis of HRV was used to assess the ANS activity and was performed using the Fast Fourier Transform. The spectral analysis incorporated a sliding 256 points Hanning window and a Coarse-Graining Spectral Analysis algorithm (50).

Frequency domain variables included: low-frequency power (LF) calculated in the band from 0.05 to 0.15 Hz, high-frequency power (HF) calculated in the band from 0.15 to 0.50 Hz, and the LF/HF ratio. Time domain variables included: rMSSD, standard deviation of R-R intervals (SDNN), and ratio of SDNN to rMSSD. For the subsequent statistical analysis, the average HR and HRV values were calculated based on the values extracted from each phase (Preliminary, Hypoxia, and Recovery).

Maximal Oxygen Uptake Determination

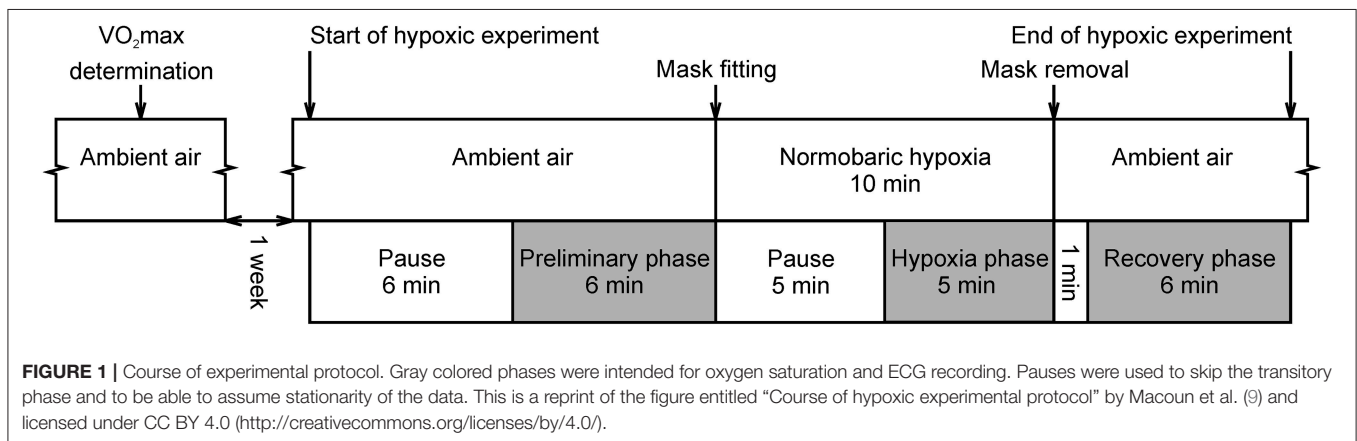
VO₂max, as a global indicator of physical fitness, and maximum heart rate (HRmax), were measured in normoxia during an incremental running test on the treadmill (Lode Valiant Plus,

TABLE 1 | Anthropological and physiological characteristics of studied groups.

	Females	Males	p-value	ES	ES rating
n	30	28			
Age (years)	23.8 ± 1.8	23.7 ± 1.7	0.812	0.06	Trivial
Weight (kg)	60.8 ± 8.5	78.4 ± 7.9	<0.001	-2.16	Large
Height (cm)	167.0 ± 5.6	180.3 ± 7.2	<0.001	-2.07	Large
BMI (kg.m ⁻²)	21.8 ± 3.0	24.2 ± 2.1	0.001	-0.90	Large
Fat (%)	20.5 ± 6.2	12.6 ± 4.8	<0.001	1.41	Large
FFM (%)	79.5 ± 6.2	87.4 ± 4.8	<0.001	-1.44	Large
VO ₂ max (ml.kg ⁻¹ .min ⁻¹)	45.1 ± 8.7	59.0 ± 7.8	<0.001	-1.67	Large
HRmax (beats.min ⁻¹)	191.3 ± 8.1	190.0 ± 6.9	0.490	0.18	Trivial
VC (l)	4.35 ± 0.64	6.18 ± 0.70	<0.001	-2.74	Large

Values are presented as mean ± SD.

ES, effect size (Cohen's d); n, sample size; BMI, body mass index; FFM, fat free mass; VO₂max, maximal oxygen uptake; HRmax, maximal heart rate; VC, vital capacity.



Groningen, Netherlands). The protocol consisted of a 4 min warm-up (2 min at 8 km.h⁻¹ for males, and 7 km.h⁻¹ for females with 0% elevation and then 2 min at the same speed at 5% elevation) followed by an increase in speed to 10 km.h⁻¹ for males and 9 km.h⁻¹ for females at 5% elevation for 1 min. From this point, at each minute, the speed was increased by 1 km.h⁻¹, keeping elevation the same, up to 16 km.h⁻¹ for males and 13 km.h⁻¹ for females. The speed was then maintained and only the elevation increased by 2.5% per minute until exhaustion. Ventilation and gas exchange were recorded continuously (breath by breath) with 30 s averaging and analyzed by Blue Cherry software (Geratherm Respiratory, Bad Kissinger, Germany). The criteria for attaining VO₂max was defined as reaching one of the following criteria: (a) respiratory exchange ratio of >1.11 (51); (b) VO₂ plateau defined as no increase in VO₂ in response to an increase in work rate (52). VO₂max was considered the highest VO₂ value in the final 30 s of the test (53). HR response was measured continuously using a chest strap (Polar, Kempele, Finland).

Statistical Analysis

All data are presented as mean ± standard deviation. Normality of distribution was checked using the Kolmogorov-Smirnov test. Skewed probability distributions of HRV indexes (LF, HF, LF/HF,

SDNN, rMSSD, and SDNN/rMSSD) were corrected applying a natural logarithm (Ln). Comparisons between the sexes for anthropological and physiological characteristics were performed using the two-sample *t*-test. A 2(sex) × 3(phase) analysis of variance (ANOVA) for repeated measures was used to evaluate the effect of hypoxia on selected variables. When the ANOVA revealed a significant effect, multiple comparisons via the Fisher's LSD *post-hoc* test were performed.

To achieve results similar to Woorons et al. (17), four groups were created from our dataset. Group of females with low aerobic capacity (FL) included 7 females with the lowest VO₂max values and group of females with high aerobic capacity (FH) included 7 females with the highest VO₂max values. Likewise, groups of males with low (ML, *n* = 7) and high aerobic capacity (MH, *n* = 7) were created. Differences in oxygen desaturation ($\Delta\text{SpO}_2 = \text{SpO}_2_{\text{Hypoxia}} - \text{SpO}_2_{\text{Preliminary}}$) between groups were evaluated using a 2(sex) × 2(low vs. high VO₂max) ANOVA and Fisher's LSD *post-hoc* tests.

Effect size was calculated as standardized mean difference (Cohen's *d*) according the formula (54) $d = \frac{(m_F - m_M)}{SD_p}$ where m_F and m_M are means of females and males, respectively. Pooled standard deviation was calculated as follows (54) $SD_p = \sqrt{\frac{(n_F - 1) \sum_{i=1}^k SD_{Fi}^2 + (n_M - 1) \sum_{i=1}^k SD_{Mi}^2}{(n_F + n_M - 2)k}}$ where n_F , n_M are sample sizes,

SD_F , SD_M are standard deviations of the females and males in i -th phase, and $k = 3$ is number of phases, respectively. To calculate effect sizes of pairwise differences between FL, FH, ML, and MH, pooled standard deviation was calculated as root-mean-square of standard deviations of all four groups. The following threshold values for effect size were adopted (55): <0.2 (trivial), ≥ 0.2 (small), ≥ 0.5 (medium), ≥ 0.8 (large).

A linear relationship between ΔSpO_2 and normoxic VO_2max was evaluated by means of Pearson's correlation coefficient (r). The null hypothesis that regression lines calculated separately for females and males would be identical was tested using a general linear model. A linear basic model contained two regression lines $\Delta SpO_2 = a_F + b_F VO_2max$ and $\Delta SpO_2 = a_M + b_M VO_2max$ for females and males, respectively. A linear submodel contained one regression line $\Delta SpO_2 = a + b VO_2max$ valid for both sexes. The magnitude of r was interpreted according following thresholds (55): <0.1 (trivial), ≥ 0.1 (small), ≥ 0.3 (medium), ≥ 0.5 (large).

For all tests, $p < 0.05$ was considered statistically significant. Statistical analyses were performed using STATISTICA 12.0 (StatSoft, Tulsa, OK, United States) and MATLAB 8.4 (MathWorks, Natick, MA, United States).

A sensitivity analysis was performed using G*Power version 3.1.9.2 software (56). The calculation was performed for a two-sample t -test for a statistical significance of 0.05, power of 0.80, and sample size of 30 and 28. The result was that the minimal detectable effect size would be $d = 0.75$. Therefore, only a large (>0.8) effect size for the population would be sufficiently detected.

RESULTS

Anthropological and physiological characteristics of studied groups are presented in **Table 1**. Importantly, females compared with males showed a significantly higher percentage of body fat, lower VO_2max , and lower vital capacity (VC).

Means and standard deviations of SpO_2 , HR, and HRV indexes in the Preliminary, Hypoxia, and Recovery phases are shown in **Tables 2–4**, respectively. Significance differences between the sexes and also between the phases are depicted in **Figure 2**. In the Preliminary phase, there was a significant sex difference for HR (**Table 2**). In the Hypoxia phase, SpO_2 decreased significantly in both sexes (both $p < 0.001$), however, there was no significant difference between sexes during this phase ($p = 0.376$, **Table 3**). HR increased significantly in both sexes (both $p < 0.001$) and the sex difference that presented in the Preliminary phase was not present in the Hypoxia phase ($p = 0.164$). Vagal related indexes (Ln HF and Ln rMSSD) decreased significantly in both sexes (all $p < 0.001$) but there was no significant difference between the sexes during the Hypoxia phase (Ln HF: $p = 0.358$; Ln rMSSD: $p = 0.590$). The index of sympathovagal balance, Ln LF/HF, did not change significantly in females ($p = 0.106$) but increased significantly in males ($p < 0.001$) and there was a significant difference between sexes in the Hypoxia phase ($p = 0.001$). The time-domain surrogate for sympathovagal balance Ln SDNN/rMSSD increased significantly in both sexes (both $p < 0.001$) and there was a significant sex difference in the Hypoxia phase ($p = 0.006$). Ln LF decreased significantly in both sexes (females: $p < 0.001$; males: $p = 0.012$)

and there was a significant sex difference in the Hypoxia phase ($p = 0.016$). Ln SDNN decreased significantly in both sexes (both $p < 0.001$) and there was no significant sex difference in the Hypoxia phase ($p = 0.295$).

During the Recovery phase, there was no significant difference between the sexes (**Table 4**). However, differences in dynamics between Preliminary and Recovery phases were found as follow. In females, SpO_2 recovered during the Recovery phase to value not significantly ($p > 0.064$) different from the Preliminary value, however, SpO_2 in males did not fully recover and remain significantly ($p = 0.017$) decreased compared to the Preliminary value. HR during the Recovery phase decreased significantly in both sexes (females: $p < 0.001$; males: $p = 0.011$) below the Preliminary values. In both sexes, vagal related indexes (Ln HF and Ln rMSSD) recovered to values not significantly (all $p > 0.263$) different from the Preliminary values. In both sexes, Ln LF/HF recovered to values not significantly (females: $p = 0.060$; males: $p = 0.131$) different from the Preliminary values. Ln SDNN/rMSSD in females recovered to value not significantly ($p = 0.122$) different from the Preliminary value, however, the index in males did not fully recover and remain significantly ($p < 0.001$) elevated compared to the Preliminary value. In males, indexes Ln LF and Ln SDNN recovered to values not significantly (Ln LF: $p = 0.361$; Ln SDNN: $p = 0.211$) different from the Preliminary values. However, in females, the indexes during the Recovery phase increased significantly (Ln LF: $p = 0.014$; Ln SDNN: $p = 0.025$) above the Preliminary values.

Regression analysis (**Figure 3**) showed no linear relationship between ΔSpO_2 and VO_2max ($p = 0.952$, $r = 0.01$, trivial effect) for females. However, there was a significant linear relationship ($p = 0.017$, $r = -0.45$, medium effect) for males. The difference between regression lines for females and males was significant ($p = 0.024$).

For the females, VO_2max was $36.2 \pm 3.9 \text{ ml.kg}^{-1}.\text{min}^{-1}$ for FL and $57.0 \pm 8.3 \text{ ml.kg}^{-1}.\text{min}^{-1}$ for FH. There was no significant difference (**Figure 4**, $p = 0.659$, $d = 0.24$, small effect) in ΔSpO_2 between FL ($-27.9 \pm 5.7\%$) and FH ($-29.4 \pm 6.7\%$). For the males, VO_2max was $49.5 \pm 4.9 \text{ ml.kg}^{-1}.\text{min}^{-1}$ for ML and $68.4 \pm 5.2 \text{ ml.kg}^{-1}.\text{min}^{-1}$ for MH. ΔSpO_2 for ML ($-20.0 \pm 8.0\%$) was significantly less steep ($p = 0.019$, $d = 1.34$, large effect) compared with MH ($-28.4 \pm 3.6\%$). Regarding sex differences, ΔSpO_2 for ML was significantly less steep ($p = 0.027$, $d = 1.26$, large effect) compared with FL. However, the difference between MH and FH was not significant ($p = 0.773$, $d = 0.16$, trivial effect).

DISCUSSION

The primary purpose of this study was to assess whether the autonomic activity and SpO_2 responses to 10 min resting normobaric hypoxia ($FiO_2 = 9.6\%$, simulated altitude $\sim 6,200$ m) were similar in age matched males and females. The secondary aim was to determine if there were sex-related differences in the association between VO_2max and the SpO_2 response to hypoxia. The primary novel findings of the study were as follows: (a) there were significant sex differences in autonomic cardiac control during the hypoxia period, with the males displaying a similar desaturation level but a relatively higher sympathetic

TABLE 2 | Comparison of oxygen saturation, heart rate, and HRV indexes between sexes in Preliminary phase.

	Females	Males	p-value	ES	ES rating
SpO ₂ (%)	98.3 ± 0.8	96.9 ± 1.1	0.240	0.31	Small
HR (beats.min ⁻¹)	67 ± 11	61 ± 9	0.038	0.56	Medium
Ln LF (ms ²)	5.9 ± 1.5	6.5 ± 1.0	0.092	-0.45	Small
Ln HF (ms ²)	6.9 ± 1.0	7.0 ± 0.9	0.708	-0.10	Trivial
Ln LF/HF	-1.0 ± 1.4	-0.6 ± 1.2	0.188	-0.35	Small
Ln SDNN (ms)	4.10 ± 0.43	4.24 ± 0.37	0.236	-0.31	Small
Ln rMSSD (ms)	3.94 ± 0.55	4.06 ± 0.50	0.486	-0.18	Trivial
Ln SDNN/rMSSD	0.16 ± 0.26	0.18 ± 0.25	0.716	-0.10	Trivial
LF (ms ²)	1,003 ± 1,655	1,148 ± 1,760			
HF (ms ²)	1,556 ± 1,534	1,547 ± 1,249			
LF/HF	1.2 ± 2.6	1.2 ± 2.0			
SDNN (ms)	66 ± 28	75 ± 29			
rMSSD (ms)	59 ± 30	65 ± 32			
SDNN/rMSSD	1.21 ± 0.31	1.24 ± 0.34			

Values are presented as mean ± SD.

ES, effect size (Cohen's d); SpO₂, oxygen saturation; HR, heart rate; Ln, natural logarithm; LF, low-frequency power; HF, high-frequency power; LF/HF, ratio of low-frequency to high-frequency power; SDNN, standard deviation of RR intervals; rMSSD, square root of the mean of the squares of the successive differences; SDNN/rMSSD, ratio of SDNN to rMSSD.

TABLE 3 | Comparison of oxygen saturation, heart rate, and HRV indexes between sexes in Hypoxia phase.

	Females	Males	p-value	ES	ES rating
SpO ₂ (%)	70.8 ± 7.1	71.9 ± 7.5	0.376	-0.23	Small
HR (beats.min ⁻¹)	83 ± 14	80 ± 11	0.164	0.37	Small
Ln LF (ms ²)	5.1 ± 1.4	5.9 ± 1.5	0.016	-0.65	Medium
Ln HF (ms ²)	5.7 ± 1.5	5.4 ± 1.7	0.358	0.24	Small
Ln LF/HF	-0.6 ± 1.4	0.5 ± 1.3	0.001	-0.87	Large
Ln SDNN (ms)	3.59 ± 0.53	3.72 ± 0.60	0.295	-0.28	Small
Ln rMSSD (ms)	3.18 ± 0.73	3.09 ± 0.86	0.590	0.14	Trivial
Ln SDNN/rMSSD	0.41 ± 0.31	0.63 ± 0.32	0.006	-0.73	Medium
LF (ms ²)	501 ± 1,268	1,011 ± 1,628			
HF (ms ²)	656 ± 650	762 ± 1,297			
LF/HF	1.5 ± 2.6	3.4 ± 5.3			
SDNN (ms)	42 ± 22	50 ± 35			
rMSSD (ms)	31 ± 22	33 ± 33			
SDNN/rMSSD	1.58 ± 0.51	1.96 ± 0.59			

Values are presented as mean ± SD.

ES, effect size (Cohen's d); SpO₂, oxygen saturation; HR, heart rate; Ln, natural logarithm; LF, low-frequency power; HF, high-frequency power; LF/HF, ratio of low-frequency to high-frequency power; SDNN, standard deviation of RR intervals; rMSSD, square root of the mean of the squares of the successive differences; SDNN/rMSSD, ratio of SDNN to rMSSD.

stimulation (LF/HF) compared with the females; (b) there was a significantly ($p = 0.024$) different association between ΔSpO_2 and normoxic VO₂max level between males and females during resting normobaric hypoxia exposure. Specifically, the ΔSpO_2 response to hypoxia indicated no association to VO₂max level in females but it was moderately correlated with VO₂max in males, and (c) males who exhibited lower aerobic capacity demonstrated smaller desaturation in resting hypoxia compared with females with low aerobic capacity.

Based on our statistical analysis in the Preliminary phase, there were no significant differences between males and females in

either SpO₂ nor in HRV variables, except lower resting HR in males. In support of this finding, it was previously demonstrated that males have a lower resting HR compared with females, due to larger cardiac chamber size and higher resting stroke volume in males (57, 58). Moreover, a lower resting HR is widely accepted as typical sign of cardiovascular system adaptation to endurance training (59, 60), and the males demonstrated a significantly higher aerobic performance compared with females in the present study. In this study, SpO₂ and vagally-related variables (HF and rMSSD) dropped in similar fashion in both groups with no differences between groups during the 10 min of resting

TABLE 4 | Comparison of oxygen saturation, heart rate, and HRV indexes between sexes in Recovery phase.

	Females	Males	p-value	ES	ES rating
SpO ₂ (%)	96.3 ± 2.5	94.2 ± 2.6	0.081	0.46	Small
HR (beats.min ⁻¹)	63 ± 10	58 ± 8	0.140	0.40	Small
Ln LF (ms ²)	6.5 ± 1.2	6.7 ± 0.9	0.510	-0.17	Trivial
Ln HF (ms ²)	7.0 ± 1.0	6.9 ± 1.0	0.637	0.12	Trivial
Ln LF/HF	-0.5 ± 1.3	-0.2 ± 1.1	0.275	-0.29	Small
Ln SDNN (ms)	4.29 ± 0.41	4.35 ± 0.39	0.592	-0.14	Trivial
Ln rMSSD (ms)	4.05 ± 0.55	3.98 ± 0.54	0.660	0.12	Trivial
Ln SDNN/rMSSD	0.23 ± 0.34	0.37 ± 0.30	0.079	-0.47	Small
LF (ms ²)	1,270 ± 1,655	1,141 ± 1,009			
HF (ms ²)	1,671 ± 1,608	1,536 ± 1,578			
LF/HF	1.3 ± 2.6	1.5 ± 1.8			
SDNN (ms)	79 ± 31	83 ± 31			
rMSSD (ms)	66 ± 32	62 ± 34			
SDNN/rMSSD	1.34 ± 0.52	1.51 ± 0.44			

Values are presented as mean ± SD.

ES, effect size (Cohen's *d*); SpO₂, oxygen saturation; HR, heart rate; Ln, natural logarithm; LF, low-frequency power; HF, high-frequency power; LF/HF, ratio of low-frequency to high-frequency power; SDNN, standard deviation of RR intervals; rMSSD, square root of the mean of the squares of the successive differences; SDNN/rMSSD, ratio of SDNN to rMSSD.

hypoxia exposure. However, despite similar HRV LF and LF/HF variables during the Preliminary phase, LF was significantly lower in females compared with males during hypoxia. Consequently, the increased LF/HF ratio, considered as sympathovagal balance index (31), indicated a relatively higher sympathetic involvement in cardiovascular control during hypoxia in males compared with females. A similar response to the LF/HF ratio during hypoxia was seen in the SDNN/rMSSD ratio, that is currently accepted as an alternative index of sympathovagal balance, derived from time domain analysis by some authors (34, 61, 62). The functional changes found in autonomic cardiac activity were also reflected in the higher HR response during hypoxia in males compared with females.

Wadhwa et al. (40), who assessed effect of sex-related differences in HRV in response to intermittent normobaric hypoxia exposure (FiO₂ = 8.0%), demonstrated a similar decline in vagal activity when the final and initial hypoxic periods were compared. However, a more pronounced reduction in vagal activity together with the progressive shift of the sympathovagal balance to sympathetic dominance was evident in males but not in females. In a recently published meta-analysis on gender related HRV levels in resting normoxia, it was shown that although healthy females have a lower mean R-R interval together with lower global autonomic cardiac activity compared with age matched males at rest, females maintain significantly greater HF and less LF power that is further reflected by a lower LF/HF ratio. This was proposed as a potential cardio-protective regulatory effect of the relatively higher cardiac vagal involvement in cardiovascular control that could be related to ovarian hormones and/or oxytocin (39). Based on our results, maintaining balance between vagal and sympathetic activity during resting normoxia, and within short-term acute normobaric hypoxia, seem to be more important for females compared with males

irrespective of whether global autonomic cardiac activity is lower in females during hypoxia exposure. Our findings may reflect an estrogen related attenuation in sympathoadrenal stress response (63) that may provide a protective effect on the cardiovascular system when under environmental stress such as hypoxia. This proposal is indirectly supported by Huikuri et al. (64) who demonstrated that post-menopausal females with estrogen replacement therapy showed significantly higher baroreflex sensitivity and total HRV compared with age-matched females without hormone treatment. Moreover, when sex-related responses to upright posture were compared, the females had a significantly attenuated increase in HR, and a smaller decrease in the HF component. Boos et al. (41) demonstrated a significant reduction in vagal activity due to an increasing hypobaric hypoxia during ascent up to an altitude of 5,140 m amongst males and females. Compared with the present study, at altitude, the males exhibit significantly higher vagally-related HRV variables and higher global HRV than females, while no significant differences in sympathovagal balance were reported between genders. In this case, a lower HRV in females especially at altitudes of 4,600 and 5,140 m may have been associated with elevated fatigue, potentially due to a lower cardiorespiratory fitness, during the strenuous ascents in the females. To explain these different findings between Boos et al. (41) and the present study, it is proposed that our results specifically reflect an acute autonomic cardiac response to hypoxia in unacclimatized persons, whereas Boos et al. (41) described chronic changes in autonomic cardiac activity which may mirror an influence of several factors, for instance, an individual course of acclimatization to altitude.

Despite no significant ($p = 0.081$) sex-related difference in SpO₂ being found during recovery in the present study, there was a significant delay in SpO₂ return to baseline in the males compared with the females, whose SpO₂ returned

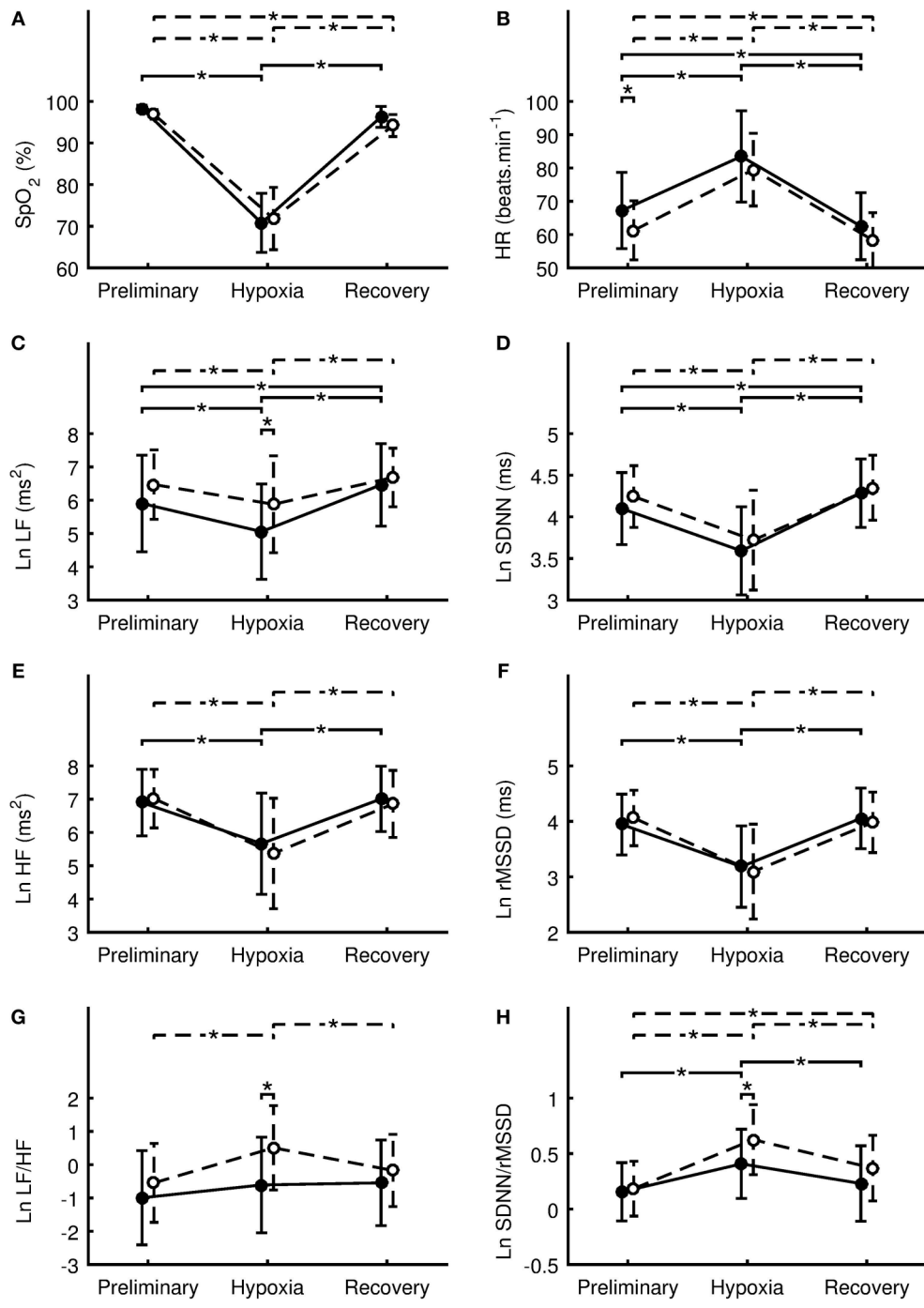
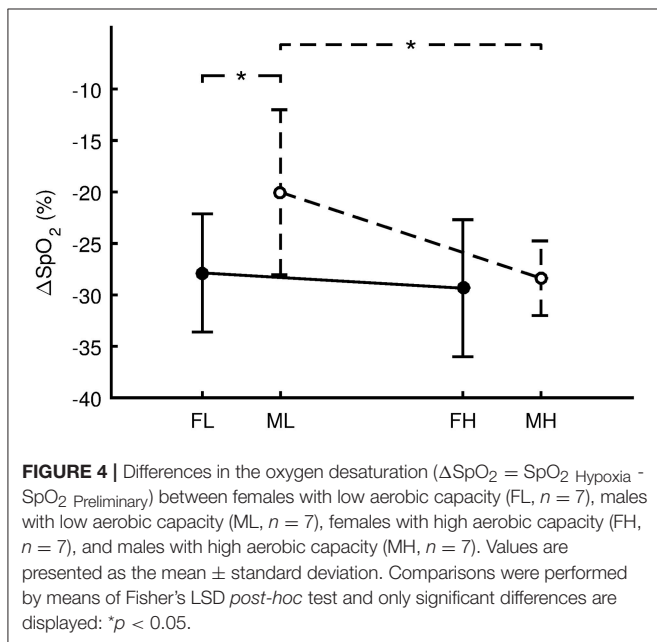
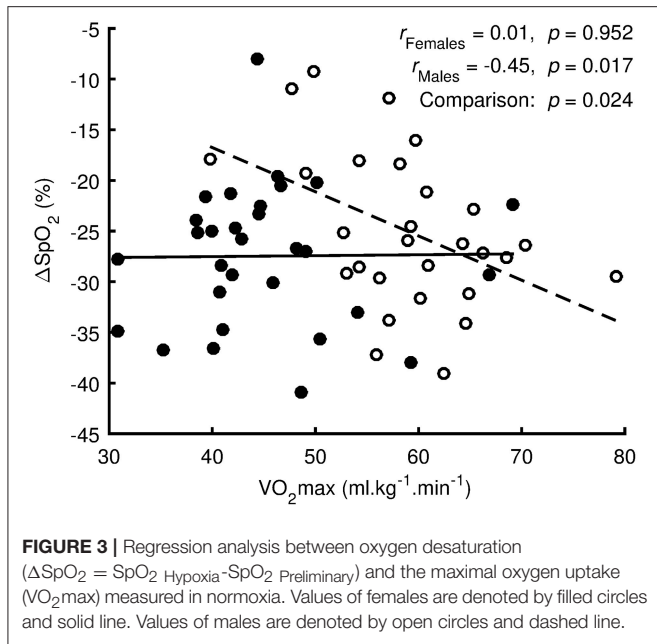


FIGURE 2 | Differences between sexes in the oxygen saturation (A), heart rate (B), natural logarithm of low frequency power (C), natural logarithm of standard deviation of RR intervals (D), natural logarithm of high-frequency power (E), natural logarithm of square root of the mean of the squares of the successive differences (F), natural logarithm of ratio of low-frequency to high-frequency power (G), natural logarithm of ratio of SDNN to rMSSD (H). Values of females are denoted by filled circles and solid line. Values of males are denoted by open circles and dashed line. Values are presented as the mean ± standard deviation. Comparisons were performed by means of Fisher's LSD *post-hoc* test and only significant differences are displayed: **p* < 0.05.

to preliminary values rapidly once hypoxia was removed. We propose that for the males, the slower SpO₂ return to baseline during the recovery phase was because of persistent sympathetic dominance during recovery compared with the preliminary

phase. Our results support the findings of Jones et al. (65), who assessed sympathetic response via muscle sympathetic nerve activity during 15 min of normobaric hypoxia (FiO₂ = 10%) and following 10 min recovery. They reported that sex appears to



contribute to the interindividual variability in the sympathetic cardiovascular response to a hypoxia environment. Females exhibited a decline in sympathetic activity in the first minute of recovery post-hypoxia, compared with males, where the decrease in sympathetic activity post-hypoxia took up to 6 min.

Following the suppression in autonomic regulation of cardiac activity during the hypoxia stress in the present study, there appeared to be a “supercompensation phenomenon” in overall autonomic cardiac activity during the recovery phase, in the females, but not in the males. However, there was a significant decrease in the mean HR compared to baseline values in both sexes. In light of this, Roche et al. (66) who assessed change in

both baroreflex sensitivity (BRS) and HRV in response to 15 min hypoxia, ($\text{FiO}_2 = 11\%$) reported that a relative bradycardia during 20 min normoxic recovery was modulated throughout improved vagal activity together with transitory significant overactivity of the spontaneous BRS. Unfortunately, Roche et al. (66) did not examine sex differences. In contrast to Roche et al. (66), Halliwill et al. (67) reported that acute exposure to hypoxia reset baroreflex control of both HR and sympathetic activity to higher pressures without changes in BRS.

To the best of our knowledge, this is the first study to show a significant ($p = 0.024$) sex-related difference in ΔSpO_2 is related to normoxic VO_2max level between males and females during short-term, acute, resting normobaric hypoxia exposure. Previously, Macoun et al. (9), who assessed ΔSpO_2 response in relation to individual aerobic fitness during similar hypoxia conditions (10 min; $\text{FiO}_2 = 9.6\%$; simulated altitude $\sim 6,200$ m), reported a negative correlation between ΔSpO_2 and VO_2max ($r = -0.45$; $p = 0.017$) in males. A negative effect of a higher VO_2max on desaturation, especially during exercise, in endurance well-trained, males has previously been reported (8, 16, 17, 20). Researchers ascribed this relationship to a number of factors, including, possible blunted chemoreceptor sensitivity (68), resulting in an insufficient ventilatory response (relative hypoventilation) to severe hypoxia in well-trained endurance athletes at rest (17), but especially during exercise in moderate, normobaric hypoxia (17). In contrast to the males, based on our regression analysis, there was no relationship ($r = 0.01$, $p = 0.952$) between VO_2max level achieved in a normoxic environment and the ΔSpO_2 response during acute resting, normobaric, hypoxia exposure in our cohort of females. Our results are supported by previously published study (18), where authors reported that there were no significant differences between aerobically well-trained and sedentary females for resting SpO_2 at different levels of normobaric, hypoxia environment ($\text{FiO}_2 = 0.187, 0.154, \text{ and } 0.117$). However, once the SpO_2 response was assessed during exercise under hypoxia conditions, a similar influence of higher VO_2max on desaturation level was identified in aerobically well-trained females, probably due to diffusion limitation (18). Former studies have repeatedly shown that the AHVR, a vital body response for homeostatic SpO_2 adjustment during hypoxia (12), depends on the VO_2max level in males (8, 16, 17), whereas in females the factors that contribute to AHVR during hypoxia have yet to be clarified (69, 70). It has been shown that AHVR varies depending on the menstrual cycle phase (71). For instance, progesterone, which peaks during the luteal phase (72), was found to stimulate AHVR (73) via central (74) and peripheral (75) receptor induced-mechanisms. Based on self-reports, all our female subjects participated in the hypoxia exposure during the follicular phase, when, according to Guenette et al. (70), AHVR is not different between trained and untrained females.

The lack of correlation between VO_2max and ΔSpO_2 in females, despite the comparable desaturation level among our males and females at similar simulated altitudes, is a novel finding. The result implies that whilst males with lower aerobic capacity demonstrated less desaturation in acute resting hypoxia, the females, irrespective of their aerobic capacity had low SpO_2 .

Therefore, while lower aerobic capacity in males seemed to be protective, this is not the case for females. A possible explanation for this finding is that these females exhibit insufficient AHVR because of an altered sympathoadrenal system activation that may have been modulated by the level of estrogen (39). In this context, Lusina et al. (76) reported, that following intermittent hypoxic training, the rise in sympathetic activity was strongly related to the change in AHVR ($r = 0.79$, $p < 0.05$) suggesting that sympathetic and ventilatory responses may have a common central control. A second explanation for the sex-related difference was discussed in a review by Harms (71) who showed that females exhibit lower lung diffusion capacity compared to age- and height-matched males due to both smaller diffusion surfaces (77), and smaller airways diameter relative to lung size (78). In case of similar ventilation, females may exhibit lower SpO₂ compared to males. A further explanation may be related to potentially lower hemoglobin levels in the females. Low hemoglobin is associated with an increased level of 2,3-diphosphoglycerate (DPG), due to the decrease in oxygen carrying capacity (79). This results in a shift in oxyhemoglobin dissociation curve to the right, decreasing the affinity of oxygen to the hemoglobin, and reducing the SpO₂ (79). This shift is exaggerated at high altitude and females may be more sensitive to this shift, irrespective of VO₂max. Supporting this idea is research (80) demonstrating that oxyhemoglobin dissociation curve is different (at the same temperature and pH) in the two sexes and that females present less hemoglobin affinity for oxygen, with 2,3-DPG levels 2 mmol/g of hemoglobin higher compared with males. However, these reasons are currently speculative and further study is required with the measurement of additional physiological data that could help explain the mechanism behind this sex difference.

From a practical perspective, we propose that lower aerobic capacity may represent a temporary advantage, particularly in males, who are performing a rapid, passive ascent to high altitude without previous hypoxia exposure. In contrast, males with a high aerobic capacity ($>65 \text{ ml.kg}^{-1}.\text{min}^{-1}$) and females in the follicular phase of the menstrual cycle, may be more vulnerable to a higher desaturation during an acute hypoxia exposure, and consequently, these subjects may be considered to be at a greater risk of developing AMS. These suggestions are supported by Karinen et al. (15) who reported that subjects who manifested AMS symptoms exhibited both a greater decline in SpO₂ in hypoxia and a higher VO₂max compared with subjects who were free from AMS symptoms. Similarly, Álvarez-Herms et al. (1) recently showed that higher appearance in AMS symptoms was scored by professional compared to amateur endurance-trained athletes who performed an altitude training camp probably due to higher training doses in professional athletes.

In individuals previously highly sensitive to acute hypoxia exposure, a smaller decrease in both SpO₂ and HRV was found after specialized pre-acclimatization using normobaric, hypoxic, intermittent training (6). Thus, in order to avoid a progressive desaturation and potentially AMS when exposed to altitude, hypoxic acclimation training may represent a promising strategy for both females and aerobically fit males who plan to use passive

transport to altitude without following staged acclimatization that occurs during active ascent to altitude.

A main limitation of this study was that, during hypoxia, subjects wore a face mask, and therefore, it was not possible to appropriately assess AHVR that typically occurs within 5 min of hypoxia exposure (12). A knowledge of the AHVR may have helped explain the VO₂max vs. SpO₂ association difference between males and females. The diagnostics system (DiANS PF8) used to determine HRV changes during the different study phases only provided data about changes in BF. Future research should include complete ventilatory response assessment (e.g., minute ventilation, dead space analysis, PetCO₂) and measurements of A-a gradient calculation, hemoglobin concentration, pO₂, 2,3-DPG levels, and sex hormone levels. In addition, continual monitoring of blood pressure and/or BRS (66, 67) may be beneficial for providing a more complex view of the autonomic regulation of the cardiopulmonary system during a resting acute, normobaric hypoxia. Another limitation of this study was that it could be considered as underpowered based on the sensitivity analysis.

CONCLUSION

Despite finding similar oxygen desaturation levels and vagal withdrawal between genders during hypoxia; females demonstrated a relatively lower sympathetic response to the resting hypoxia exposure, compared with males. Delayed return in SpO₂ to its baseline during recovery after hypoxia exposure may be because of prolonged sympathetic stimulation in the males, but not the females. Moreover, there was a sex-related difference in the resting, acute, hypoxia response, relating to the association between SpO₂ levels, and maximal aerobic capacity. Specifically, resting VO₂max in females was not associated with resting desaturation levels, whereas in males, VO₂max was associated with the SpO₂ response.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

MB contributed to the design of the study. MB and JK performed data collection. JK performed the statistical analysis. MB wrote the first draft of the manuscript. MB, JK, and AM wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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