Effects of the menstrual cycle on physiological responses to high-altitude in eumenorrheic women

A master's thesis in Human Movement and Sport Sciences
Training and Performance option

submitted by

Guia Tagliapietra

to
University of Lausanne, Switzerland
Faculty of Social and Political Sciences
Institute of Sport Sciences

Director: Prof. **Grégoire Millet**
Expert: Dr. **Antoine Raberin**

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Abstract

An increasing number of women travel to high-altitude for work, leisure, military deployments and sport. There is a growing awareness that sex hormones may have an impact on the cardiorespiratory system, which plays a central role in successful acclimatization to high-altitude. Eumenorrheic women are subject to natural hormonal fluctuations across the menstrual cycle. Yet, the impact of ovarian hormones on pathophysiological responses to high-altitude remains underexplored. The aim of the present study was to investigate the effects the menstrual cycle on respiratory and cardiovascular responses to high-altitude, both at rest and during exercise, and on acute mountain sickness (AMS) prevalence.

Sixteen eumenorrheic women first underwent physiological measurements at rest, i.e., spirometry, chemosensitivity, vascular occlusion and cognitive tests, as well as during submaximal intensity exercise on a cycle ergometer (gas exchange, heart rate recovery) at low altitude; followed by a crossover study design involving one-night stay and physiological assessments at rest and during exercise at the Torino Hut (3375 m) during both the early-follicular (EF) and the mid-luteal (ML) phases. The calendar method was used to monitor participants' menstrual cycles over a 6 month-period. Hormone concentrations were verified via blood sample analyses.

At rest, we demonstrated a higher forced vital capacity (3.71 ± 0.44 vs. 3.65 ± 0.42 L, p = 0.033), peak expiratory flow (7.33 ± 1.34 vs. 7.06 ± 0.87 $L \cdot s^{-1}$, p = 0.046), tidal volume (0.81 ± 0.22 vs. 0.71 ± 0.19 L, p = 0.026), ventilation (15.2 ± 1.9 vs. 13.2 ± 2.5 $L \cdot min^{-1}$, p = 0.039) and a lower post-vascular-occlusion tissue saturation index peak (TSI_{peak}, 67.4 ± 3.6 vs. 68.7 ± 2.5%, p = 0.047) during EF compared to ML at high-altitude. However, these small differences observed at rest were abolished by exercise. Moreover, no significant difference was observed in AMS prevalence between EF and ML either 6 h (19% vs. 25%) or 15 h (37% vs. 19%) post-arrival at 3375 m. Furthermore, the number of lapses in the perceptual vigilance task was significantly lower during EF compared to ML at high-altitude (0.313 ± 0.602 vs. 1.188 ± 2.198).

In conclusion, despite slight differences in pulmonary function, ventilation and TSI_{peak} between EF and ML, other near-infrared spectroscopy derived measurements of resting muscle oxygen consumption and microvascular reactivity, oxygen uptake kinetics, cycling efficiency, heart rate variability and heart rate recovery remained consistent across the menstrual cycle during acute high-altitude exposure. Ovarian hormone differences between EF and ML had small or insignificant effects on acclimatization to high-altitude and, consequently, on AMS, sleep

quality, submaximal exercise and cognitive function in healthy females. Therefore, an individualized approach tailored to each woman's specific responses to hypoxic environments across the menstrual cycle may be more beneficial in optimizing high-altitude sojourns and exercise than relying on general guidelines.

1. Introduction

An increasing number of people are exposed to high altitude for work, leisure, air travel, mountain tourism, sport, athletic competitions, military deployments or altitude training camps. With over 500 million people, representing approximately 7% of the global population (Tremblay & Ainslie, 2021), inhabiting regions above 1500 m, there is a growing emphasis on the understanding of (patho) physiological responses to high-altitude.

Hypoxic environments are characterised by a diminished oxygen (O_2) availability due to reductions in barometric pressure (P_b) (hypobaric hypoxia) or in ambient O_2 concentration associated with a lower inspired O_2 fraction (F_iO_2) (normobaric hypoxia), which lead to a decline in the partial pressure of oxygen (PO_2) (Conkin & Wessel, 2008). A drop in the partial pressure of inspired oxygen (P_iO_2 < 150 mmHg), as experienced at high-altitude (e.g., at the summit of Mt. Everest (8848 m), P_b = 253 mmHg; P_iO_2 = 50 mmHg) or in hypobaric/hypoxic chambers, diminishes the driving force for pulmonary gas exchange (Cornwell et al., 2021), thereby altering the O_2 cascade at various stages, starting from ambient air down to alveoli, arteries and mitochondria. As a result, O_2 supply to the tissues may be affected.

Acute hypobaric hypoxia exposure evokes adaptive responses in humans (**Figure 1**), including increased minute ventilation ($\dot{V}_E$), heart rate (HR) and cardiac output (Burtscher et al., 2022). These mechanisms are triggered by carotid body chemoreceptors which mediate sympathetic activation. Up to 3500 m the increase in resting minute V̇E is primarily explained by the augmentation of tidal volume (V_T) (Cogo, 2011). Moreover, acute exposure to high-altitude elicits pulmonary vasoconstriction and during the first hours of exposure nitric-oxide-mediated systemic arterial vasodilatation to support tissue perfusion and O_2 delivery (Bärtsch & Gibbs, 2007). Particularly, pulmonary vasoconstriction plays a crucial role in the pathophysiology of high-altitude pulmonary edema (HAPE) by contributing to the elevation of microvascular hydrostatic pressures, resulting in alterations in the permeability of the alveolar capillary barrier and subsequent mechanical damage leading to capillary leakage (Swenson & Bärtsch, 2012).

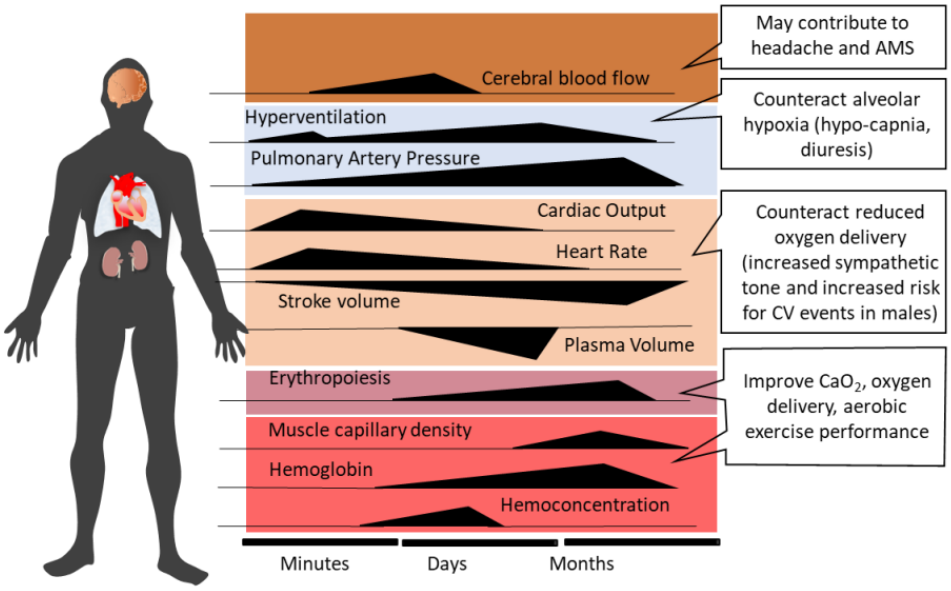


Figure 1. Physiological responses to hypoxia (modified from Mallet et al., 2023). AMS, acute mountain sickness; CV, cardiovascular; CaO_2, arterial oxygen content.

Several studies have observed different physiological responses between hypobaric hypoxia (HH) and normobaric hypoxia (NH) exposure, primarily attributed to the distinct partial pressure levels that characterize each condition (Coppel et al., 2015; DiPasquale et al., 2015; Millet & Debevec, 2020; Roach et al., 1996). Particularly, $\dot{V}_E$, tidal volume (V_t), end tidal partial pressure of oxygen ($P_{ET}O_2$) and of carbon dioxide ($P_{ET}CO_2$) were lower in HH than NH, while resting HR was higher in HH. Furthermore, pre-acclimatization in NH seemed to be less effective than in HH (Fulco et al., 2013). It is well-established that acute, intermittent and chronic exposure to hypoxia induce an increased production of reactive oxygen species (Mrakic-Sposta et al., 2021; Pialoux et al., 2009). However, markers of oxidative stress have been reported to be higher in HH than NH (Faiss et al., 2013).

Unacclimatized persons who rapidly ascend to high-altitude (e.g., to 2500 m or above) are at risk of developing high-altitude illnesses (HAIs). The adverse effects of exposure to high-altitude include acute mountain sickness (AMS), cerebral and/or pulmonary edema (Hackett, 1999; Swenson & Bärtsch, 2012). AMS is characterized by headache symptoms coupled with gastrointestinal issues (e.g., poor appetite, nausea and/or vomiting), fatigue/weakness, and/or dizziness/light-headedness (Roach et al., 2018). It has been observed that AMS symptoms are exacerbated at real high-altitude compared to NH (Millet et al., 2012). Moreover, the prevalence of AMS increases with altitude (i.e., 10-25% at 2500 m and 50-85% at ~ 5000 m) (Bärtsch &

Swenson, 2013). However, the impact of HH may vary between individuals, based on medical history, risk factors, gender and medications (Richalet et al., 2012).

Since 2019, the number of studies on the topics of menstrual cycle and exercise have considerably increased. Despite growing interest on the subject, females remain significantly underrepresented within medicine, sport and exercise research (Costello et al., 2014; Cowley et al., 2021; Nuzzo & Deaner, 2024). However, the participation rates of women in mountain activities are noteworthy, i.e., 33% of American Alpine Club outdoor climbers, 40% of Everest trekkers and over 40% of Swiss Alpine Club members (Horakova et al., 2023). Moreover, the proportion of female athletes to be taking part in the forthcoming Olympic Winter Games in 2026 is estimated to be 47% (Pellegrini et al., 2023). Amongst the extensive body of literature regarding the specific responses to hypoxia (Bärtsch & Saltin, 2008; Mallet et al., 2021), most studies were conducted on men. Results of these interventions are not equally applicable to women given the anatomical, physiological and endocrinological differences between sexes. Thus, exploring female-related physiological responses at high-altitude becomes a compelling question, given the rising participation of women in both recreational and competitive mountain sports, including the Winter Olympics which are often held at altitude (e.g., Cortina, Italy, 2026).

Particularly, females not taking hormonal contraceptives are subject to cyclic fluctuations of reproductive hormones, i.e., estrogen and progesterone during different phases of the menstrual cycle (Davis-Wilson & Hackney, 2017). These rhythmic and recurring endogenous hormonal changes regulate various physiological processes, including the maturation of ovarian follicles, ovulation, and the preparation of endometrium for potential pregnancy (Reed & Carr, 2000). In absence of fertilization, ovarian hormone levels decrease, causing the shedding of the endometrial wall and a new ovarian cycle begins with the onset of menstrual bleeding. Subsequently, estrogen levels rise during the follicular phase, reaching their peak just prior to the luteinizing hormone (LH) surge that triggers ovulation followed by the luteal phase, when both estrogen and progesterone, first increase and then decrease in the absence of fertilization (**Figure 2**) (Janse De Jonge et al., 2019). The menstrual cycle typically consists of a complex interplay between the hypothalamus, the anterior-pituitary gland, the ovaries and the uterus.

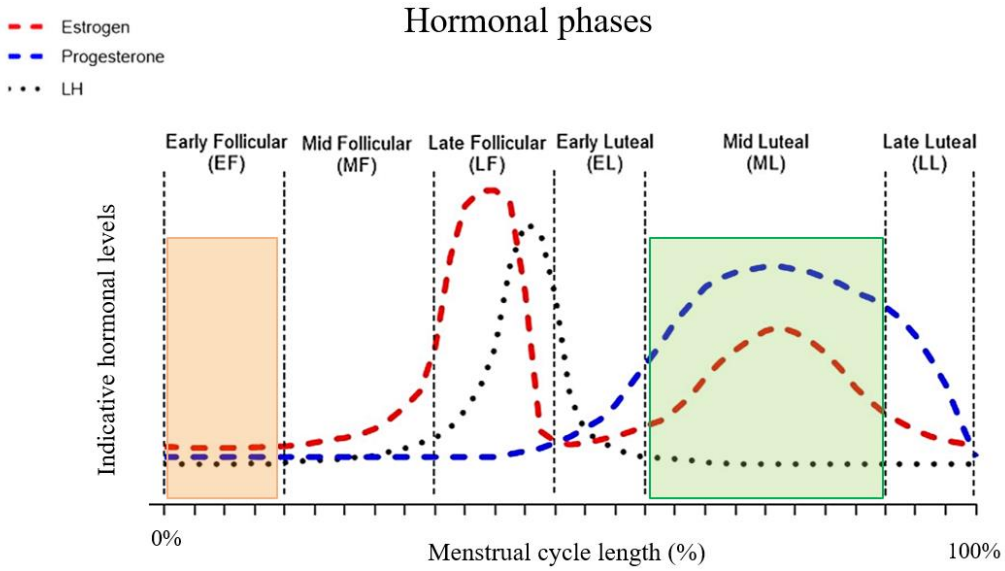


Figure 2. Graphical representation of ovarian hormone fluctuations during the eumenorrheic menstrual cycle and the corresponding phases. Dashed lines depict two key reproductive hormones in women, i.e., estrogen and progesterone. The early-follicular (EF) phase, denoted in orange, is characterized by low hormonal concentrations whereas the mid-luteal (ML) phase, in green, is marked by elevated levels of both estrogen and progesterone. Modified from DAM and colleagues (2022).

Furthermore, ovarian hormones may influence respiratory, metabolic, cardiovascular and neurologic systems (Sathish et al., 2015), and potentially acclimatization to high-altitude and exercise. Particularly, studies on animal models indicate that progesterone has an impact on pulmonary function, potentially enhancing bronchial smooth muscle relaxation (Eliyahu et al., 2023; Foster et al., 1983). Despite these observations, the impact of hormonal fluctuations on airflow and pulmonary volumes evaluated by spirometry in eumenorrheic women remains a subject of debate. Indeed, conflicting results are found in the literature, with some studies indicating higher values during the luteal phase (da Silva et al., 2006; Rajesh et al., 2000), whereas others have reported no significant changes (Chen & Tang, 1989; Das, 1998). During the luteal phase, when both estrogen and progesterone concentrations are elevated, body-core temperature and resting minute $\dot{V}_E$ tend to be higher compared to the follicular phase in normoxic conditions (Das, 1998; Schoene et al., 1981; Slatkovska et al. 2006).

Specifically, progesterone is known to have a stimulatory effect on respiratory function, i.e., increasing the rate and depth of breathing (Bairam et al., 2015). Even if the exact mechanism in not fully understood yet, this ovarian hormone may act on central and/or peripheral chemoreceptor or on the respiratory centers in the brainstem, modifying CO_2

sensitivity or the firing rate of medullary inspiratory neurons (Loeppky et al., 2001). Furthermore, it has been shown that respiratory response to progesterone is mediated by an estrogen-dependent progesterone receptor in the hypothalamus (Bayliss & Millhorn, 1992). Evidence suggests that estrogen may enhance the effects of progesterone on $\dot{V}_E$ (Behan & Wenninger, 2008) and mitigate pulmonary artery vasoconstriction under normoxic and hypoxic conditions (Lahm et al., 2007).

While some studies have reported a higher hypoxic ventilatory response (HVR) during the luteal phase compared the follicular phase (Schoene et al., 1981; White et al., 1983), other investigations failed to identify significant differences (Beidleman et al., 1999; Regensteiner et al., 1990). The effects of the menstrual cycle phase on lung function at high altitude have not been extensively studied. To date, only one study, conducted in a HH chamber, examined this aspect, and found no influence of the cycle phase on forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) (Beidleman et al., 1999). Moreover, it remains unclear whether the menstrual cycle affects resting $\dot{V}_E$ at high-altitude (Raberin et al., 2023). Similarly, research findings diverge on the impact of ovarian hormones on $\dot{V}_E$ during maximal or submaximal exercise both in normoxic and hypoxic conditions (Beidleman et al., 1999; Richalet et al., 2020; Schoene et al., 1981; Takase et al., 2002; Williams & Krahenbuhl, 1997). Two studies observed a higher oxygen saturation (SpO_2) in the mid-luteal phase during exercise in hypoxic conditions (Beidleman et al., 1999; Richalet et al., 2020). However, oxygen uptake ($\dot{V}O_2$) during maximal or submaximal exercise remained unchanged across the menstrual cycle in both normoxic and hypoxic conditions (Beidleman et al., 1999; De Souza et al., 1990). Similarly, $\dot{V}O_2$ kinetics are unaffected by the menstrual cycle phase at sea level (Gurd et al., 2007). Another parameter that seems to be impacted by the menstrual cycle in normoxia is running economy (i.e. energy expenditure per unit of distance) which is lower in the luteal than in the follicular phase (Goldsmith & Glaister, 2020). On the contrary, contrasting results are found concerning cycling efficiency, which remains unchanged across the menstrual cycle (Dombovy et al., 1987) or decreases during the luteal phase (Hessemer & Brück, 1985).

Concerning the cardiac outcomes, while BP seems to be higher at the onset of menstruation, HR tends to increase during the luteal phase (Barba-Moreno et al., 2022; Choi et al., 2013). Furthermore, available data indicate small variations in heart rate variability (HRV) between the hormonal phases, with a slight decrease associated with elevated progesterone levels of the luteal phase (Schmalenberger et al., 2020). It appears that hormonal modulation of cardiac vagal activity occurs independently of changes in breathing rates throughout the menstrual cycle (Tenan et al., 2014). However, other examinations of parasympathetic tone

markers of HRV and of heart rate recovery (HRR) indices revealed no discernible differences between the cycle phases (Yazar & Yazici, 2016). Whether these menstrual-cycle induced modulations are preserved at high-altitude is even less evident. An investigation led in acute HH conditions observed consistent catecholamine levels across the menstrual cycle, but higher BP and HR during the luteal phase (Mazzeo et al., 1998), while no differences between cycle phases was found in the hypoxic cardiac response at exercise (Richalet et al., 2020).

In female adult rats, estradiol was shown to provide a protective effect against oxidative stress and cardiorespiratory disorders, including elevated blood pressure, heart rate, sleep apneas, induced by chronic intermittent hypoxia (CIH) (Laouafa et al., 2017). Specifically, estrogen demonstrates antioxidant properties (Karowicz-Bilinska et al., 2008), while progesterone mitigates oxidative stress induced by CIH in ovariectomized female rats (Joseph et al., 2020).

In normoxia, the menstrual cycle appears to have no effect on microvascular function (Williams et al., 2020). Nevertheless, $\dot{V}O_2$ kinetics, HRV, cycling efficiency, microvascular function and HRR, have not been explored yet at real high-altitude during different phases of the menstrual cycle.

It has been suggested that there may be a potential reduction in AMS during the luteal phase (Burtscher et al., 2023); however, the current level of evidence was reported by these later authors as low and speculative, necessitating further investigation. A recent study, observed no significant difference in AMS occurrence between women in the follicular and in the luteal groups (Gardner et al., 2024). Currently, no studies have investigated AMS prevalence and severity during both the early-follicular (EF) and mid-luteal (ML) phases using a within-subject design.

Finally, multiple studies conducted on healthy women have demonstrated a hormonal influence on visuospatial, fine motor and articulatory skills, as well as in attention, concentration, verbal memory, visual memory, working memory, and reaction time, as evaluated using neuropsychological tests under normoxic conditions (Souza et al., 2012). However, conflicting findings exist, with some investigations suggesting consistent memory performance, visuospatial abilities and attention during the menstrual cycle (Hatta & Nagaya, 2009; Mordecai et al., 2008). Moreover, the impact of hormonal fluctuations on cognitive function at high-altitude remains an unexplored area of research, despite the well-established association between high-altitude environments and alterations in cognitive functions (Bahrke & Shukitt-Hale, 1993).

Not only is there a lack of comprehensive data on female-specific physiological responses to high-altitude (Burtscher et al., 2023), but also practical guidelines tailored to females partaking in mountain tourism or exercising at high-altitude are limited (Derstine et al., 2023). Also because the accurate assessment of menstrual cycle phases and the direct measurement of ovarian hormones are sometimes sub-optimally carried (Gargaglioni et al., 2019). Studies on women should implement improved methodological control to ensure standardized and high-quality research designs (Elliott-Sale et al., 2021), thereby preventing precipitated or unreliable conclusions concerning the effects of the menstrual cycle (D'Souza et al., 2023).

To our knowledge, only a handful of studies have tested women at real high-altitude during different phases of the menstrual cycle (Gardner et al., 2024; Muza et al., 2001). However, these studies employed a between-subjects design. Therefore, the present investigation aims to assess if there are any pathophysiological differences, both at rest and during exercise at high altitude in eumenorrheic women between two distinct phases of their menstrual cycle: the early-follicular (EF) and the mid-luteal (ML). Firstly, we hypothesize that there may be slight differences in the ventilatory responses between the two phases, whereas no significant differences are expected in cycling efficiency, $\dot{V}O_2$ kinetics, HRV, HRR, resting muscle oxygen consumption, microvascular reactivity and cognitive function. Secondly, we hypothesize that AMS prevalence and symptom scores will not differ between EF and ML.

2. Methods

2.1. Participants

Sixteen healthy eumenorrheic women volunteered and gave written informed consent to participate in the study. All participants were not taking any medication (e.g., acetazolamide) and were free from any cardiorespiratory, neurological and haematological diseases. The subjects were recruited via advertisement (Appendix 1) posted on the local newspapers in Aosta Valley, on social media and displayed in libraries and universities.

They were selected according to the following inclusion criteria: between 18 and 43 yr, with regular menstruation, without reported menstrual irregularities, not taking any hormonal contraceptive for at least three months prior to participation, with a cycle length between 21 and 35 days, physically active and a body mass index (BMI) $\leq$ 27 $kg \cdot m^{-2}$. Over a 6-month period, menstrual cycle related inclusion criteria for all participants were monitored through

individual interviews conducted via telephone or email and verified during the initial visit at low altitude prior to their inclusion in the study. During each trial, hormone concentrations were confirmed via blood sample analyses. Exclusion criteria included: use of medications that could influence hormonal response, alcohol consumption, intensive physical activity within 24 h prior to the tests and prolonged altitude/hypoxia exposure prior to the study, i.e., more than one night at ≥ 2000 m. The experimental protocol was approved by the Ethical Committee of the Local Health Unit of the Aosta Valley Region. The study was conducted in compliance with the guidelines outlined in the Declaration of Helsinki.

2.2. Experimental design and ascent protocol

Each participant took part in three experimental trials (**Figure 1**). They initially underwent baseline assessments at low altitude (1224 m; Courmayeur, Italy; P_b ~663 ± 0.9 mmHg; P_iO_2 = 128 ± 0.3 mmHg), followed by a high-altitude (3375 m; Torino hut, Aosta Valley, Italy, on the Mont Blanc chain; P_b ~508 ± 5.8 mmHg; P_iO_2 = 96 ± 1 mmHg) crossover randomized study involving one-night stays and evaluations during the EF (day 3 ± 1) and the ML (~75% of the menstrual cycle length). On average, the duration between the two phases at high-altitude was 15 ± 9 days. At the visit conducted at low altitude, the sixteen participants were stratified into two subgroups based on their ovarian hormone concentrations: nine were attributed to the follicular phase subgroup (F) and seven to the luteal phase subgroup (L) (**Figure 3**). Subjects reached Torino hut by cable car in 15-20 min. Low-altitude measurements were performed before the high-altitude exposure, ensuring that subjects were familiarized with the testing protocol and pre-emptively addressing any potential carryover effects of altitude exposure on the control assessments.

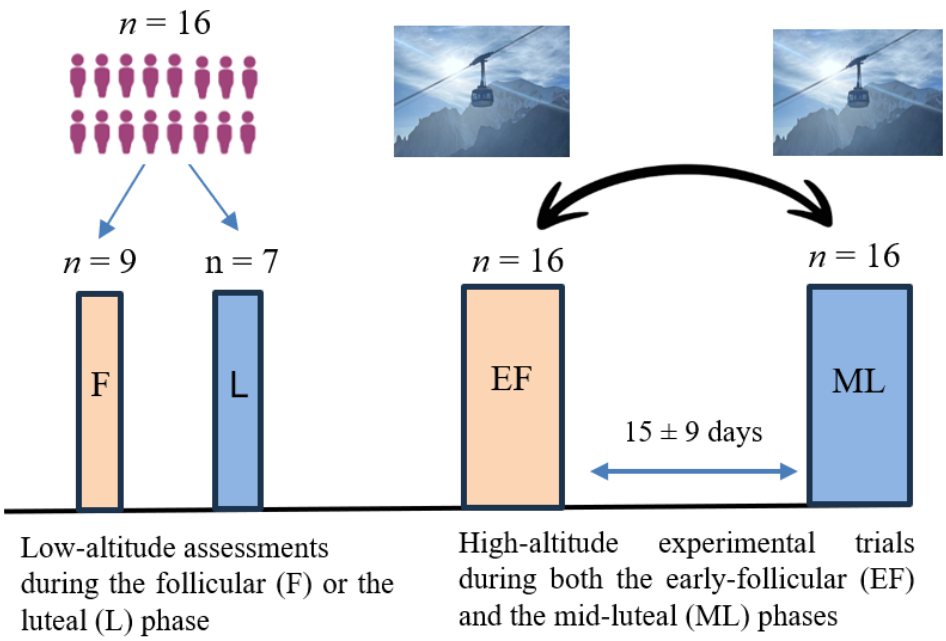


Figure 3. Study design.

2.3. Experimental protocol

Upon the initial visit conducted at low altitude, urine and blood samples were collected, and subjects' hydration status via urine specific gravity (USG) was immediately determined from the urine samples. Then, participants underwent HRV, cognition, spirometry, acute transient hypoxic chemosensitivity, vascular occlusion (VOT), submaximal intensity cycling exercise and HRR tests (see below for details of each test). BP and HR were measured at rest on the left arm in a sitting position both at low altitude and 15 h after arrival at high-altitude using a digital sphygmomanometer (M2, Omron Healthcare, Hoofddorp, The Netherlands). During the second and third visits, spirometry tests were performed upon reaching high-altitude, at 3:00 p.m., and 16 h after, at 7:00 a.m.. In addition to this, participants filled in the Lake Louise score (LLS) and performed cognitive tests 6 h post-arrival at high-altitude. Polysomnography (PSG) measurements were also conducted as part of the study protocol at high-altitude; however, they are not going to be displayed and discussed in the present study. The following morning, upon awakening, at 6:00 a.m., subjects' saliva and urine samples were collected. Then, participants underwent the HRV test and filled in the LLS and the Groningen Sleep Quality Scale (GSQS). Blood samples were collected 16 h post-arrival at high-altitude. VOT and submaximal intensity cycling exercise on an E100 cycle ergometer (Cosmed, Rome, Italy) immediately followed by HRR were conducted 17 h after arrival at Torino hut. SpO_2 was assessed at rest (post 15 h) and during exercise (post 17 h) using a pulse oximeter (WristOx

3150, Nonin Medical Inc., Plymouth, MN, USA) placed on the women's earlobe. All experiments were conducted at the same time of the day for all the participants by the same investigator and with the same devices to minimize the methodological variability. Detailed descriptions of the general experimental protocol of the study are shown in **Figure 4** and **Figure 5**.

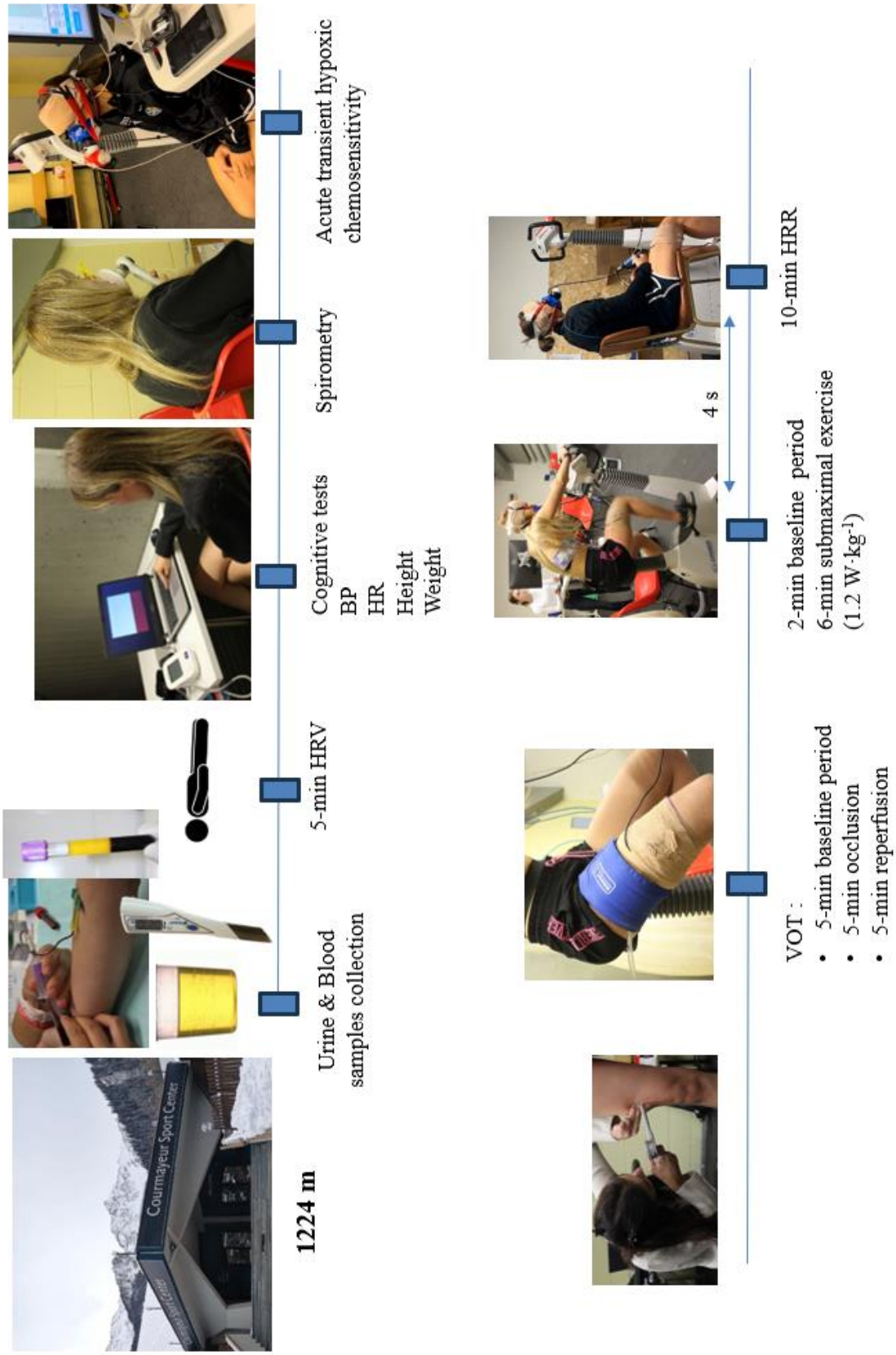


Figure 4. Study protocol at low altitude.

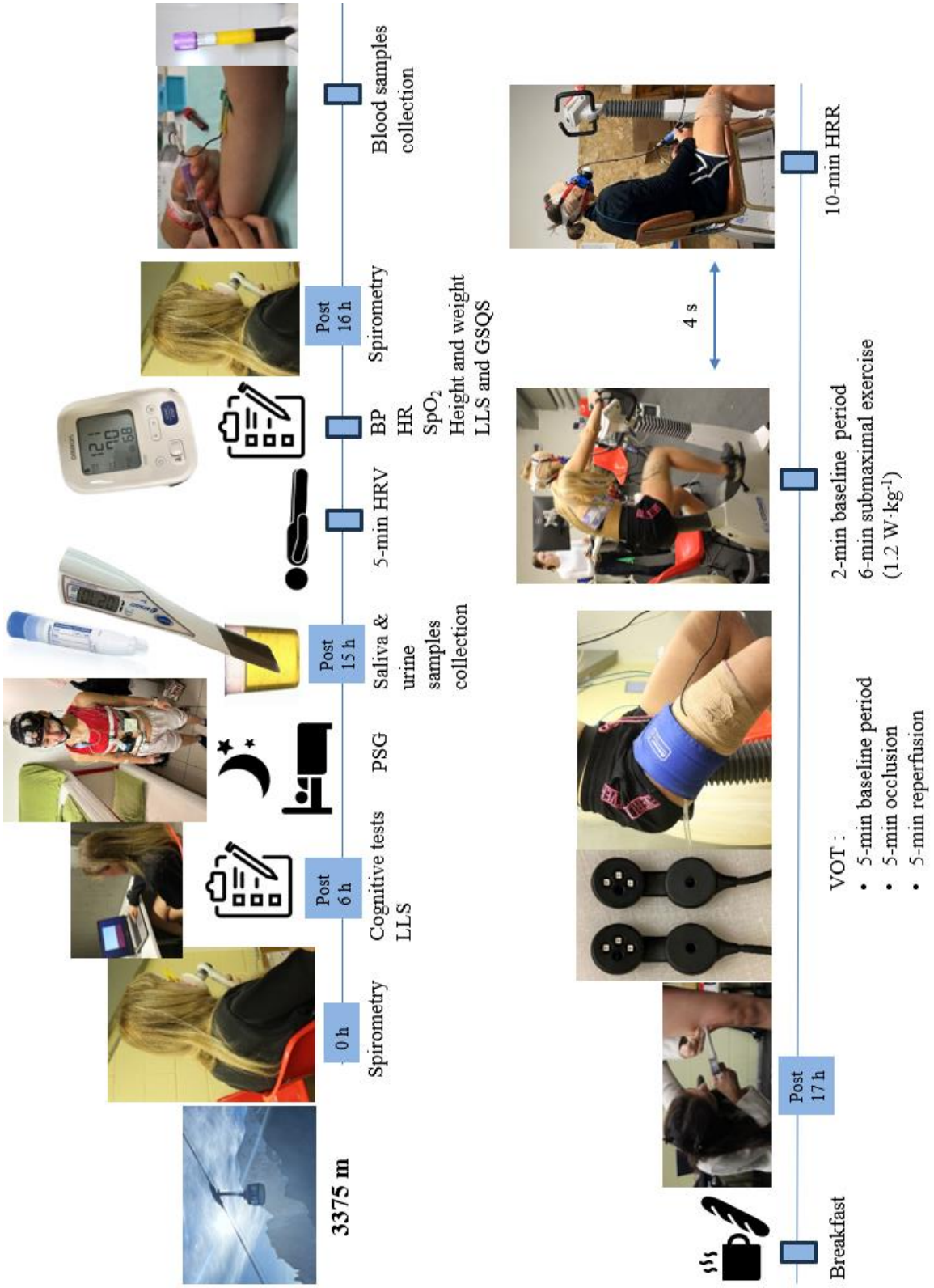


Figure 5. Study protocol at high-altitude.

2.4. Measurements

2.4.1. Menstrual cycle phase assessment

Over a 6-month period, participants' menstrual cycles were monitored using a calendar-based method. While this approach provides a general overview, additional retrospective confirmations through blood samples analyses were conducted to ensure accuracy in determining the correct phase. It is important to note that even among eumenorrheic women, menstrual cycle lengths differ from one another (Fehring et al., 2006). Considering that the follicular phase contributes the most to this variability (Reed & Carr, 2000), the proportion of the follicular phase represented by the third day of the cycle was assessed using the following equation:

$$\frac{x}{\text{follicular phase}} = \frac{21.4}{100}$$

where x represents the corresponding third day of cycles shorter or longer than the conventional 28-day menstrual cycle; the follicular phase, the mean length of the follicular phases monitored over the six months for each participant, and 21.4, expressed in %, indicates the proportion of the entire menstrual cycle that the third day represents (Janse De Jonge et al., 2019). By accounting for the variability of the follicular phase over multiple cycles, the risk of error was reduced. Experimental trials during the mid-luteal phase were performed for each participant at approximately 75% into the mean of their cycle lengths recorded over the 6-month period (Romero-Moraleda et al., 2019). This timing was chosen to take into account the variation in cycle lengths within and between women and to ensure consistency across participants.

2.4.2. Blood samples

At low and high altitude, 6 mL of venous blood were obtained from the antecubital vein with the participants in a seated position. Subsequently, blood samples were centrifuged (10 min at 3500 rpm) and the obtained plasma and serum were aliquoted into microtubes and kept frozen at -80°C until analysis. Serum concentrations of estradiol (E2) and progesterone (P4) were measured to verify correct menstrual phase identification. E2 and P4 levels were quantified using a competitive enzyme-linked immunosorbent kit (E2 ELISA kit, MyBioSource®, San Diego, CA, USA and P4 ELISA kit, Abnova®, Taipei City, Taiwan, respectively). In addition to this, Sandwich enzyme-linked immunosorbent kits were employed

to evaluate C-reactive protein (CRP) concentrations (CRP ELISA kit, Abnova®, Taipei City, Taiwan). Oxidative stress markers, including advanced oxidation protein products (AOPP), catalase, GPx (glutathione peroxidase), myeloperoxidase (MPO), nitrites (NO^{2-}), total nitrite and nitrate (NOx), superoxide dismutase (SOD), and xanthine oxidase (XO), were determined using the methodologies outlined by Manferdelli and colleagues (2023). Furthermore, the ferric-reducing antioxidant power (FRAP) and malondialdehyde (MDA) were measured following the procedures previously described (Martin et al., 2020).

2.4.3. Hydration status

At low altitude and 16 h after arrival at high altitude, urine specific gravity (USG) of human urine was measured to determine hydration levels using a digital refractometer (PAL 10-s, Atago Co., Tokyo, Japan). Briefly, by measuring the refractive index of a substance, the refractometer provides information about its concentration. Participants were provided with a sterile container for collecting their urine and afterwards the key START on the refractometer was pressed and the tip of the device was dipped within 5 s into the sample. Prior to each experiment, calibration was conducted using distilled water maintained at the same temperature as the urine samples, in accordance with the manufacturer's guidelines. In individuals with normal kidney function, urine specific gravity ranges from 1.002 to 1.030. Values greater than 1.020 indicate a mild dehydration, with higher measurements indicating increased dehydration status. The refractometer used exhibits an accuracy of ± 0.0010 and a resolution of 0.0001.

2.4.4. Cortisol indices

Upon awakening, participants were asked to rinse their mouth with water and soak three cotton swabs (Salivette; Sarstedt, Nümbrecht, Germany) with saliva by gently moving them around in the mouth for 2 min without chewing (S1: 0 min, S2: 30 min, S3: 45 min). During sampling procedures participants were refrained from consuming any liquids other than water and from food intake. Upon collection, samples were immediately stored at -20°C, then they were transferred to a long-term storage at -80°C, before being transported to the laboratory for analysis. Cortisol concentrations were measured using a competitive enzyme-linked immunosorbent kit (Salivary cortisol ELISA kit, ELISA kit, Abnova®, Taipei City, Taiwan). As previously proposed (Estoppey et al., 2019), two cortisol indices were calculated: first sample

on awakening (S1) and total post awaking cortisol levels (area with respect to ground, AUC-G={[(S1 + S2)/2] x 30} + {[(S2 + S3)/2] x 15}).

2.4.5. Acute transient hypoxic chemosensitivity

To evaluate the acute HVR participants were seated and asked to breathe normally. Following a 3-min baseline period, they were exposed to 10 periods of pure nitrogen inhalation in a single-blind setup (Solaiman et al., 2014). Each phase comprised 1–8 consecutive breaths in a randomized order aiming to elicit a diverse spectrum of arterial oxygen desaturations (~99% to 62%) (**Figure 6**). The shifts from normoxic air to 100% nitrogen breathing were achieved using a three-way T-shaped valve (2100 series, Hans Rudolph, Kansas City, MO). Successive nitrogen exposures were spaced by ~2 min or until respiratory parameters returned to baseline. Throughout the procedure, pulmonary $\dot{V}_E$ and SpO_2 were continuously monitored by a metabolic cart (Ergocard Professional, Medisoft, Sorinnes, Belgium) and a pulse oximeter (WristOx 3150, Nonin Medical Inc, Plymouth, MN) placed on the earlobe. The lowest SpO_2 value measured within the 30 s after each period of 100% nitrogen inhalation was plotted against the corresponding peak in $\dot{V}_E$ (**Figure 7**).

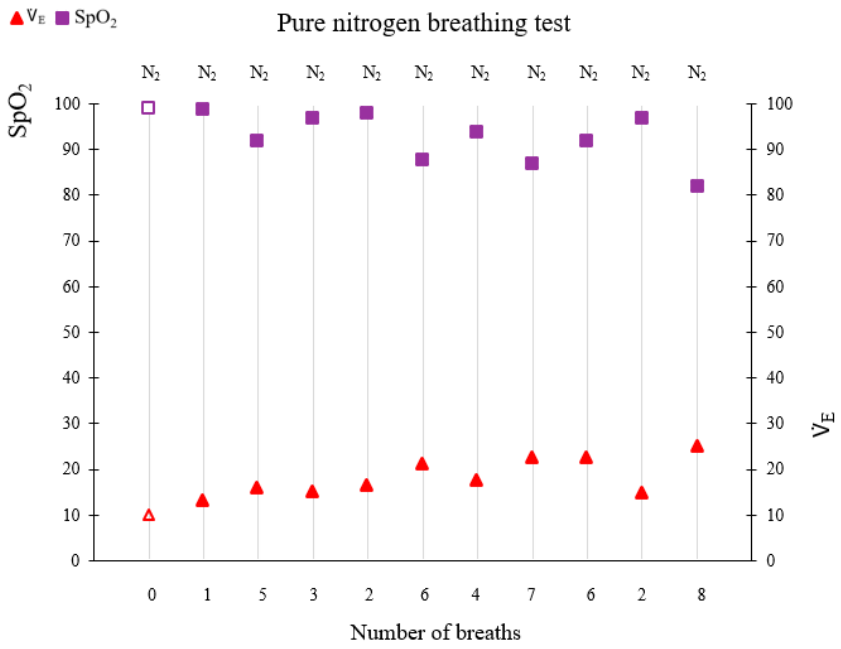


Figure 6. Graphical representation of the lowest peripheral oxygen saturation (SpO_2) values, represented by purple squares, recorded within the 30 s after each period of pure nitrogen inhalation (N_2) and the corresponding peaks in pulmonary ventilation ($\dot{V}_E$), indicated by red triangles, of a representative participant. The empty square and triangle represent baseline values.

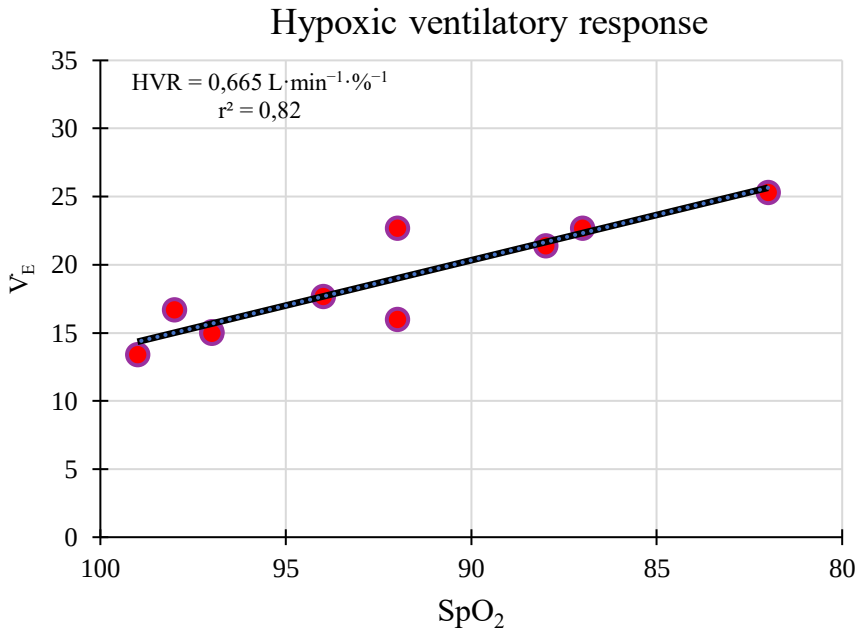


Figure 7. Graphical representation of the changes in pulmonary ventilation ($\dot{V}_E$) in response to alteration in peripheral oxygen saturation (SpO2). The slope of this relationship represents the hypoxic ventilatory response of a representative subject.

2.4.6. Pulmonary function

Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FEV_1/FVC ratio, forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25-75\%}$) and peak expiratory flow (PEF) were obtained using a spirometer (Pony FX; Cosmed, Rome, Italy), according to standardized procedures (Miller et al., 2005). Participants were seated during the measurements. Lung function parameters are expressed in both absolute terms and as percentage of predicted values calculated using the Global Lung Function Initiative 2012 equations (Quanjer et al., 2012).

2.4.7. Ventilatory and pulmonary gas exchange parameters

Respiratory frequency (R_f), V_T, $\dot{V}_E$, $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$) and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) were continuously monitored, breath by breath, using a metabolic cart (Quark, Cosmed, Rome, Italy) at rest and during exercise (**Figure 8**). The ventilatory equivalents for O_2 and CO_2 were expressed as $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$, respectively. **Figure 9** illustrates the experimental setup. Rest was defined as sitting on the bike

without movement. Prior to each experiment, the device was calibrated using a 3-L syringe at two distinct flow rates in accordance with the manufacturer's guidelines. Subsequently, O_2 and CO_2 analyzers were calibrated using gas mixtures of known concentrations. Mean values of respiratory variables were calculated as the average over the final 60 s of both the resting period and the exercise bout. Power output was predetermined and set at 1.2 $W \cdot kg^{-1}$. Ventilation data included n = 16 subjects, while gas exchange parameters encompassed only 13 subjects.

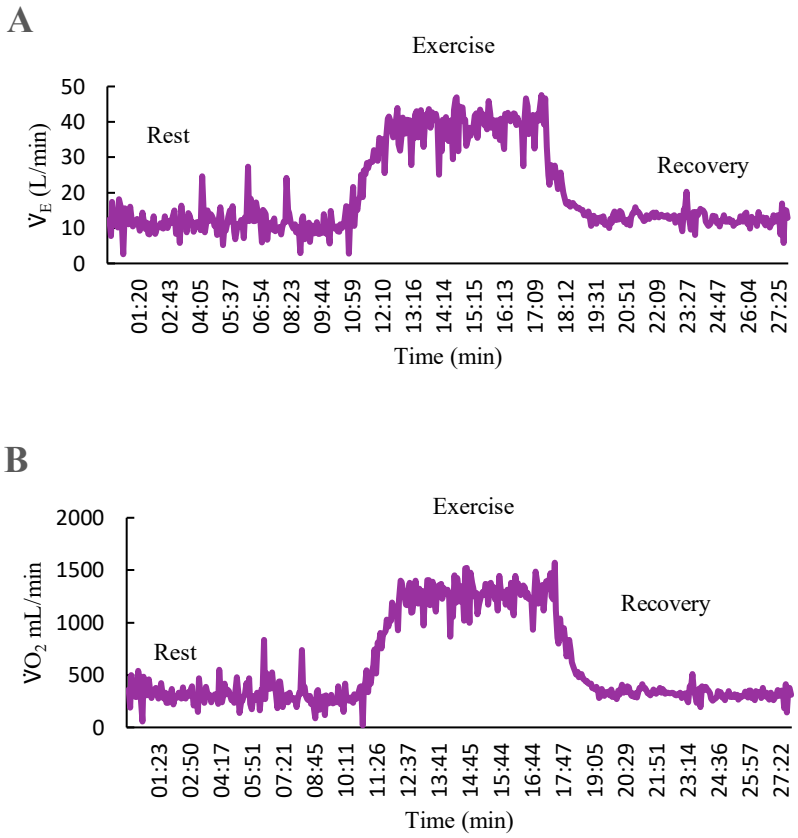


Figure 8. Graphical representation of breath-by-breath monitoring of minute ventilation ($\dot{V}_E$, panel A) and oxygen uptake ($\dot{V}O_2$, Panel B) at rest and during submaximal intensity exercise of a representative subject.

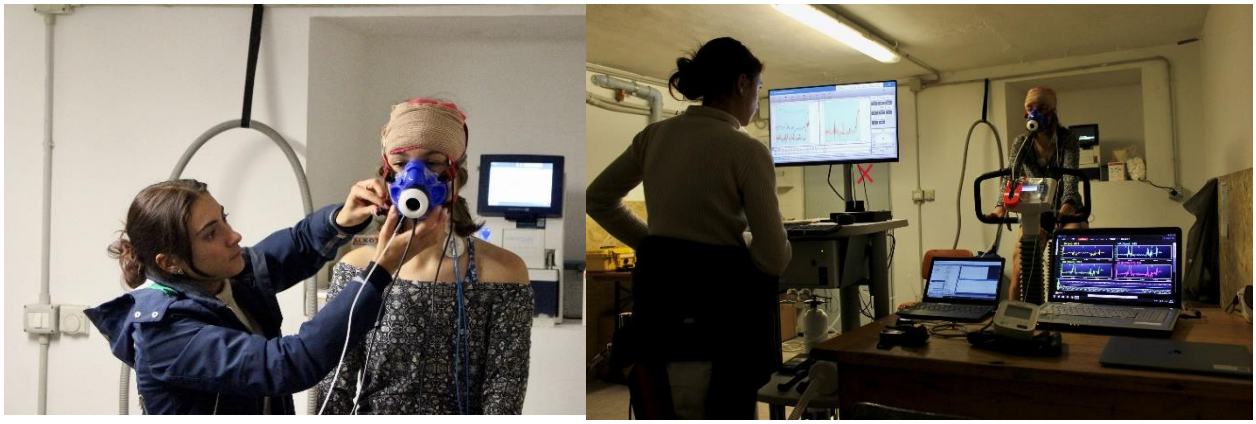


Figure 9. Experimental setup for the breath-by-breath measurements of gas exchange employed both at rest and during exercise.

2.4.8. Cycling efficiency

During a 6-min steady-state submaximal cycling exercise, net efficiency was calculated as described by Reger and colleagues (2013), using the following equation:

$$\frac{W}{E - E_{rest}} \times 100$$

where W is the mechanical power and E- E_{rest} represents the metabolic power above rest.

Mean values were derived from the last 60 s of exercise. Overall, the present data include n = 13 subjects.

2.4.9. Oxygen uptake kinetics of the on- and off-phases of submaximal exercise

The $\dot{V}O_2$ data were resampled at 1 Hz and then fitted with a mono-exponential function using a computerized non-linear regression technique to model the kinetics of both the on- and off-transients of $\dot{V}O_2$, as previously described (Borrani et al., 2001; Perrey et al., 2002). Analyses were conducted on MATLAB (v. R2019a, MathWorks, Natick, MA, USA). The parameters of interest were the amplitude (A), calculated as the difference in $\dot{V}O_2$ values between the baseline and the steady-state and the time constant (τ) representing the time from the onset of exercise to the point where the $\dot{V}O_2$ curve reaches 63% of its final amplitude during the on-phase and the τ of the exponential decay after exercise cessation during the off-phase (**Figure 10**). *A* reflects the magnitude of the increase/decrease in $\dot{V}O2$ to exercise. τ is a measure of the speed with which metabolic adjustments occur in response to changes in energy demands:

smaller values of τ indicate faster $\dot{V}O_2$ kinetics. Overall, the presented data include n = 13 subjects.

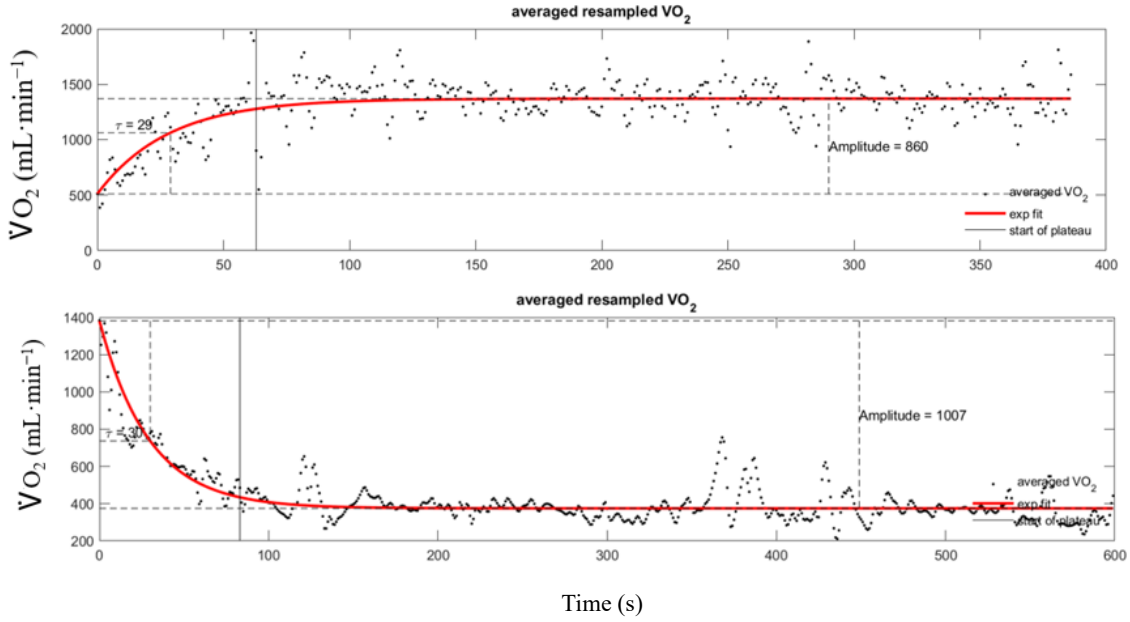


Figure 10. Model of on-transient (top panel) and off-transient (bottom panel) $\dot{V}O_2$ kinetics for a representative participant. Black data points represent the measured $\dot{V}O_2$ values, while the red line indicates the model fit to the data. The vertical line marks the onset of $\dot{V}O_2$ steady-state, and the horizontal dashed lines delineate the baseline and $\dot{V}O_2$ steady-state levels. Amplitude changes are indicated for both on- and off-transient phases. τ, the time constant characterizing the rate at which $\dot{V}O_2$ approaches 63% of the final amplitude during the on- and off-phases of exercise, respectively.

2.4.10. Heart rate variability

HRV was measured 15 h after reaching high altitude, upon awakening, while lying supine, with an empty bladder, and following an 11-hour fasting period. Participants were instructed to breathe naturally. The inter-beat interval (R-R interval) was recorded for 5 min using a chest belt (Polar H10, Kempele, Finland) connected to the mobile app known as Polar Sensor Logger (v. 0.25, Jukka Happonen, Helsinki, Finland) which operates via the Polar SDK (v. 3.3.2). HRV analyses were conducted using MATLAB (v. R2019a, MathWorks, Natick, MA, USA) employing a fast Fourier transform and the Welch power spectral density estimate in the low-frequency (LF, 0.04–0.15 Hz; in ms^2) and high-frequency bands (HF, 0.15–0.40 Hz; in ms^2) and the root mean square of the successive differences (RMSSD; in ms), following the guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Heart Rate Variability: Standards of measurement,

physiological interpretation, and clinical use, 1996). In addition to this, HRV analyses included the normalized HF (nHF, calculated as the HF/(LF+HF) ratio) and LF (nLF, LF/(LF+HF)) powers. The low- and high-frequency bands are illustrated in **Figure 11**. LF encompasses information pertaining to both the sympathetic and parasympathetic branches. On the contrary, HF corresponds to the parasympathetic activity. The RMSSD is a time-domain measure that quantifies variations in heart rate, primarily reflecting parasympathetic nervous system activity.

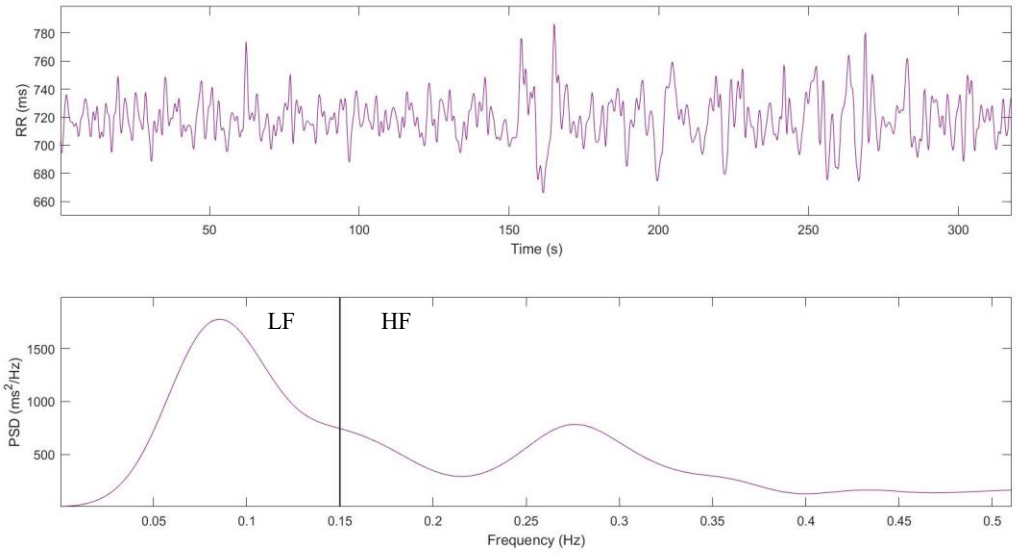


Figure 11. Graphical representation of the inter-beat interval (RR) in the top panel, along with the low-frequency (LF) and high-frequency (HF) bands depicted in the bottom panels. The data is illustrated for a representative subject, with a vertical line demarcating the division between the frequency bands. PSD, power spectral density.

2.4.11. Heart rate recovery

Postexercise HRR was evaluated with the participant in a passive seated position on a chair positioned adjacent to the cycle ergometer. The assessment commenced immediately after the conclusion of the 6-min submaximal exercise test, and the transition from exercise to sitting was completed in less than 4 s. The analyses of HRR were conducted on MATLAB (v. R2019a, MathWorks, Natick, MA, USA) and involved multiple procedures (**Figure 12**), i.e., calculation of the delta between the final HR at exercise completion and HR recorded 1 min (ΔHR1min), 2min (ΔHR2min) and 3min (ΔHR3min) after, acquisition of the time constant of the HR decay by fitting the 10-min post-exercise HRR to a first-order exponential decay curve (HRRτ) and the semilogarithmic regression analysis of the first 30 s of HRR (T30), as previously proposed (Buchheit et al., 2008). The RMSSD was computed for 30-s successive windows ($RMSSD_{30s}$)

through the 10-min recovery phase, serving as index of the parasympathetic reactivation post-exercise, and area under the curve (AUC, in s^2) was determined by the logarithmic trapezoidal method providing an overall magnitude of RMSSD across the time (**Figure 13**). HRV analyses conducted during the final 5 min of the recovery period included: $RMSSD_{5–10min}$, the power density in the LF band and the HF band and the normalized HF ($HFnu_{5–10min}$, calculated as the HF/(LF+HF) ratio) and LF ($LFnu_{5–10min}$, LF/(LF+HF)) powers. Overall, the presented data include n = 9 female subjects.

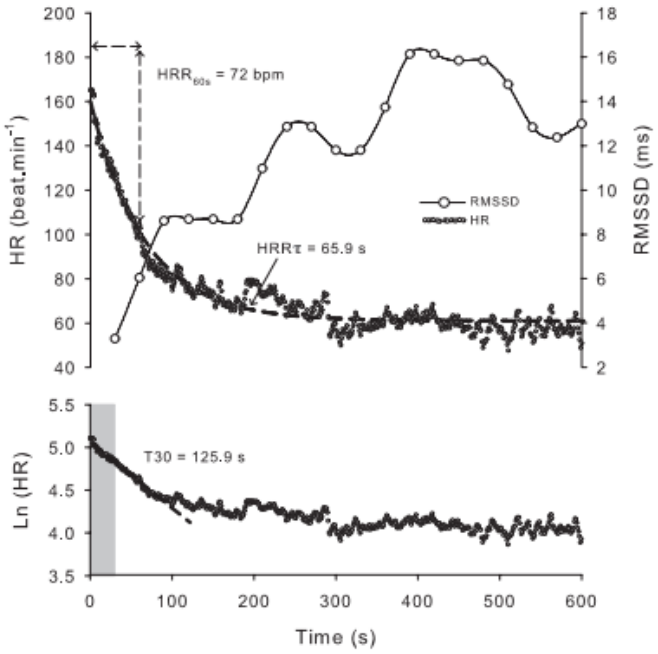


Figure 12. Graphical representation of the methodology employed to assess heart rate recovery (HRR) indices and parasympathetic reactivation in a single subject following submaximal exercise. Gray shading represents initial 30-s period from which T30 is calculated. HRR_{60s}, HR recorded following 60 s of recovery; HRRτ, postexercise HRR into a first-order exponential decay curve; RMSSD, root mean square of successive R-R interval differences (30-s windows); bpm, beats/min. From Buchheit et al. (2007).

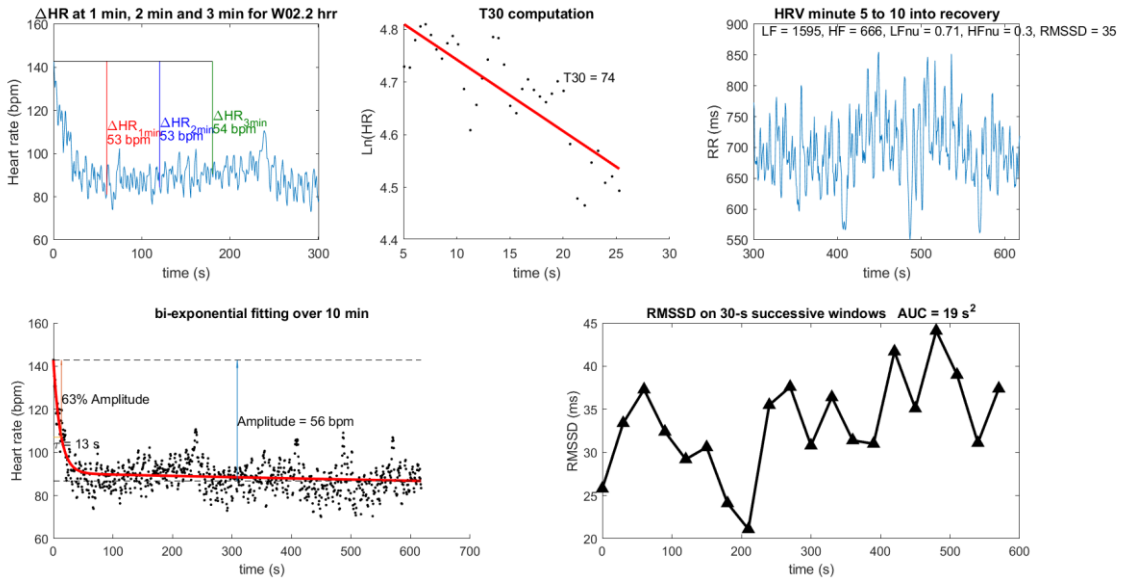


Figure 13. Example of the assessment of heart rate recovery (HRR) parameters of a representative participant following submaximal exercise (see main text for details).

2.4.12. Vascular occlusion test

At low altitude and 17h after arrival at 3375 m, participants underwent a 5-min period of tissue ischemia triggered by femoral artery occlusion, followed by 5 min of vascular reperfusion whilst seated in a resting position on a cycle ergometer (McLay et al., 2016). Following a 5-min baseline period, during which the tissue saturation index (TSI) signal remained stable for at least 30 s (<2% variation), occlusion was induced by the inflation of a pneumatic cuff placed on the proximal part of the thigh and connected to an automatic rapid inflation system (HokansonE20 AG101, Bellevue, WA, USA). Cuff pressure was regulated to 300 mmHg and maintained for the full 5-min period of ischemia. Oxygenation changes in the vastus lateralis muscle were continuously monitored with a NIRS device (Portalite, Artinis Medical Systems, Elst, The Netherlands), comprising three dual-wavelength (760 and 850 nm) light transmitters-channels and one receiving optode. The receiver-transmitters distances adopted were 29, 35 and 41 mm. The NIRS probe was consistently positioned by the same investigator on the lower third of the vastus lateralis muscle (~10 cm above the knee joint) of the right thigh and secured in place using double-sided tape. Marks made on the skin facilitated accurate re-application at the same location during subsequent examinations. A small towel and elastic bandages were wrapped around the probe and the thigh to minimize interference from ambient light and movement. The preparation of the shaved skin over the target muscle area

included cleaning. Adipose tissue thickness (ATT) at the site of the NIRS probe was assessed using a skinfold calliper. NIRS data were recorded at 50 Hz and exported at 2 Hz. Baseline TSI (%) was determined as the 30 s average before cuff occlusion. The desaturation rate (%·s^{-1}), computed by linear regression of the initial 60 s decrease in TSI signal following cuff inflation, was considered as an index of resting skeletal muscle oxidative metabolism, as previously suggested (Manferdelli et al., 2023; McLay et al., 2016; Rasica et al., 2022). The reperfusion rate (%·s^{-1}) was calculated as the linear increase in TSI signal during the initial 10 s following cuff deflation. The difference between the highest and the lowest TSI value during reperfusion and occlusion (TSI_{peak} and TSI_{min}, respectively, %) was used to evaluate the amplitude of TSI change (A_{TSI}). The time taken for the TSI signal to return to baseline ($t_{baseline}$) after cuff release was also assessed. Selected NIRS-VOT parameters under analysis are shown in **Figure 14** Overall, the presented data include n = 14 subjects.

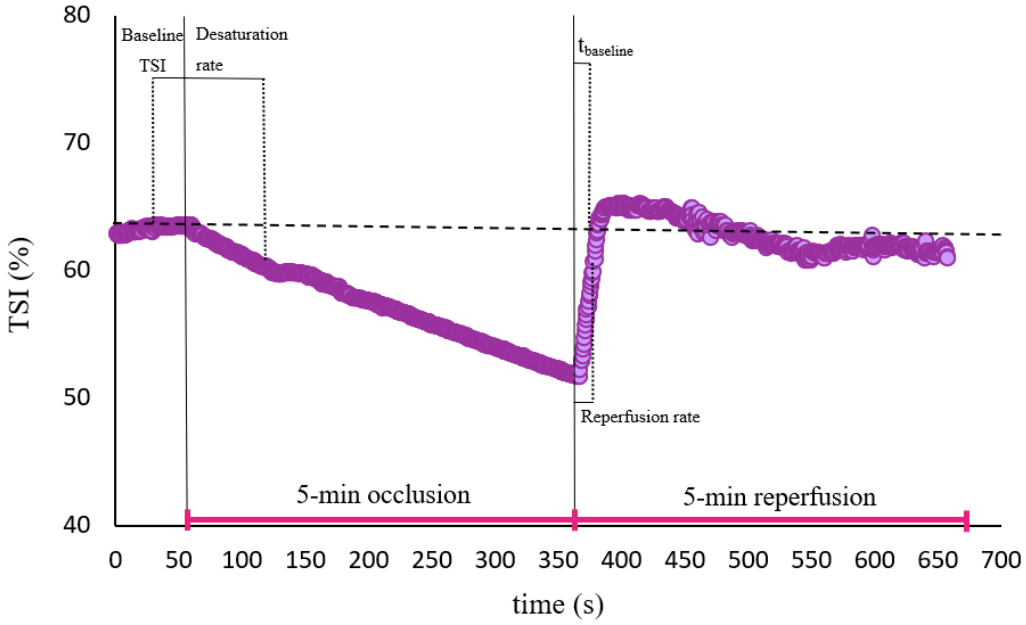


Figure 14. Graphical representation of the fluctuations in tissue saturation index (TSI) observed during a vascular occlusion test (VOT) of a representative participant. Occlusion phase followed by reperfusion bout are depicted with vertical lines indicating cuff inflation and release. Baseline TSI is represented by a horizontal dashed line. $t_{baseline}$, time required for the TSI signal to return to baseline values after cuff deflation (see main text for details).

2.4.13. Acute mountain sickness and sleep quality

Symptoms of acute mountain sickness (AMS) were assessed 6 h post-arrival at high-altitude and in the morning after awakening using the Lake Louise score (LLS) (Roach et al.,

2018). A total score ≥ 3 in the presence of headache together with at least one other symptom, i.e., gastrointestinal, fatigue and/or weakness and dizziness/light-headedness, was considered diagnostic for AMS. The prevalence of AMS was calculated as the proportion of individuals with AMS to the total number of subjects exposed to 3375 m, expressed as a percentage in each phase of the menstrual cycle.

Subjective sleep quality was assessed each morning using the Groningen sleep quality scale (GSQS-15; Meijman et al., 1990). The scores of this questionnaire span from 0 to 14, with a higher value indicating poorer sleep the night before. When sleep is unrestricted, subjects score is between 0 and 2. A score ≥ 6 indicates disturbed sleep (Jafarian et al., 2008). A maximum score of 14 points indicates poor sleep the night before.

2.4.14. Cognitive function

Participants completed a battery of neuropsychological tests using the Psychology Experimental Building Language (PEBL, v. 2.1) software (Mueller & Piper, 2014) installed on a laptop, in a quiet room at low altitude and 6 h post-arrival at high-altitude. The cognitive evaluation included the Digit span test, the Trail-making test (TMT), the Psychomotor vigilance task (PVT) and the Time wall task (**Figure 15**). The Digit Span test evaluates short-term and working memory, with the participant required to recall correctly the longest sequence of numbers displayed on the computer screen (Woods et al., 2011). According to Miller (1956), individuals typically have a short-term memory capacity limited to 7 ± 2 items. The TMT comprises two parts: A and B. TMT-A consists of 25 randomly assorted numbers that shall be interconnected in ascending order, whereas in TMT-B subjects are asked to alternate numbers and letters. In addition to direct scores derived from total time of task completion, two derived scores were calculated: difference score (B-A) and ratio score (B/A) (Chaytor et al., 2006). Lower values of the ratio B/A, i.e., closer to 1.0, are indicative of better performance (Piper et al., 2015). Set A assesses visuoperceptual abilities, visual scanning, attention, hand-eye coordination and cognitive processing speed. Part B relies on working memory, cognitive flexibility, task-switching abilities and requires an increased motor speed and visual attention compared to part A (Gaudino et al., 1995; sanchez-cubillo et al., 2009). The ratio between completion times of set B and A provides an indicator of executive control abilities (Arbuthnott & Frank, 2000). PVT measures the ability to respond quickly to stimuli appearing at randomly-varying time intervals on the laptop screen by pressing the space bar key. Cognitive skills

required for task completion encompass reaction time, attention, concentration, sleepiness and alertness (Loh et al., 2004). Reaction times were recorded. Durations exceeding 500 ms were classified as lapses (Anderson et al., 2010). The Time-wall task requires participants to estimate the moment a moving target will reach a marked point at the bottom of the display after passing behind a wall and press the space bar on the keyboard at this exact moment. Inaccuracy was the primary dependant measure calculated as outlined by Piper and colleagues (2012):

$$\text{Inaccuracy} = \frac{\text{(Response time – correct time)}}{\text{Correct time}}$$

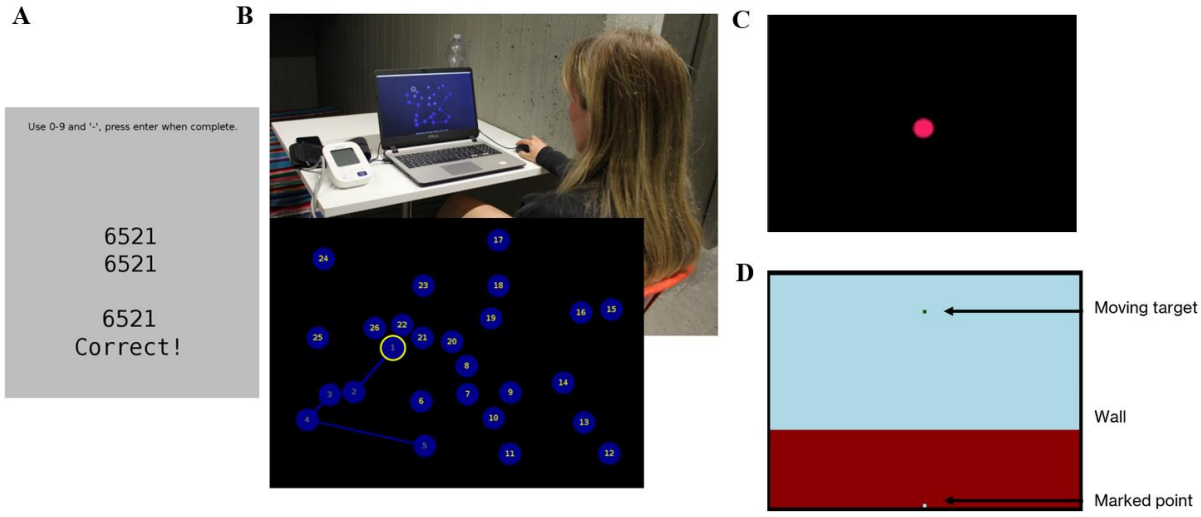


Figure 15. Assessment of cognitive function through Digit span (panel A), Trail-making (panel B), Psychomotor vigilance (panel C) and Time-wall tests and experimental setup.

2.4.15. Polysomnography

Polysomnography measurements included ten electroencephalography (EEG) electrodes, two electrooculography (EOG) electrodes, six surface electromyography (EMG) electrodes (two submental, four for right and left anterior tibialis muscle), electrocardiogram, nasal pressure, thoracic and abdominal belts for respiratory movements, body position and SpO_2. **Figure 16** illustrates placement of sleep monitoring equipment.

Figure 16. Placement of sleep monitoring equipment on participants.

2.5. Statistical analyses

A priori power analysis using G*Power software (v.3.1, G*Power software, Düsseldorf, Germany) was conducted to determine the appropriate sample size. Based on data from previous researches (Beidleman et al., 1999; Cornelli et al., 2013; Maki et al., 2002 Slatkovska et al., 2006) on the effects of menstrual cycle phase on ventilatory responses, peripheral oxygen saturation, oxidative stress and cognitive function, between 4 and 16 subjects were required to yield the targeted analysis power of $\beta = 0.8$ at $\alpha = 0.05$.

After assessing normality of data distributions with the Shapiro-Wilk test, differences between F and L groups under low altitude conditions were evaluated using a Student's t-test for unpaired data. In addition to this, Levene's test for equality of variances was employed to determine whether the assumption of equal variances was respected. When this assumption was violated, Welch's t-test was used. To determine the significance of mean differences between EF and ML during acute high-altitude exposure, a Student's t-test for paired samples was employed. When the assumption of normality was not respected, the non-parametric Wilcoxon test was applied instead. AMS prevalence was analyzed using McNemar's test. Furthermore,

changes in pulmonary function between the two phases of the menstrual cycle during high-altitude exposure were compared using a two-way (menstrual cycle phase x time spent at high-altitude) repeated measures ANOVA. To further explore significant effects revealed by this analysis, a Holm post hoc test was employed.

Statistical significance was established at $p < 0.05$. Quantitative variables are presented as mean ± SD, with the exception of figures, which display mean ± SEM. Qualitative variables are reported as *n* (%). All measurements and analyses were conducted on the 16 participants, except mentioned otherwise in methods, tables or figures. All statistical analyses were performed using Jamovi (v.2.3.18, Jamovi software, Sydney, Australia).

3. Results

Participants' characteristics are displayed in **Table 1**.

Table 1. Baseline participants' selected physical characteristics.

Participants' characteristics	
Age (years)	33 ± 7
Height (cm)	166 ± 7
Body mass (kg)	60 ± 10
Body mass index ($kg \cdot m^{-2}$)	22.7 ± 3.2
Cycle length (days)	27 ± 2

Values are Mean ± SD. N = 16.

3.1. Blood, urine and saliva analyses

Mean estradiol and progesterone levels, presented in **Table 2**, are within previously reported normal ranges for eumenorrheic women (Elliott-Sale et al., 2021; Schaumberg et al., 2017). Progesterone demonstrated significantly higher levels in L compared to F ($p = 0.002$) and during ML compared to EF ($p < 0.001$).

Table 2. Serum concentrations of progesterone and estradiol under the four experimental trials.

	F LA	L LA	EF HA	ML HA
Estradiol ($ng \cdot L^{-1}$)	45.3 ± 22.2	38.1 ± 31.4	46.9 ± 24.5	56.7 ± 23.1
Progesterone ($ng \cdot mL^{-1}$)	3.72 ± 1.49	**7.89 ± 2.50** [a]	3.81 ± 2.01	**7.86 ± 2.59** [ab]

Values are Mean ± SD. F LA, follicular phase subgroup at low altitude; L LA, luteal phase subgroup at low altitude; EF HA, early-follicular phase at high-altitude; ML HA, mid-luteal phase at high-altitude. Significant differences are reported as follows: ^{a} *from F;* ^{b} *from EF. N = 16 at low altitude and n = 15 at high-altitude, respectively.*

Markers of oxidative stress (**Table 3**) and of inflammatory state, hemolysis and iron metabolism (**Table 4**) were similar between F and L subgroups at low-altitude as well as between EF and ML phases at high-altitude. However, total protein levels were significantly different between EF and ML ($p = 0.010$).

Table 3. Oxidative stress biomarkers in the four conditions.

	F LA	L LA	EF HA	ML HA
AOPP (μmol·L^{-1})	260 ± 158	248 ± 87	193 ± 83	169 ± 35
Catalase (μmol·L^{-1}·min^{-1})	1.55 ± 0.39	1.30 ± 0.61	1.34 ± 1.18	0.96 ± ± 0.27
FRAP (μmol·L^{-1})	647 ± 48	640 ± 37	605 ± 72	635 ± 51
GPx (μmol·L^{-1}·min^{-1})	89 ± 8	92 ± 8	92 ± 17	88 ± 11
MDA (μmol·L^{-1})	13.2 ± 2.3	14.0 ± 0.3	13.1 ± 4.2	13.0 ± 1.9
MPO (μmol·L^{-1}·min^{-1})	62.2 ± 11.1	60.9 ± 17.5	42.8 ± 13.8	49.1 ± 9.9
NO_2^- (μmol·L^{-1})	12.5 ± 4.5	13.4 ± 4.8	7.0 ± 1.1	8.2 ± 1.9
Nytrotyrosine (μmol·L^{-1})	65.7 ± 47.2	60.4 ± 24.2	49.6 ± 27.3	42.5 ± 10.8
NOx (μmol·L^{-1})	55.1 ± 26.0	46.8 ± 15.9	38.5 ± 11.3	33.2 ± 5.4
SOD (μmol·L^{-1}·min^{-1})	14.1 ± 5.6	15.3 ± 1.8	13.9 ± 2.5	14.7 ± 2.5
XO (μmol·L^{-1}·min^{-1})	0.075 ± 0.046	0.099 ± 0.053	0.142 ± 0.077	0.172 ± 0.087

Values are Mean ± SD. F LA, follicular phase subgroup at low altitude; L LA, luteal phase subgroup at low altitude; EF HA, early-follicular phase at high-altitude; ML HA, mid-luteal phase at high-altitude; AOPP, advanced oxidation protein products; FRAP, Ferric reducing antioxidant power; GPx, glutathione peroxidase; MDA,

malondialdehyde; MPO, myeloperoxidase; $NO2^{-}$, nitrite; NOx, total nitrite and nitrate; SOD, superoxide dismutase; XO, xanthine oxidase. N = 15.

Table 4. Inflammatory, hemolytic and iron metabolism biomarkers in the four conditions.

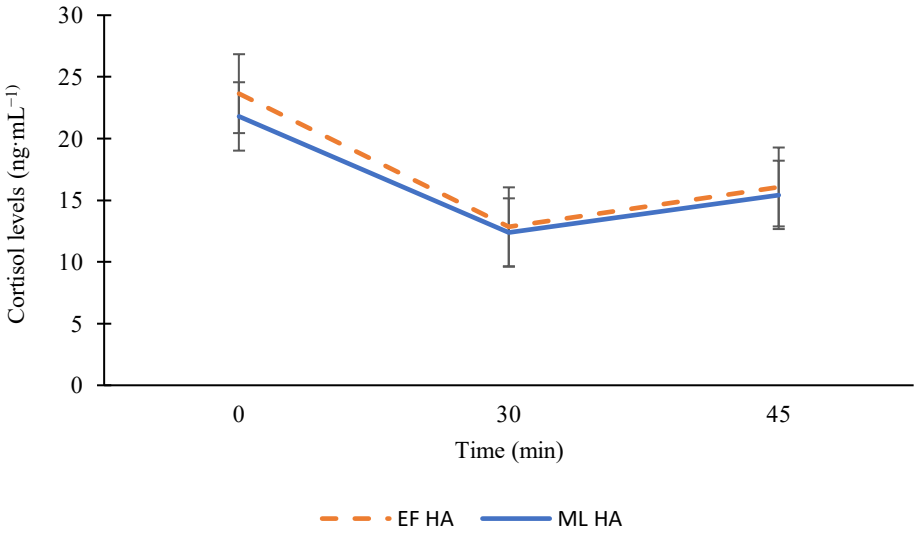
	F LA	L LA	EF HA	ML HA
CC16 ($ng \cdot mL^{-1}$)	7.36 ± 3.02	6.27 ± 3.00	5.68 ± 2.97	6.96 ± 1.98
CRP ($\mu g \cdot mL^{-1}$)	2.67 ± 1.99	2.90 ± 2.09	2.57 ± 1.83	2.58 ± 1.93
IL-6 ($ng \cdot mL^{-1}$)	5.84 ± 5.12	11.8 ± 6.3	23.2 ± 13.8	13.8 ± 12.2
Fibrogen ($mg \cdot dL^{-1}$)	203 ± 68	186 ± 79	232 ± 65	216 ± 111
Haptoglobin ($ng \cdot mL^{-1}$)	91.6 ± 51.2	96.2 ± 54.2	96.3 ± 50.5	92.4 ± 52.4
Total protein ($g \cdot L^{-1}$)	76.2 ± 3.4	77.7 ± 2.5	75.5 ± 2.0	**79.6 ± 4.9** [b]
Hepcidin ($ng \cdot mL^{-1}$)	14.9 ± 4.5	15.1 ± 4.2	16.2 ± 5.0	17.1 ± 3.5
Erythroferrone ($ng \cdot L^{-1}$)	3.83 ± 1.39	4.68 ± 1.99	7.61 ± 4.08	6.68 ± 2.90

Values are Mean ± SD. F LA, follicular phase subgroup at low altitude; L LA, luteal phase subgroup at low altitude; EF HA, early-follicular phase at high-altitude; ML HA mid-luteal phase at high-altitude; CC16, Clara cell 16–kDa protein; CRP, C-reactive Protein; IL-6, interleukin 6. Significant differences are reported as follows: ***b*** *between EF and ML. N = 15.*

At low altitude, hydration status did not significantly differ between participants in F and L subgroups (F vs. L; 1.013 ± 0.008 vs. 1.009 ± 0.004; $p = 0.244$). Subjects' urine specific gravity was similar between EF and ML phases at high-altitude (EF vs. ML; 1.018 ± 0.004 vs. 1.017 ± 0.006; p = 0.851).

At high-altitude, post-awakening cortisol levels (S1) were comparable between EF and ML (23.6 ± 30.4 vs. 21.8 ± 25.4 $ng \cdot mL^{-1}$; $p = 0.821$, respectively). Similarly, cortisol AUC-G was not significantly different between the two phases of the menstrual cycle at high-altitude (EF vs. ML; 867 ± 976 vs. 823 ± 858 $ng \cdot mL^{-1} \cdot min$; $p = 0.999$). **Figure 17** illustrates post-awakening cortisol levels at specific time points during acute high-altitude exposure.

Figure 17. Post-awakening cortisol levels at high altitude 0, 30 and 45 min post-awakening.



Values are Mean ± SEM. EF, early-follicular; ML, mid-luteal; HA, high-altitude. N = 16.

3.2. Resting measurements at low altitude

Table 5 and **6** display key respiratory and cardiovascular parameters evaluated at rest in a cohort of 16 subjects, with the results of subsequent analyses conducted for F and L subgroups at low altitude. Notably, the percent difference between F and L subgroups was 20% for the HR and 86% for LF. However, no significant differences were observed between F and L in the other variables investigated, including in the LF/HF ratio (2.24 ± 1.86 vs. 1.26 ± 0.85; $p = 0.241$, respectively).

Table 5. Lung function, ventilation, gas exchange parameters and peripheral oxygen saturation at rest at low altitude.

	Pooled	F	L	*p*-value
FVC (L)	3.74 ± 0.43	3.64 ± 0.37	3.88 ± 0.41	$p = 0.246$
FVC (% predicted)	97.4 ± 8.5	92.1 ± 10.1	100 ± 9.5	$p = 0.128$
FEV_1 (L)	3.10 ± 0.32	3.01 ± 0.32	3.13 ± 0.35	$p = 0.466$
FEV_1 (% predicted)	96.8 ± 8.5	92.6 ± 14.5	95.9 ± 9.2	$p = 0.608$
FEV_1/FVC (%)	83.3 ± 8.0	82.8 ± 5.7	81.4 ± 10.1	$p = 0.729$
FEV_1/FVC (% predicted)	99.4 ± 9.0	99.7 ± 8.0	95.9 ± 10.1	$p = 0.412$
$FEF_{25-75\%}$ ($L \cdot s^{-1}$)	3.35 ± 0.79	3.20 ± 0.73	3.24 ± 0.97	$p = 0.914$
$FEF_{25-75\%}$ (% predicted)	97.2 ± 23.0	94.4 ± 28.9	89.6 ± 22.5	$p = 0.719$
PEF ($L \cdot s^{-1}$)	6.32 ± 1.02	6.38 ± 0.85	6.18 ± 1.16	$p = 0.837$
Rf (breaths·min^{-1})	18.0 ± 2.7	17.6 ± 3.1	18.4 ± 2.2	$p = 0.553$
V_T (L)	0.646 ± 0.120	0.628 ± 0.126	0.671 ± 0.115	$p = 0.606$

$\dot{V}_E$ (L·min^{-1})	10.8 ± 1.8	10.3 ± 2.2	11.4 ± 1.0	p = 0.260
$\dot{V}O_2$ (mL·min^{-1})	345 ± 62	328 ± 69	367 ± 48	p = 0.219
$\dot{V}O_2$ (mL·min^{-1}·Kg^{-1})	5.57 ± 1.20	5.19 ± 1.23	6.06 ± 1.04	p = 0.160
$\dot{V}CO_2$ (mL·min^{-1})	278 ± 53	267 ± 61	292 ± 40	p = 0.371
$\dot{V}_E/\dot{V}O_2$	28.2 ± 3.2	28.1 ± 3.5	28.2 ± 3.1	p = 0.933
$\dot{V}_E/\dot{V}CO_2$	34.8 ± 4.1	34.4 ± 4.1	35.3 ± 4.4	p = 0.681
$P_{ET}CO_2$ (mmHg)	30.6 ± 2.7	30.9 ± 2.8	30.3 ± 2.8	p = 0.665
SpO_2 (%)	98.9 ± 0.9	98.8 ± 0.9	99.0 ± 1.0	p = 0.810

Values are Mean ± SD. F, follicular phase subgroup; L, luteal phase subgroup; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of forced vital capacity; PEF, peak expiratory flow; Rf, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for O_2; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for CO_2; $P_{ET}CO_2$, end tidal partial pressure of carbon dioxide; SpO_2, peripheral oxygen saturation. N = 16 when pooled, n = 9 in the follicular subgroup and n = 7 in the luteal subgroup.

Table 6. Hemodynamics, heart rate variability and microvascular function at rest at low altitude.

	Pooled	F	L	p-value
HR (bpm)	63.7 ± 10.6	59.1 ± 9.0	72.1 ± 8.3	***p* = 0.020**
SBP (mmHg)	96.7 ± 13.7	96.0 ± 16.7	96.1 ± 6.0	p = 0.983
DBP (mmHg)	67.0 ± 6.9	67.0 ± 7.0	65.4 ± 7.1	p = 0.426
RMSSD (ms)	42.3 ± 25.8	50.3 ± 28.2	34.4 ± 22.4	p = 0.265
LF (ms^2)	961 ± 694	1375 ± 589	547 ± 544	***p* = 0.018**
HF (ms^2)	916 ± 568	1183 ± 1128	648 ± 621	p = 0.293
nLF (n.u.)	0.554 ± 0.180	0.606 ± 0.186	0.503 ± 0.170	p = 0.302
nHF (n.u.)	0.446 ± 0.180	0.394 ± 0.186	0.497 ± 0.170	p = 0.302
Baseline TSI (%)	64.8 ± 4.1	65.0 ± 3.6	64.5 ± 4.9	p = 0.844
Desaturation rate (%·s^{-1})	-0.085 ± 0.041	-0.091 ± 0.055	-0.078 ± 0.024	p = 0.937
Reperfusion rate (%·s^{-1})	0.894 ± 0.319	0.825 ± 0.262	0.963 ± 0.380	p = 0.481
Norm reperfusion rate (s^{-1})	0.018 ± 0.008	0.016 ± 0.007	0.019 ± 0.010	p = 0.580
TSI_{min} (%)	52.8 ± 6.3	52.7 ± 6.6	52.9 ± 6.6	p = 0.942

Values are Mean ± SD. p value in bold indicates statistical significance. F, follicular phase subgroup; L, luteal phase subgroup; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; RMSSD, root-mean-square difference of successive normal R–R intervals; LF, power in low frequency range; HF, power in high frequency range; nLF, normalized LF power; nHF, normalized HF power; baseline TSI, baseline tissue saturation index; norm reperfusion rate, normalised reperfusion rate; TSI_{min}, minimum tissue saturation index value reached during the occlusion phase. N = 16 when pooled, n = 9 in the follicular phase subgroup and n = 7 in the mid-luteal phase subgroup.

3.3. Submaximal exercise tests at low altitude

Table 7 presents the cardiorespiratory variables of interest measured during exercise and immediately thereafter at low altitude. However, HR during the submaximal intensity exercise was similar between F and L subgroups (132 ± 21 vs. 144 ± 18 bpm; $p = 0.266$, respectively). Post-exercise, the ΔHR exhibited a percent difference between F and L subgroups of 21.1% at 1 min, 23.4% at 2 min and of 21.7% at 3 min into the recovery phase. In addition to this, a percent difference of 89.4 % was observed between the two groups in the HF. Also, $LFnu_{5–10min}$ and $HFnu_{5–10min}$ were significantly different between F and L phases. No differences were observed in the other cardiorespiratory parameters.

Table 7. Selected cardiorespiratory variables assessed during and immediately after the submaximal exercise at low altitude.

	Pooled	F	L	p-value
Rf (breaths·min^{-1})	30.3 ± 4.8	29.2 ± 3.0	31.7 ± 6.8	$p = 0.338$
V_T (L)	1.49 ± 0.26	1.05 ± 0.31	1.49 ± 0.23	$p = 0.959$
$\dot{V}_E$ (L·min^{-1})	42.7 ± 8.5	43.1 ± 8.7	42.1 ± 9.9	$p = 0.845$
$\dot{V}O_2$ (mL·min^{-1})	1529 ± 298	1499 ± 250	1428 ± 147	$p = 0.519$
$\dot{V}O_2$ (mL·min^{-1}·Kg^{-1})	23.6 ± 2.8	23.6 ± 3.4	23.6 ± 2.4	$p = 0.134$
$\dot{V}CO_2$ (mL·min^{-1})	1467 ± 201	1403 ± 299	1314 ± 208	$p = 0.513$
$\dot{V}_E/\dot{V}O_2$	28.7 ± 4.7	27.3 ± 2.2	30.5 ± 6.8	$p = 0.758$
$\dot{V}_E/\dot{V}CO_2$	31.0 ± 4.5	29.3 ± 1.5	33.1 ± 6.4	$p = 0.606$
$P_{ET}CO_2$ (mmHg)	36.7 ± 4.7	38.2 ± 2.1	34.7 ± 6.6	$p = 0.606$
SpO_2 (%)	94.1 ± 5.7	92.2 ± 6.4	96.3 ± 4.7	$p = 0.190$
CE (%)	20.1 ± 1.9	20.2 ± 1.7	19.9 ± 2.3	$p = 0.824$
RPE	10.7 ± 2.6	10.0 ± 3.0	11.6 ± 1.7	$p = 0.238$
VAS legs	29.1 ± 24.6	25.8 ± 20.9	33.4 ± 29.9	$p = 0.556$
VAS respiration	36.8 ± 24.5	36.6 ± 24.6	37.0 ± 26.6	$p = 0.975$
Aon (mL·min^{-1})	1018 ± 141	1107 ± 200	977 ± 152	$p = 0.636$
τon (s)	32.2 ± 6.2	30.8 ± 5.9	33.9 ± 6.6	$p = 0.207$
Aoff (mL·min^{-1})	1089 ± 150	1116 ± 190	1061 ± 105	$p = 0.825$
τoff (s)	40.1 ± 6.9	40.1 ± 6.5	38.1 ± 4.8	$p = 0.745$
ΔHR1min (bpm)	42.3 ± 8.0	46.5 ± 7.8	37.6 ± 5.4	**$p = 0.025$**
ΔHR2min (bpm)	50.9 ± 8.9	56.4 ± 5.8	44.6 ± 7.6	**$p = 0.005$**
ΔHR3min (bpm)	53.4 ± 9.6	58.8 ± 5.4	47.3 ± 9.9	**$p = 0.014$**
HRRτ (s)	46.6 ± 24.0	46.9 ± 19.5	46.3 ± 30.6	$p = 0.962$
A (bpm)	57.7 ± 11.3	62.6 ± 7.5	52.0 ± 12.7	$p = 0.066$
T30 (s)	128 ± 43	121 ± 48	137 ± 39	$p = 0.494$

AUC (s^2)	13.9 ± 8.8	15.8 ± 5.7	11.9 ± 11.5	p = 0.182
$RMSSD_{5–10min}$ (ms)	23.9 ± 14.8	27.0 ± 9.3	20.4 ± 19.6	p = 0.440
$LF_{5–10min}$ (ms^2)	417 ± 304	446 ± 224	373 ± 418	p = 0.704
$HF_{5–10min}$ (ms^2)	185 ± 145	246 ± 129	94 ± 126	**p = 0.041**
$LFnu_{5–10min}$ (n.u.)	0.717 ± 0.177	0.629 ± 0.173	0.848 ± 0.072	**p = 0.012**
$HFnu_{5–10min}$ (n.u.)	0.283 ± 0.177	0.371 ± 0.173	0.152 ± 0.072	**p = 0.012**

Values are Mean ± SD. p value in bold indicates statistical significance. F, follicular phase subgroup; L, luteal phase subgroup; Rf, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E/\dot{V}O_2$, respiratory equivalent for O_2; $\dot{V}_E/\dot{V}CO_2$, respiratory equivalent for CO_2; $P_{ET}CO_2$, end tidal partial pressure of carbon dioxide; SpO_2, peripheral oxygen saturation; CE, cycling efficiency; RPE, rate of perceived exertion; VAS legs, visual analogue scale for interpretation of leg pain; VAS respiration, visual analogue scale for interpretation of breathing difficulty; A_{on}, amplitude of $\dot{V}O_2$ values during the on-transient response; τ_{on}, time constant of the on-transient pulmonary $\dot{V}O_2$ kinetics; A_{off}, amplitude of $\dot{V}O_2$ values during the off-transient response; τ_{off}, time constant of the off-transient pulmonary $\dot{V}O_2$ kinetics; ΔHR_{1min}, absolute difference between the final heart rate at exercise completion and the heart rate recorded after 1min of recovery; ΔHR_{2min}, absolute difference between the final heart rate at exercise completion and the heart rate recorded after 2min of recovery; ΔHR_{3min}, absolute difference between the final heart rate at exercise completion and the heart rate recorded after 3min of recovery; HRRτ, time constant of the heart rate decay; A, amplitude of the peak-to-baseline heart rate difference; T30, short-term heart rate time constant; AUC, the area under the curve; $RMSSD_{5–10min}$, root-mean-square difference of successive normal R–R intervals; $LF_{5–10min}$, power in low frequency range; $HF_{5–10min}$, power in high frequency range; $LFnu_{5–10min}$, normalized LF power; $HFnu_{5–10min}$, normalized HF power. N = 16 when pooled, n = 9 in the follicular phase subgroup and n = 7 in the mid-luteal phase subgroup.

3.4. Acute transient hypoxic chemosensitivity

A significant difference was observed in the HVR between the F (0.298 ± 0.049 $L \cdot min^{-1} \cdot \%^{-1}$) and L phase subgroups (0.497 ± 0.139 $L \cdot min^{-1} \cdot \%^{-1}$) at low altitude ($p$ = 0.001).

3.5. Ventilatory responses at high-altitude

Results obtained from the pulmonary function test conducted upon arrival at high-altitude are reported in **Table 8**, while **Table 9** displays the same variables evaluated 16 h after arrival at high-altitude. The percent differences between F and L subgroups were 1.63% and 3.75% for FVC and PEF values, respectively. However, no significant cycle-phase differences were found between EF and ML in the other variables assessed using the spirometry test.

Table 8. Pulmonary function upon arrival at high-altitude.

Upon arrival at high-altitude	EF	ML	*p*-value
FVC (L)	3.71 ± 0.44	3.65 ± 0.42	***p* = 0.033**
FVC (% predicted)	94.3 ± 11.1	93.0 ± 10.8	***p* = 0.035**
FEV_1 (L)	3.10 ± 0.39	3.10 ± 0.31	*p* = 0.960
FEV_1 (% predicted)	94.7 ± 12.9	95.1 ± 11.9	*p* = 0.789
FEV_1/FVC (%)	84.0 ± 7.8	85.2 ± 5.9	*p* = 0.288
FEV_1/FVC (% predicted)	100.1 ± 8.7	100.4 ± 8.0	*p* = 0.752
$FEF_{25-75\%}$ ($L \cdot s^{-1}$)	3.48 ±0.98	3.51 ± 0.80	*p* = 0.778
$FEF_{25-75\%}$ (% predicted)	97.9 ± 27.2	101.1 ± 27.1	*p* = 0.351
PEF ($L \cdot s^{-1}$)	7.33 ± 1.34	7.06 ± 0.87	***p* = 0.046**

Values are Mean ± SD. p value in bold indicates statistical significance. EF, early-follicular; ML, mid-luteal; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of forced vital capacity; PEF, peak expiratory flow. N = 16.

Table 9. Pulmonary function 16 h post-arrival at high-altitude.

16 h post-arrival at high-altitude	EF	ML	*p*-value
FVC (L)	3.65 ± 0.46	3.66 ± 0.39	*p* = 0.824
FVC (% predicted)	93.1 ± 11.6	93.3 ± 10.7	*p* = 0.879
FEV_1 (L)	2.99 ± 0.42	3.02 ± 0.35	*p* = 0.371
FEV_1 (% predicted)	91.6 ± 14.4	92.6 ± 12.5	*p* = 0.302
FEV_1/FVC (%)	82.3 ± 10.2	82.9 ± 7.4	*p* = 0.551
FEV_1/FVC (% predicted)	98.1 ± 11.9	98.6 ± 8.4	*p* = 0.917
$FEF_{25-75\%}$ ($L \cdot s^{-1}$)	3.14 ±0.98	3.17 ± 0.86	*p* = 0.618
$FEF_{25-75\%}$ (% predicted)	82.9 ± 33.5	90.3 ± 25.3	*p* = 0.313
PEF ($L \cdot s^{-1}$)	7.03 ± 1.16	6.88 ± 1.05	*p* = 0.536

Values are Mean ± SD. EF, early-follicular; ML, mid-luteal; FVC, forced vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of forced vital capacity; PEF, peak expiratory flow. N = 16.

Table 10 displays the results of two-ways repeated measures ANOVA for lung function tests conducted at high-altitude upon arrival and 16 h after during both EF and ML. A main effect of time spent at high-altitude was observed in FEV_1 and $FEF_{25-75\%}$, when both are expressed as absolute and predicted values, as well as in the FEV_1/FVC ratio and PEF, when expressed as absolute values. However, there was no significant effect observed for either the menstrual

phase or the interaction between menstrual phase and time spent at high-altitude on the measured lung function variables.

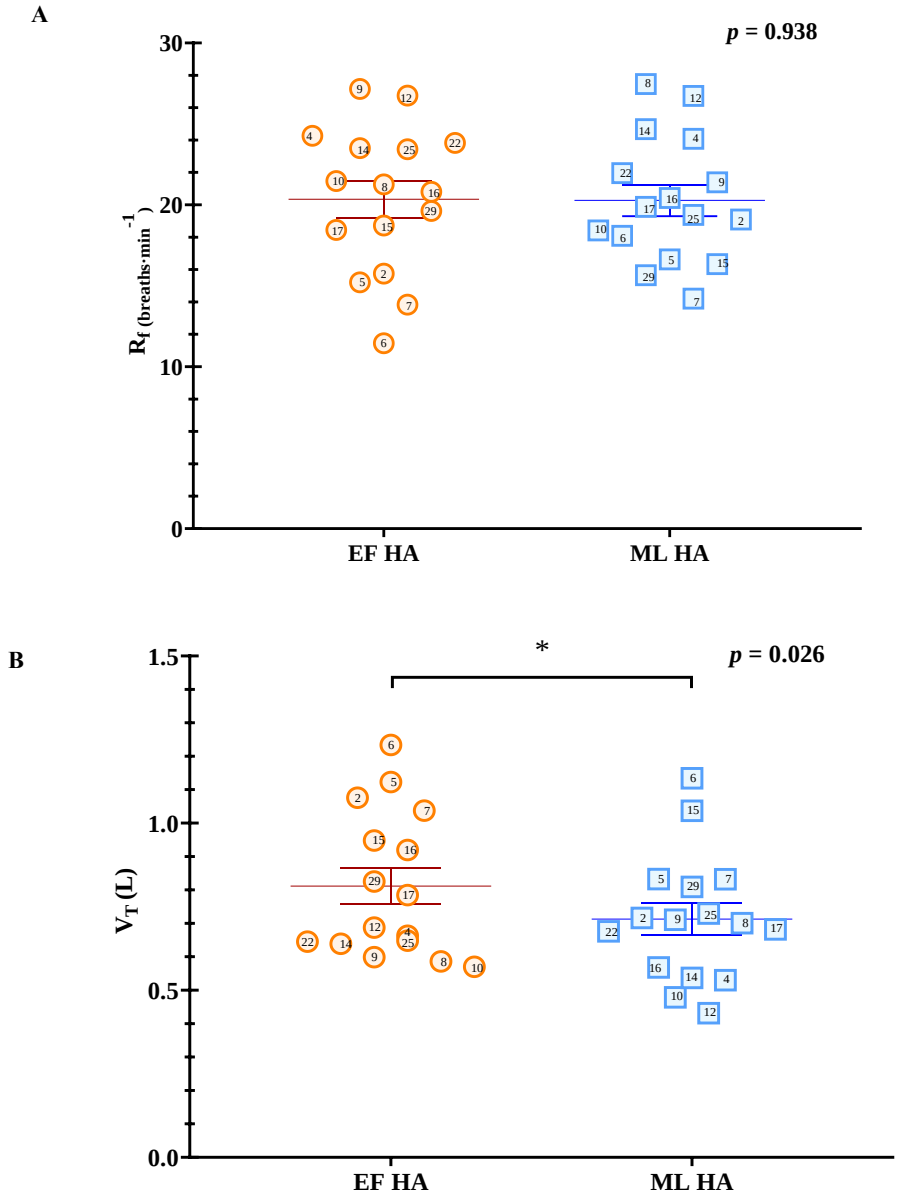
Table 10. Pulmonary function assessed upon arrival at high-altitude and 16 h after.

	EF upon arrival at high-altitude	EF 16 h after	ML upon arrival at high-altitude	ML 16 h after	Main effect (menstrual cycle phase)	Main effect (time spent at high-altitude)	Interaction Effect (menstrual cycle phase * Time)
FVC (L)	3.71 ± 0.44	3.65 ± 0.46	3.65 ± 0.42	3.66 ± 0.39	$p = 0.367$	$p = 0.285$	$p = 0.050$
FVC (% predicted)	94.3 ± 11.1	93.1 ± 11.5	93.0 ± 10.8	93.2 ± 10.7	$p = 0.344$	$p = 0.392$	$p = 0.053$
FEV_1 (L)	3.10 ± 0.39	2.99 ± 0.42	3.10 ± 0.31	3.02 ± 0.35	$p = 0.649$	$\mathbf{p = 0.001}$	$p = 0.294$
FEV_1 (% predicted)	94.7 ± 12.9	91.6 ± 14.4	95.1 ± 11.9	92.6 ± 12.4	$p = 0.488$	$\mathbf{p = 0.001}$	$p = 0.436$
FEV_1/FVC (%)	84.0 ± 7.82	82.3 ± 10.24	85.2 ± 5.92	82.9 ± 7.37	$p = 0.328$	$\mathbf{p = 0.020}$	$p = 0.577$
FEV_1/FVC (% predicted)	100 ± 9	98 ± 12	100 ± 8	99 ± 8	$p = 0.752$	$p = 0.134$	$p = 0.879$
$FEF_{25-75\%}$ ($L \cdot s^{-1}$)	3.48 ±0.98	3.14 ± 0.98	3.51 ± 0.80	3.17 ± 0.86	$p = 0.611$	$\mathbf{p = 0.000}$	$p = 0.981$
$FEF_{25-75\%}$ (% predicted)	97.9 ± 27.2	82.0 ± 33.5	101.1 ± 27.1	90.3 ± 25.3	$p = 0.135$	$\mathbf{p = 0.007}$	$p = 0.613$
PEF ($L \cdot s^{-1}$)	7.33 ± 1.34	7.03 ± 1.16	7.06 ± 0.87	6.88 ± 1.05	$p = 0.158$	$\mathbf{p = 0.013}$	$p = 0.525$

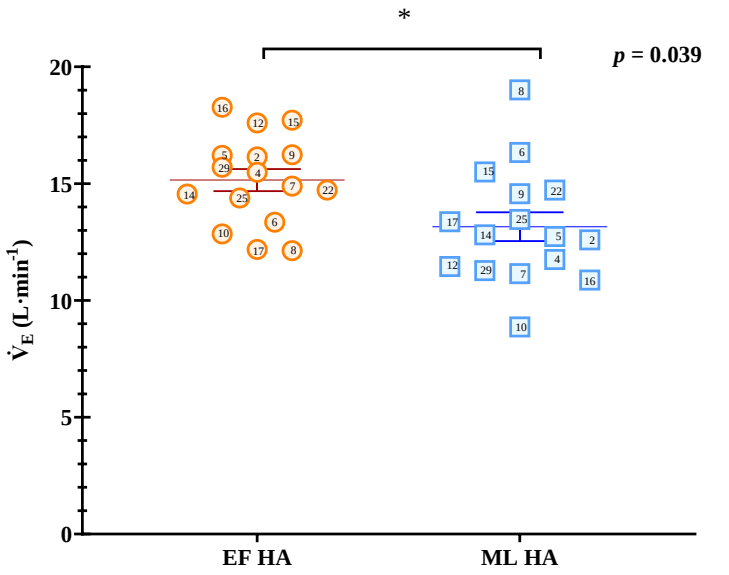
Values are Mean ± SD. p value in bold indicates statistical significance. EF, early-follicular; ML, mid-luteal; FVC, forced vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; $FEF_{25-75\%}$, forced expiratory flow at 25-75% of forced vital capacity; PEF, peak expiratory flow. N = 16.

The respiratory variables measured during resting conditions 17 h post-arrival at high-altitude are represented as function of the menstrual cycle phase in **Figure 18**. Resting minute $\dot{V}_E$ was significantly higher in EF than in ML (15.2 ± 1.9 vs. 13.2 ± 2.5 L·min^{-1}; p = 0.039). On the contrary, no significant differences were observed between EF and ML in SpO_2 levels (92.4 ± 2.3 vs. 91.2 ± 3.1%; p = 0.169), $\dot{V}_E/\dot{V}O_2$ (37.3 ± 6.9 vs. 35.2 ± 5.8; p = 0.607) and $\dot{V}_E/\dot{V}CO_2$ (42.3 ± 7.2 vs. 40.6 ± 5.3; p = 0.570) values.

Figure 18. Selected resting respiratory parameters measured 17 h post-arrival at high-altitude.



C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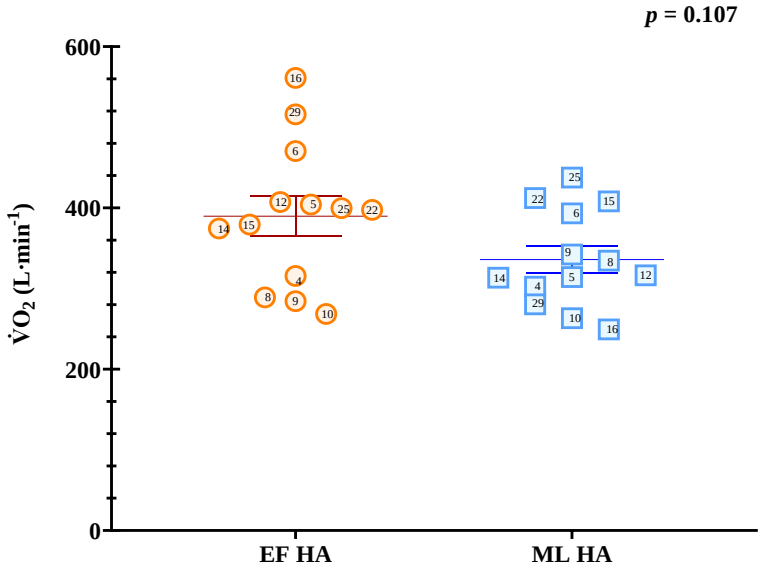
15
10
5
0
V̇E (L·min-1)
EF HA
ML HA

D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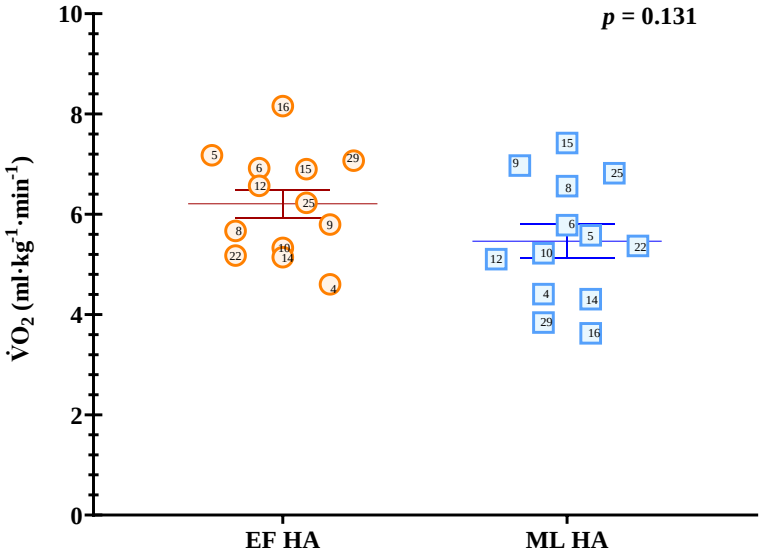
600
400
200
0
V̇O2 (L·min-1)
EF HA
ML HA

E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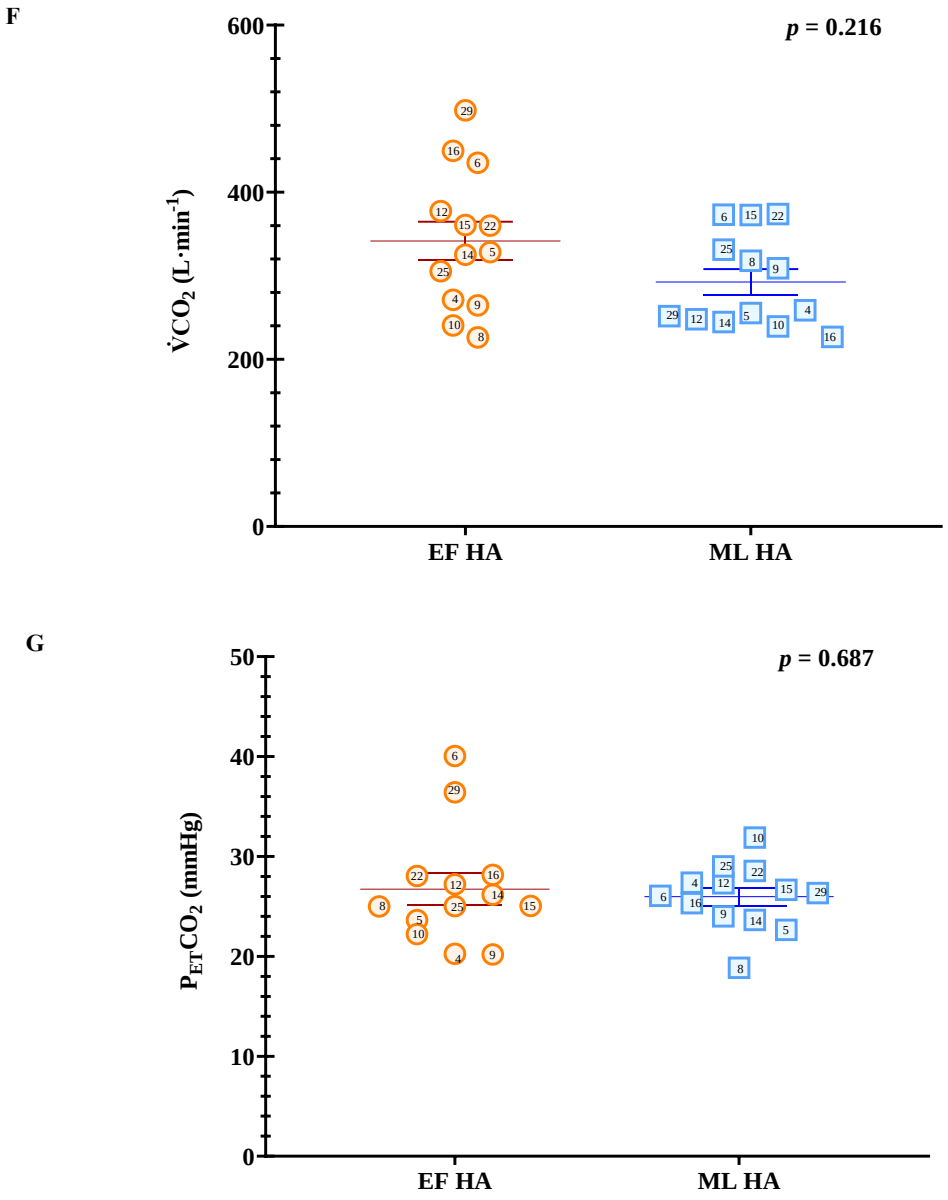
10
8
6
4
2
0
V̇O2 (ml·kg-1·min-1)
EF HA
ML HA



*Values are Mean ± SEM. Orange dots indicate the early-follicular (EF) phase while blue squares represent the mid-luteal (ML) phase. HA, high-altitude; Rf, respiratory frequency (Panel A); V_T, tidal volume (Panel B); $\dot{V}_E$, minute ventilation (Panel C); $\dot{V}O_2$, oxygen uptake (Panel D and E); $\dot{V}CO_2$, carbon dioxide production (Panel F); $P_{ET}CO_2$, end tidal partial pressure of carbon dioxide (G). * Significant between-phase difference ($p < 0.05$). N = 16 for ventilatory parameters and n = 13 for gas exchange variables.*

3.6 Physiological responses to exercise at high-altitude

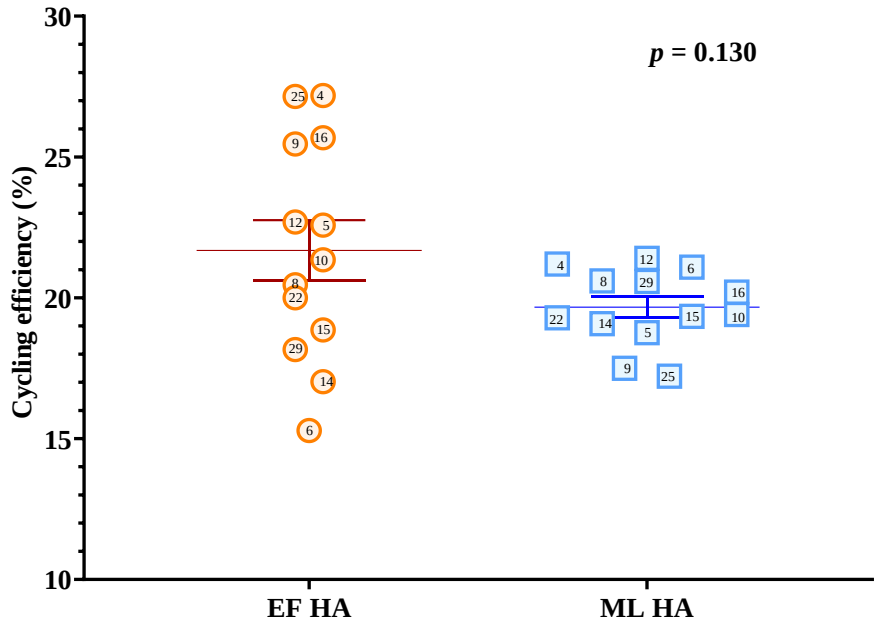
Ventilatory responses and gas exchange values measured during submaximal intensity exercise, performed 17 h after arrival at high altitude, were unaffected by cycle phase (**Table 11**). HR (147 ± 13 vs. 146 ± 15 bpm; p = 0.521) and RPE (11.9 ± 2.0 vs. 11.8 ± 1.5; $p = 0.774$) values did not significantly differ between EF and ML, respectively. Similarly, cycling efficiency remained consistent between EF and ML (**Figure 19**).

Table 11. Selected respiratory variables during the submaximal exercise 17 h post-arrival at high-altitude.

Variables during exercise	EF	ML	*p*-value
Rf (breaths·min^{-1})	32.6 ± 5.5	32.3 ± 5.5	$p = 0.650$
V_T (L)	1.67 ± 0.31	1.68 ± 0.35	$p = 0.736$
$\dot{V}_E$ (L·min^{-1})	53.9 ± 13.2	53.5 ± 13.4	$p = 0.661$
$\dot{V}O_2$ (mL·min^{-1})	1274 ± 390	1353 ± 237	$p = 0.763$
$\dot{V}O_2$ (mL·min^{-1}·Kg^{-1})	20.5 ± 5.5	21.9 ± 3.6	$p = 0.340$
$\dot{V}CO_2$ (mL·min^{-1})	1384 ± 381	1388 ± 216	$p = 0.939$
$\dot{V}_E/\dot{V}O_2$	39.0 ± 9.4	37.2 ± 6.4	$p = 0.368$
$\dot{V}_E/\dot{V}CO_2$	38.5 ± 8.8	37.2 ± 4.8	$p = 0.430$
$P_{ET}CO_2$ (mmHg)	30.4 ± 6.0	30.7 ± 4.1	$p = 0.834$
SpO_2 (mmHg)	87.2 ± 5.7	89.0 ± 4.0	$p = 0.528$

Values are Mean ± SD. Rf, respiratory frequency; $\dot{V}_T$, tidal volume; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$ carbon dioxide production; $\dot{V}E/\dot{V}O_2$, ventilatory equivalent for O_2; $\dot{V}E/\dot{V}CO_2$, ventilatory equivalent for CO_2; $P_{ET}CO_2$, end tidal partial pressure of carbon dioxide; SpO_2, peripheral oxygen saturation. N = 16 for ventilatory parameters and n = 13 for gas exchange variables.

Figure 19. Cycling efficiency during the submaximal intensity exercise performed 17 h after arrival at high altitude.



Values are Mean ± SEM. Orange dots indicate the early-follicular (EF) phase while blue squares represent the mid-luteal (ML) phase. HA, high-altitude. N = 13.

At high-altitude, τ_{on} and τ_{off}, characterizing the $\dot{V}O_2$ kinetics during the on- and off-phases of submaximal exercise, exhibited no significant differences between EF and ML (**Table 12**).

Table 12. Oxygen uptake kinetics parameters during on- and off-transient phases of submaximal exercise 17 h post-arrival at high-altitude.

	EF	ML	p-value
A_{on} (mL·min^{-1})	916 ± 256	996 ± 115	**p = 0.043**
τ_{on} (s)	36.4 ± 7.7	35.3 ± 7.4	p = 0.160
A_{off} (mL·min^{-1})	991 ± 248	1006 ± 134	p = 0.935
τ_{off} (s)	45.1 ± 7.8	45.9 ± 6.8	p = 0.442

Values are Mean ± SD. A_{on}, amplitude of oxygen uptake values during the on-transient response; τ_{on}, time constant of the on-transient pulmonary oxygen uptake kinetics; A_{off}, amplitude of oxygen uptake values during the off-transient response; τ_{off}, time constant of the off-transient pulmonary oxygen uptake kinetics. N = 13.

3.7. Cardiovascular responses at high-altitude

No significant differences were observed between EF and ML in HR (75 ± 14 vs. 79 ± 13 bpm; $p = 0.346$), SBP (104 ± 11 vs. 99 ± 7 mmHg; p = 0.095) and DBP (74 ± 7 vs. 73 ± 6 mmHg; $p = 0.562$), respectively. Similarly, HRV (**Table 13)** and HRR parameters (**Table 14)** did not differ between the two phases of the menstrual cycle. TSI_{peak} was lower during EF compared to ML. However, the other metrics of resting muscle oxygen consumption and microvascular reactivity shown in **Table 15** were similar between the two phases of the menstrual cycle.

Table 13. Heart rate variability (HRV) parameters in time and frequency domains in supine position 15 h post-arrival at high-altitude.

	EF	ML	*p*-value
HR (bpm)	70.4 ± 12.0	72.0 ± 9.7	$p = 0.356$
RMSSD (ms)	42.7 ± 27.1	36.0 ± 18.7	$p = 0.154$
LF (ms^2)	1182 ± 1605	914 ± 1029	$p = 0.632$
HF (ms^2)	776 ± 647	620 ± 592	$p = 0.326$
nLF (n.u.)	0.558 ± 0.189	0.547 ± 0.226	$p = 0.899$
nHF (n.u.)	0.442 ± 0.189	0.453 ± 0.226	$p = 0.899$

Values are Mean ± SD. RMSSD, root-mean-square difference of successive normal R–R intervals; LF, power in low frequency range; HF, power in high frequency range; nLF, normalized LF power; nHF, normalized HF power. N = 16.

Table 14. Postexercise heart rate recovery parameters in time and frequency domains 17 h post-arrival at high-altitude.

Variables post-exercise	EF	ML	p-value
ΔHR1min (bpm)	40.5 ± 8.0	37.8 ± 5.1	p = 0.623
ΔHR2min (bpm)	49.8 ± 6.1	46.0 ± 6.8	p = 0.195
ΔHR3min (bpm)	54.1 ± 7.1	49.2 ± 8.9	p = 0.199
HRRτ (s)	48.1 ± 26.5	44.2 ± 23.9	p = 0.341
A (bpm)	56.4 ± 8.1	51.2 ± 10.1	p = 0.082
T30 (s)	157 ± 88	171 ± 79	p = 0.695
AUC (s^2)	9.2 ± 6.5	8.1 ± 4.4	p = 0.727
$RMSSD_{5–10min}$ (ms)	16.2 ± 11.1	15.2 ± 8.6	p = 0.979
$LF_{5–10min}$ (ms^2)	370 ± 465	333 ± 255	p = 0.721
$HF_{5–10min}$ (ms^2)	150 ± 186	124 ± 163	p = 0.905
$LFnu_{5–10min}$ (n.u.)	0.759 ± 0.143	0.771 ± 0.161	p = 0.620
$HFnu_{5–10min}$ (n.u.)	0.241 ± 0.143	0.229 ± 0.161	p = 0.620

Values are Mean ± SD. ΔHR1min, absolute difference between the final heart rate at exercise completion and the heart rate recorded after 1min of recovery; ΔHR2min, absolute difference between the final heart rate at exercise completion and the heart rate recorded after 2min of recovery; ΔHR3min, absolute difference between the final heart rate at exercise completion and the heart rate recorded after 3min of recovery; HRRτ, time constant of the heart rate decay; A, amplitude of the peak-to-baseline heart rate difference; T30, short-term heart rate time constant; AUC, the area under the curve; $RMSSD_{5–10min}$, root-mean-square difference of successive normal R–R intervals; $LF_{5–10min}$, power in low frequency range; $HF_{5–10min}$, power in high frequency range; $LFnu_{5–10min}$, normalized LF power; $HFnu_{5–10min}$, normalized HF power. N = 9.

Table 15. NIRS-VOT derived parameters 17 h post-arrival at high-altitude.

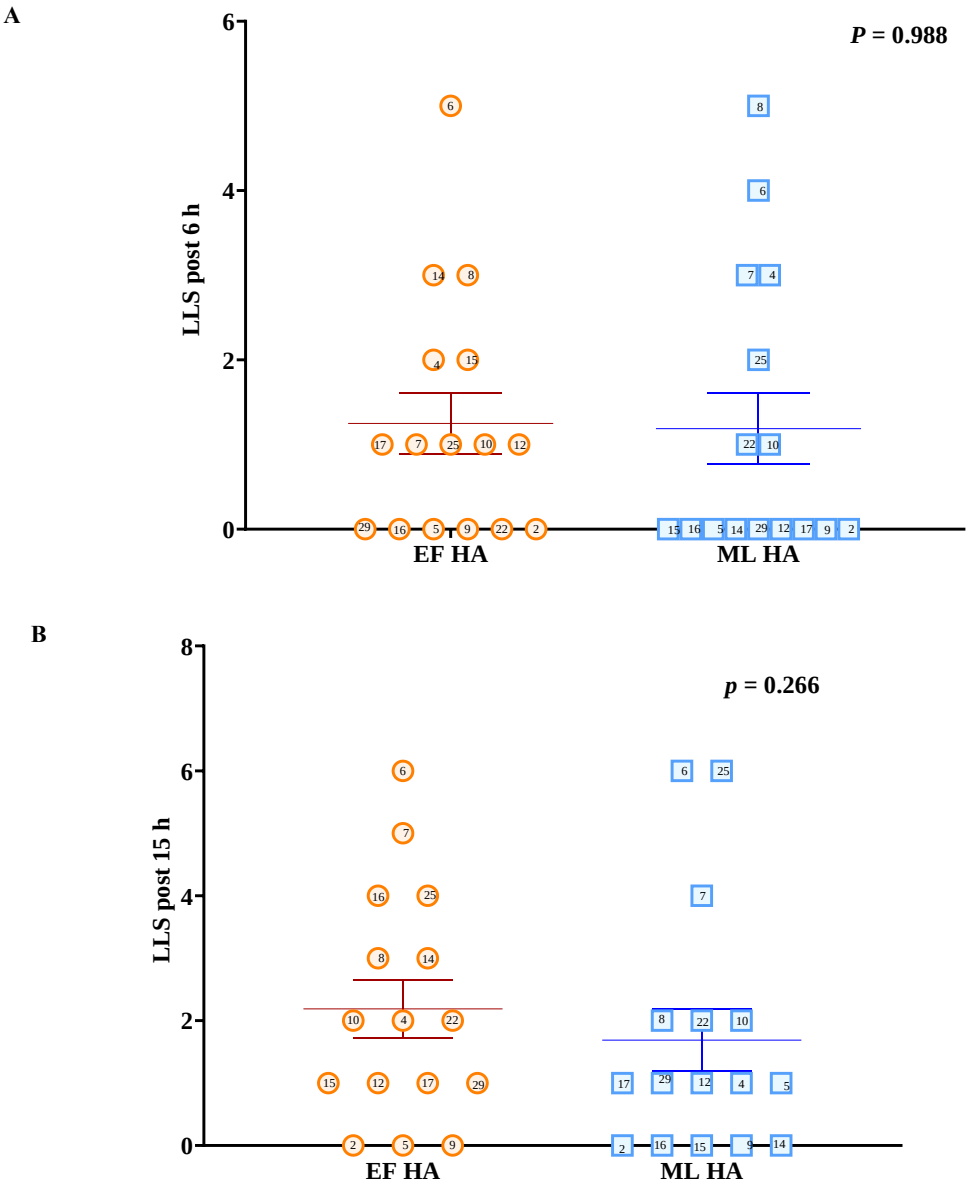
	EF	ML	p-value
Baseline TSI (%)	65.2 ± 4.4	66.2 ± 2.6	p = 0.309
Desaturation rate (%·s^{-1})	-0.083 ± 0.056	-0.076 ± 0.040	p = 0.635
Reperfusion rate (%·s^{-1})	0.589 ± 0.432	0.695 ± 0.261	p = 0.484
Norm reperfusion rate (s^{-1})	0.011 ± 0.069	0.013 ± 0.068	p = 0.853
TSI_{min} (%)	53.2 ± 6.3	54.2 ± 3.9	p = 0.394
TSI_{peak} (%)	67.4 ± 3.6	68.7 ± 2.5	**p = 0.047**
ATSI (%)	17.7 ± 4.6	15.5 ± 4.5	p = 0.345
$t_{baseline}$ (s)	16.4 ± 6.5	22.7 ± 20.3	p = 0.871

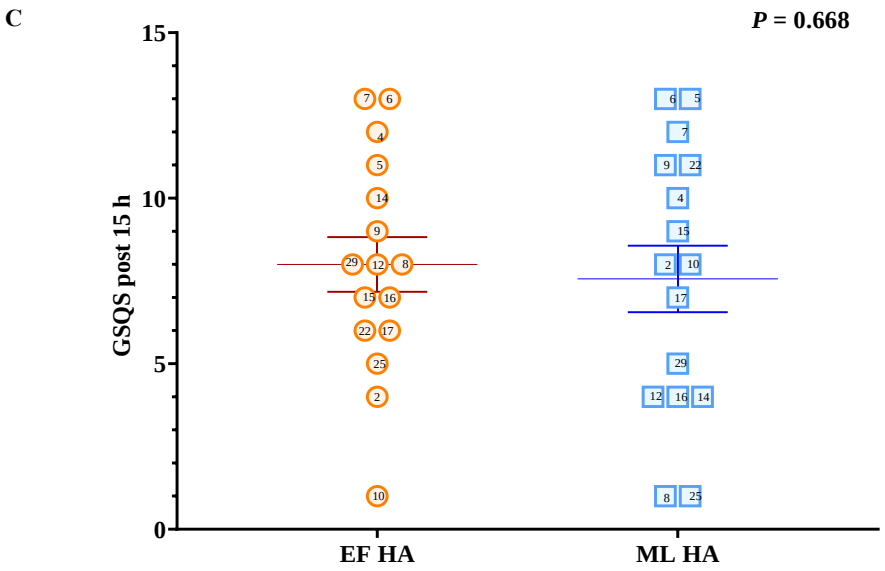
Values are Mean ± SD. p value in bold indicates statistical significance. Baseline TSI, baseline tissue saturation index; norm reperfusion rate, normalised reperfusion rate; TSI_{min}, minimum tissue saturation index value reached during the occlusion phase; TSI_{peak}, peak tissue saturation index value reached during the occlusion phase; A_{TSI}, amplitude of tissue saturation index change during the reperfusion phase; $t_{baseline}$, time to baseline. N = 14.

3.8. Acute mountain sickness and sleep quality

AMS severity and GSQS scores illustrated in **Figure 20** were similar between EF and ML. Moreover, no differences were observed between the two phases of the menstrual cycle in AMS prevalence 6 h (p = 0.564) and 15 h (p = 0.083) post-arrival at high-altitude, as indicated in **Figure 21**.

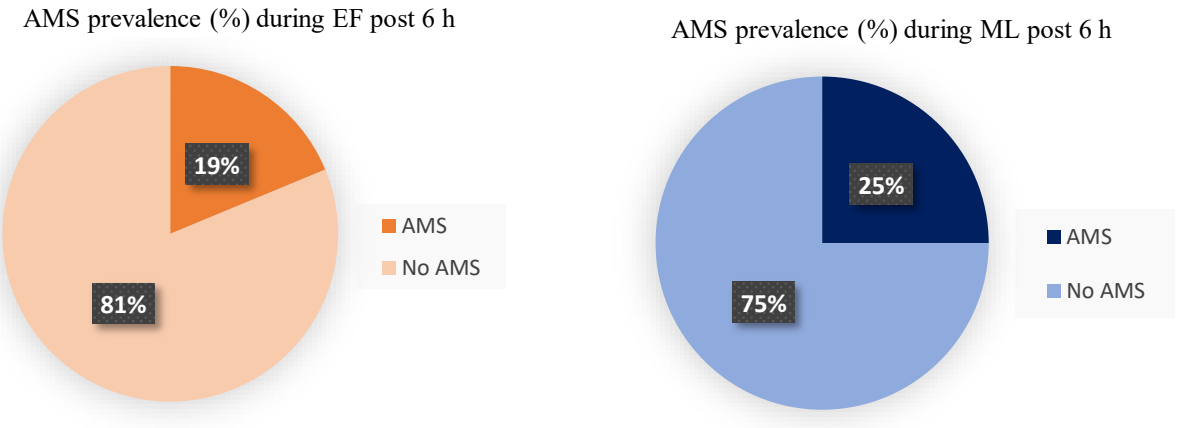
Figure 20. Individual changes in sleep quality and acute mountain sickness scores during high-altitude exposure.

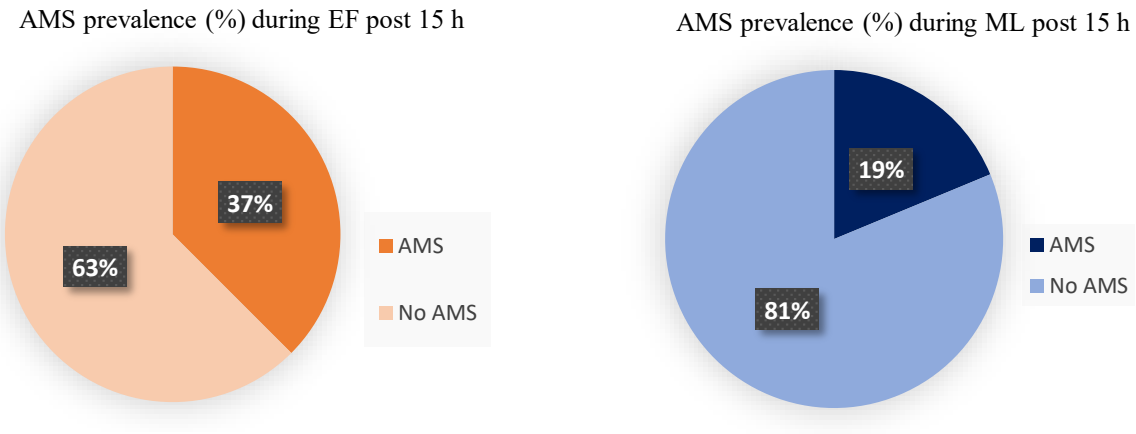




Values are Mean ± SEM. Orange dots indicate the early-follicular (EF) phase while blue squares represent the mid-luteal (ML) phase. Panel A and B show acute mountain sickness scores assessed using the Lake Louise score (LLS) respectively 6 h and 15 h post-arrival at high altitude. Panel C indicates sleep quality assessed upon awakening, 15 h post-arrival at high-altitude. HA, high-altitude. N = 16.

Figure 21. Acute mountain sickness prevalence during the early-follicular and the mid-luteal phases at different time points.





Values are expressed as n (%). AMS, Acute mountain sickness; EF, early-follicular (orange); ML, mid-luteal (blue). N = 16.

3.9. Cognitive function

Table 16 displays the results of cognitive tests performed at both low altitude and during acute high-altitude exposure. Similar outcomes were observed between EF and ML, with the exception of the number of lapses in the perceptual vigilance task, which was significantly lower during EF than ML at high-altitude.

Table 16. Cognitive test results at low and high-altitude.

	F	L	*p*-value
At low altitude :			
Digit span score	6.33 ± 1.12	6.14 ± 1.07	$p = 0.736$
TMT-A	20718 ± 3907	20274 ± 3018	$p = 0.808$
TMT-B	37812 ± 8775	41064 ± 18192	$p = 0.643$
B-A	17094 ± 9523	20790 ± 15557	$p = 0.566$
B/A	1.90 ± 0.67	1.97 ± 0.57	$p = 0.814$
PVT RT	329 ± 45	394 ± 118	$p = 0.142$
Lapses	1.11 ± 0.83	1.27 ± 0.75	$p = 0.909$
Inaccuracy	0.064 ± 0.020	0.068 ± 0.026	$p = 0.757$
	EF	ML	*p*-value
At high-altitude:			
Digit span score	6.75 ± 0.93	6.75 ± 0.93	$p = 0.999$
TMT-A	20249 ± 5408	19793 ± 3891	$p = 0.744$
TMT-B	27936 ± 5930	32251 ± 7928	$p = 0.106$

B-A	7687 ± 7180	12458 ± 7272	$p = 0.116$
B/A	1.43 ± 0.32	1.66 ± 0.44	$p = 0.127$
PVT RT	331 ± 30	345 ± 49	$p = 0.252$
Lapses	0.313 ± 0.602	1.188 ± 2.198	**$p = 0.031$**
Inaccuracy	0.050 ± 0.018	0.050 ± 0.020	$p = 0.916$

Values are Mean ± SD. p value in bold indicates statistical significance. F, follicular phase subgroup; L, luteal phase subgroup; EF, early-follicular phase; ML, mid-luteal phase; TMT-A, total time for the completion of the Trail-making test part A; TMT-B, total time for the completion of the Trail-making test part B; B-A, difference between Trail making test part B and Trail making test part A, B/A, ratio between Trail making test Part B and part A; PVT RT; perceptual vigilance task reaction time; inaccuracy, the primary dependent variable of the Time-wall task. N = 16.

4. Discussion

The aim of the present study was to investigate whether potential pathophysiological differences exist, both at rest and during exercise at high-altitude, between EF and ML phases of the menstrual cycle among eumenorrheic women. To the best of our knowledge, this is the first study showing that HRV, HRR and microvascular function are not affected by the menstrual cycle during acute high-altitude exposure, as initially hypothesized. Moreover, the respiratory parameters appear to be selectively affected by cycle phase. Particularly, spirometry test revealed higher FVC and PEF during EF than ML upon arrival at 3375 m. However, these differences were no longer significant 16 h post-arrival at Torino hut. Furthermore, a higher resting minute $\dot{V}_E$ was observed during EF compared to ML at high-altitude.

4.1. Menstrual cycle

Interestingly, ovarian hormone levels were found to fall within previously reported normal ranges for eumenorrheic women (Elliott-Sale et al., 2021; Schaumberg et al., 2017). While progesterone concentrations increased significantly during the mid-luteal phase, estradiol levels did not exhibit significant differences between the two menstrual phases investigated. Notably, due to the substantial inter- and intra-individual variability in ovarian hormone concentrations (Elliott-Sale et al., 2021), definitive cutoff values for estrogen levels corresponding to distinct menstrual phases remain undefined within sport sciences. Furthermore, progesterone thresholds used for the successful verification of the mid-luteal phase vary among studies, e.g., progesterone exceeding 6 $ng \cdot mL^{-1}$ (Schaumberg et al., 2017) or greater than 16 $nmol \cdot L^{-1}$ (Elliott-Sale et al., 2021). Moreover, no consensus has been reached

among researchers regarding the specific hormonal profiles associated with each phase of the menstrual cycle (Elliott-Sale et al., 2021). In the present investigation, participants' menstrual cycles and related symptoms were monitored over a 6-month period preceding the commencement of the tests as well as during the study. Participants were queried about the occurrence of menstrual bleeding 3 days before undergoing physiological assessments. In case of a delay, testing sessions would have been rescheduled. However, all participants underwent testing on both the 3 ± 1 day and at 75% of their menstrual cycle. Phases, i.e., F, L, EF and ML, were determined based on these data and hormone level verification through serum sample analyses.

4.2. Respiratory function at rest and during exercise

The present study observed an enhanced HVR during the luteal phase compared to the follicular phase, consistent with findings from previous research (Schoene et al., 1981; White et al., 1983). In contrast to our within-subject comparison at 3375 m, Muza and colleagues (2001) reported no significant differences in $\dot{V}_E$ at Pikes Peak (4300 m) using a between-groups design with distinct subjects for each menstrual phase. Although our study does not corroborate a higher $\dot{V}_E/\dot{V}O_2$ as reported by Takase and colleagues (2002) in a hypobaric hypoxic chamber simulating 3000 m, our investigation reveals that V_T was more elevated during EF than ML. An increase in V_E without a corresponding increase in V_E/VO_2 suggests a potential elevation in VO_2, e.g., increased metabolic demand or respiratory cost. However, in our study VO_2 values were not significantly different between EF and ML. Also, $\dot{V}_E/\dot{V}CO_2$ was consistent across EF and ML. These findings suggest that the respiratory function is minimally affected by the menstrual phase during acute high-altitude exposure, with only slight differences observed in certain parameters. Discrepancies between studies may arise from the methods used to identify cycle phases, hormone concentration variability, individual responsiveness to a given ovarian hormone level, hormone receptor expression and hormonal interactions. However, additional factors necessitate examination for their potential contributions to differences in $\dot{V}_E$ (Loeppky et al., 2001). Notably, a strong association between ROS production and the augmentation in the HVR after chronic intermittent hypoxia (CIH) has been demonstrated (Pialoux et al., 2009). Previous reports have observed a higher oxidative stress at normoxia when estrogen levels rise (Cornelli et al., 2013) or during the luteal phase (Karowicz-Bilinska et al., 2008), while others failed to find significant differences across the menstrual cycle (Ishikawa et al., 2023). To our knowledge, this study is the first to demonstrate that oxidative stress exhibits non-significant

variations across the menstrual phases at high-altitude. Furthermore, it is important to mention that multiple hormones may influence physiologic regulation of breathing (Saaresranta & Polo, 2002), e.g., thyroxine, corticotropin-releasing hormone, leptin, whereas dopamine somatostatin and neuropeptide Y have a depressing effect.

During the submaximal intensity exercise at 3375 m, no significant differences were found between EF and ML in the main ventilatory and gas exchange variables, including $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$. Possibly, exercise-induced increases in $\dot{V}_E$ might have overcome and masked any hormone-mediated potential ventilatory differences measured at rest (MacNutt et al., 2012). Interestingly, a recent study (Oliveira et al., 2024) demonstrated that hypoxia induces a greater increase in $\dot{V}_E$ during exercise compared to rest. This effect can be modulated by neural mechanisms regardless of the presence of hypoxic blood within the active skeletal muscle circulation or the release of metabolic byproducts from skeletal muscles, in the absence of humoral influences. In line with our findings, despite increased plasma progesterone in the mid-luteal phase of eight female lowlanders, $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$ were not impacted by menstrual phase during both submaximal and peak exercise performed in a hypobaric chamber simulating 4300 m (Beidleman et al., 1999). Thus, ventilatory efficiency, evaluated as $\dot{V}_E/\dot{V}CO_2$ (Petek et al., 2023), remained unaffected by the menstrual-cycle phase at high-altitude. On the contrary, Takase and colleagues found higher $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$ at peak exercise during the luteal phase, whereas no significant differences were observed during submaximal exercise in the same study.

4.3. Hemodynamic responses

The present study also investigated cardiovascular responses at rest and during exercise during EF and ML at low and high altitude. Under normoxic conditions, previous investigations have reported a higher BP at the onset of menstruation than during the late follicular phase (Choi et al., 2013; Dunne et al., 1991; Srigopika et al., 2021). Interestingly, a research on hypertensive menopausal women found a significant decrease in SBP during treatment with estradiol, while DBP remained unchanged (Jespersen et al., 1983). It seems probable that the rise in endogenous estrogen levels toward the late-follicular phase may induce a similar, if smaller, effect. Indeed, estrogen may exert vasodilatory effects (White, 2002) and modulate sympathetic nervous system tone (Wyss & Carlson, 2003). In the current study, BP did not change throughout the menstrual cycle at low altitude, consistent with findings from previous research (Thakrar, 2024), as well as at high altitude. However, it is important to be cautious

when interpreting our low altitude results due to the limitations imposed by small sample sizes. Therefore, the stable BP observed during the two phases of the menstrual cycle at high-altitude could be attributed to the counterbalancing effects of progesterone opposing estrogen's influence on vascular tone (Sarrel, 1999) and to the cycle phases examined. In fact, our study did not investigate the late-follicular phase, characterized by peak estrogen levels. Conversely, HR showed a significant rise in the luteal phase subgroup at normoxia. Similarly, Barba-Moreno and colleagues (2022) observed a higher HR during the luteal phase, both at rest and during submaximal exercise, which may be attributed to an increase in basal body temperature arising from the thermogenic effects of progesterone.

4.4. Heart rate variability

Accumulating evidence suggests changes in HRV across the menstrual cycle (Brar et al., 2015). However contrasting results are found in the literature, with some investigations reporting higher sympathetic activity (Yildirir et al., 2001) and others indicating greater parasympathetic activity (Princi et al., 2005) during the luteal phase. Regarding this, it has also been suggested that estrogen increases choline uptake and acetylcholine synthesis influencing parasympathetic tone, whereas progesterone may increase the sympathetic activity (Saeki et al., 1997). However, the effects of progesterone on the autonomic nervous system remains controversial (Salerni et al., 2015). Moreover, there is evidence supporting the cardioprotective effects of estrogen (Iorga et al., 2017). Interestingly, a recent study associated the HRV decrease during the luteal phase with progesterone peak (Schmalenberger et al., 2020). In the present study, at low altitude, a higher LF was observed in F compared L subgroups. Although, all the other parameters, including the LF/HF ratio, considered as an index sympatho-vagal balance or of sympathetic modulations (Heart Rate Variability: Standards of measurement, physiological interpretation, and clinical use, 1996) were not significantly different between F and L. While, these findings may suggest that the cycle phase has a negligible influence on HRV at low altitude, it is important to note that due to high inter-individual variations, within-subject designs are highly recommended (Laborde et al., 2017). At high-altitude, HRV, assessed using a within-subject design, remained unchanged between EF and ML. Discrepancies between studies may result from interindividual variability in HRV, methodologies used for data processing, participants' characteristics and the position adopted, i.e, supine, standing or sitting.

4.5. Heart rate recovery

HR gradually returns to baseline levels during the recovery period following exercise. The initial rapid decrease in HR post-exercise completion primarily results from parasympathetic reactivation at the sinus node, whereas the second slow HR decay phase is generally related to the sympathetic activity withdrawal (Buchheit et al., 2007). It has been demonstrated that HRR from treadmill exercise until maximal exhaustion was unaffected by menstrual phase at normoxia (Yazar & Yazici, 2016). However, HRR remains significantly under-investigated in eumenorrheic women. Regarding the influence of menstrual cycle on HRR, no interaction was found between menstrual cycle phase and recovery time for HR after high-intensity interval exercise (Benito et al., 2023). In the present study, differences in the ΔHR between the end of the exercise and specific recovery time points, i.e., 1 min, 2 min and 3 min after a submaximal exercise, have been observed between F and L subgroups at low altitude, suggesting a modulating effect of the menstrual cycle on the parasympathetic system reactivation during the recovery from a submaximal exercise. However, the HRRτ and T30 were not different between F and L. Alongside, a greater HF has been observed in F subgroup, which is indicative of increased parasympathetic activity from minute 5 to 10 into the recovery. It is important to acknowledge potential confounding factors, such as individual variability, particularly given that subjects in the two groups were different and that the sample sizes are small. At high-altitude, HRR assessed using a within-subject design, remained unchanged between EF and ML.

An investigation on sympathoadrenal responses during acute and chronic high-altitude exposure in women observed an increase in plasma catecholamine content and in norepinephrine and epinephrine urine excretion from sea level to high-altitude. However, the authors failed to find any significant differences between follicular- and luteal-phase assignments at altitude (Mazzeo et al., 1998). Similarly, a recent study revealed no differences between cycle phase in hypoxic cardiac response at exercise (Richalet et al., 2020). In the present study, menstrual cycle did not elicit any detectable change in BP, HR, HRV and HRR at high-altitude, confirming the hypotheses initially proposed.

4.6. Microvascular function

Beyond the cardiac outcomes, sex hormones are believed to modulate endothelium and vascular smooth muscle responses to stimuli by regulating vasoconstriction or vasodilation to maintain vascular tone (Orshal & Khalil, 2004). Specifically, 17β-estradiol, a type of estrogen,

has been suggested to mitigate the advancement of cardiovascular disease by exerting beneficial effects on inflammation, oxidative stress, NO bioavailability, and vascular remodelling (Green et al., 2016). However, a recent meta-analysis indicates that the menstrual cycle does not affect microvascular function at normoxia and demonstrates that the small effect of the luteal phase on the macrovascular function may be explained by progesterone's antagonistic actions on the benefits attributed to estrogen alone (Williams et al., 2020). Similarly, in the present study, aside from the higher TSI peak observed during ML compared to EF, no significant differences were found between the two menstrual phases in resting muscle oxygen consumption and microvascular reactivity indices. Indeed, even if estrogen has been shown to promote vasodilatation, progesterone exhibits vasoconstrictive properties that counteract the effects of estrogen on vascular tone (Sarrel, 1999). Moreover, emerging evidence suggests distinct regulatory mechanisms and susceptibility to hormonal influences between the macrovascular and the microvascular beds (Jekell et al., 2019; Sandoo et al., 2011) which may contribute to the absence of alterations on NIRS-VOT-derived resting muscle metabolism and relative speed of re-oxygenation. Indeed, previous studies have revealed lower endothelial NO synthase (eNOS) protein content (Laughlin et al., 2003) and reduced estrogen receptors (ER) expression (Huxley et al., 2018) in smaller vessels and in their endothelial cells compared to larger and resistance vessels. Notably, estrogen's influence on vascular function and on NO bioavailability through eNOS activation to increase NO production, primarily occurs by its binding to estrogen receptor. To our knowledge, this study is the first to compare resting muscle oxygen consumption and microvascular reactivity between two menstrual cycle phases at high-altitude.

5. Practical applications

Assessments of objective performance through anaerobic, aerobic, or strength-related tests do not consistently reveal a clear influence of the menstrual cycle phases on physical performance in normoxia (Carmichael et al., 2021; Colenso-Semple et al., 2023; McNulty et al., 2020). Most studies have reported minimal or insignificant differences in metabolic and performance variables among menstrual phases (Bemben et al., 1995; De Souza et al., 1990; D'Souza et al., 2023).

High altitude may induce AMS which can be a source of malaise and performance decrements, affecting high-altitude sojourns, activities and itineraries. It has been suggested that the cyclic fluctuations in estrogen and progesterone levels during the menstrual cycle in eumenorrheic women may have an impact on physiological responses to exercise at high-

altitude due to their stimulatory effect on $\dot{V}_E$ and potentially improve acclimatization to high-altitude during the luteal phase (Richalet et al., 2020). However, Gardner and colleagues (2024) found no differences in AMS occurrence between women in the follicular and luteal phases, classifying participants based solely on the timing of their last menstrual period and traditionally accepted cycle-phase ranges, without verifying hormone levels through blood sample analysis or controlling for each participant's individual cycle length. Moreover, Beidleman and colleagues (1999) concluded that maximal and submaximal exercise performances remain unaffected by menstrual cycle phase in a hypobaric hypoxic chamber. In line with these findings, the present study observed that fluctuations in ovarian hormones in eumenorrheic women have small or, in many cases, insignificant impacts on pathophysiological responses at rest and during exercise to terrestrial high-altitude. Consequently, there is currently very little evidence to aptly recommend a specific menstrual cycle phase for mountaineering, competition, work or any other high-altitude activities. Progressive acclimatization remains the primary advice for high-altitude travellers to avoid severe AMS. Moreover, rather than relying on general guidelines, it is advisable to adopt an individualized approach (McNulty et al., 2020; Ruiz-Alias et al., 2024), considering each woman's specific response to high-altitude across the menstrual cycle. In light of these findings, the increased representation of females in rigorous physiological research is strongly encouraged (D'Souza et al., 2023).

6. Methodological considerations

The present study was the first to investigate microvascular function, HRV, cycling efficiency, HRR and the prevalence and severity of AMS during two different phases of the menstrual cycle among eumenorrheic women at real high-altitude.

The strengths of the present study are the monitoring of the menstrual cycle over a 6-month period prior to the beginning of the study including menstrual cycle symptoms, considering variations in cycle length within and between women, verifying the menstrual cycle phase using blood analyses, testing the same subjects at terrestrial high-altitude during two different phases of the menstrual cycle and including sixteen relatively untrained women not exposed to high-altitude prior to the study such that the tests were not confounded by previous hypoxic exposure and training sessions (Pirke et al., 1990; Russell et al., 1984). Moreover, volunteers performed familiarization tests before acute exposure to high-altitude.

Nevertheless, few limitations must be acknowledged, primarily due to logistical constraints. Firstly, baseline assessments were conducted at low altitude (1224 m) rather than

sea level. Furthermore, the majority of participants were inhabitants of Aosta Valley, inhabiting altitudes ranging from 500 m to 1220 m. Some of them may have undergone an acclimatization to low altitude compared to lowlanders, potentially influencing the observed physiological responses during acute high-altitude exposure. However, the percentage of women prone to AMS in this study are in line with known prevalence at this altitude, i.e., 10-25% at 2500 m and 50-85% at ~ 5000 m (Bärtsch and Swenson, 2013), suggesting that participants were not particularly pre-acclimatized. Secondly, although the study aimed to maintain a randomized design, logistical constraints resulted in more subjects reaching high altitude first during ML phase, followed by the EF phase (10 vs. 6). Is it also important to acknowledge that fluid intake was not controlled. However, the quite constant and low values of USG suggest that the participants were not dehydrated.

7. Conclusions

Overall, the present study provides novel insights into the complex relationship between menstrual cycle phases and pathophysiological responses to acute high-altitude exposure in eumenorrheic women. Our findings showed that hormone influence on respiratory and cardiovascular responses at 3375 m are negligeable. In fact, despite a higher FVC, PEF and $\dot{V}_E$, V_T, during EF compared to ML at rest, $\dot{V}O_2$ kinetics, cycling efficiency, HRV, HRR and microvascular function remained consistent across the menstrual cycle. The hormonal variations measured between EF and ML had no significant effect on acclimatization and ultimately on AMS, exercise, sleep, cognitive function and other activities in healthy females. Further research is necessary to substantiate our findings and explore longitudinal responses to high-altitude across multiple menstrual cycles in order to investigate the intra-subject variability that was shown to be high in normoxia (Ruiz-Alias et al., 2024). Moreover, extending the duration of the exposure to high altitude beyond 18 h (e.g., 10 days) would clarify the effect of menstrual cycle on the acclimatization mechanisms. Future investigations should also examine additional factors (e.g., periodic breathing during sleep, maximal intensity exercise, nutritional supplementation as nitrate or ketones,…) that may influence physiological responses to high-altitude and performance in diverse female populations combining different environmental stressors (particularly, cold that is an important component of the high altitude conditions).

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9. Appendices

9.1 Advertisement for participant recruitment

UNIL | Université de Lausanne

WomAlt Project

Seeking female participants

Study : The effects of the menstrual cycle on physiological responses at rest and during exercise at high-altitude in eumenorrheic women.

For women between the ages of 18 and 43, able and willing to have an overnight stay and perform physiological tests at the Rifugio Torino Mont-Blanc (3375m) over the course of two distinct testing days as well as one pretest analysis at 1200m in Courmayeur

Ascent and descent by cable car Sky Way, meals and 2 overnight stays included + compensation for participating in the study + you shall also receive the results of your tests at the end of the study.

All data and personal information shall remain strictly confidential

Contacts:

guia.tagliapietra@unil.ch

Period : September / October.

Duration : 2 non-consecutive days + 1 pretest in Courmayeur