Hypoxic repeated sprint interval training improves cardiorespiratory fitness in sedentary young women

Zhaowei Kong a, On Kei Lei a, Shengyan Sun b, Lei Li c, Qingde Shi d, Haifeng Zhang e, Jinlei Nie d, *

a Faculty of Education, University of Macau, Macao, China
b Institute of Physical Education, Huzhou University, Huzhou, Zhejiang Province, China
c School of Physical Education, Ludong University, Shandong Province, China
d School of Health Sciences and Sports, Macao Polytechnic Institute, Macao, China
e College of Physical Education, Hebei Normal University, Shijiazhuang, Hebei Province, China

A R T I C L E   I N F O

Article history:
Received 8 October 2021
Received in revised form
19 January 2022
Accepted 22 January 2022
Available online 26 January 2022

Keywords:
Hypoxia
High-intensity interval training
Aerobic capacity
Metabolic health

A B S T R A C T

Objective: The purpose of this study was to investigate the effects of repeated sprint interval training (RSIT) under different hypoxic conditions in comparison with normoxic RSIT on cardiorespiratory fitness (CRF) and metabolic health in sedentary young women.

Methods: Sixty-two sedentary young women (age: 21.9 ± 2.8 years, peak oxygen uptake [\(\text{VO}_{2}\text{peak}\)] 25.9 ± 4.5 ml kg\(^{-1}\) min\(^{-1}\)) were randomized into one of the four groups, including a normoxic RSIT group (N), RSIT simulating an altitude of 2500 m (H2500), RSIT simulating an incremental altitude of 2500–3400 m (H2500–3400) and a non-exercise control group (C). The training intervention (80 × 6 s all-out cycling sprints with 9 s recovery) was performed three times/week for 4 weeks. Anthropometric measures, \(\text{VO}_{2}\text{peak}\), fasting blood glucose and lipids were assessed during the follicular phase of the menstrual cycle before and after the intervention.

Results: Compared with the control group, significant increases in \(\text{VO}_{2}\text{peak}\) were found in both hypoxic groups (H2500: +8.2%, \(p < 0.001\), \(d = 0.52\); H2500–3400: +10.9%, \(p < 0.05\), \(d = 0.99\)) but not in the N group (+3.6%, \(p > 0.05\), \(d = 0.21\)) after the intervention, whereas the two hypoxic groups had no difference in \(\text{VO}_{2}\text{peak}\). Blood glucose and lipids, and body composition remained unchanged in all groups.

Conclusion: The present study indicates that combining hypoxia with RSIT can enhance the improvement of CRF compared with normoxic RSIT alone in the sedentary young population. Yet, compared with RSIT under stable hypoxia, incremental hypoxia stress in the short-term does not additionally ameliorate CRF. © 2022 The Society of Chinese Scholars on Exercise Physiology and Fitness. Published by Elsevier (Singapore) Pte Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Low levels of physical activity are associated with a wide range of health consequences, such as all-cause mortality and cardiometabolic diseases. 1 Regular exercise is commonly recommended as an effective mean to improve health outcomes in the physically inactive population. 2 However, this recommendation is difficult to implement, as one of the frequently cited barriers is a lack of time. 3 High-intensity interval training (HIIT) protocols, therefore, are becoming increasingly popular for their advantage of time efficiency over traditional endurance training 4,5 to enhance cardiometabolic health, 6 despite the fact that the feeling of exhaustion during maximal exercise makes the Wingate-based HIIT extremely hard to adhere to. 7 Repeated sprint interval training (RSIT) is a particular form of HIIT characterized by an maximal intensity, interspersed with recovery times of less than 30 s. 8 The efficacy of RSIT in improving cardiometabolic health has been well documented. 9 Numerous studies have demonstrated that RSIT effectively improved aerobic fitness in young, healthy adults 9 and sedentary individuals, 9 decreased the risk of a complex of metabolic abnormalities such as insulin sensitivity, poor glycemic control, high blood pressure and body composition in healthy and diseased elderly, 10 overweight/obese, 9,11 and type 2 diabetes...
patients.\textsuperscript{12} However, cardiorespiratory fitness (CRF) improvement with a relatively small effect size\textsuperscript{4} may weaken the practical implications of RSIT. It would be of great interest to practitioners and researchers for providing more effective solutions to improve cardiometabolic outcomes.

Exercise under hypoxic conditions is becoming a more commonly used strategy for improving cardiometabolic outcomes. It seems that the addition of hypoxia plays an important role in the process of physiological adaptation to insufficient oxygen availability in further ameliorating the effect of physical exercise in improving physical fitness, body composition and lipid profiles.\textsuperscript{13–15} Similarly, our previous study demonstrated that training in a moderately hypoxic environment caused greater weight loss and improved blood pressure compared with normoxic training after 4 weeks of residential training.\textsuperscript{16} More recently, we found that the addition of hypoxia combined with HIIT conferred greater improvement in CRF than the normoxic protocol in sedentary overweight women.\textsuperscript{17} The key mechanisms for this improvement are associated with compensatory vasodilation,\textsuperscript{4} elevated vascular oxygen delivery,\textsuperscript{10,19} and increased glycolytic energetic metabolism and mitochondrial function.\textsuperscript{20} However, other research has yielded inconsistent results regarding the additive benefits of hypoxia on CRF and metabolic alterations. An optimal combination of time-efficient RSIT and effective dose of hypoxia is incompletely understood. For example, a similar improvement or no difference between normoxic and hypoxic groups on aerobic capacity\textsuperscript{21} and body composition\textsuperscript{17} was found following short-term RSIT in untrained population. The apparent discrepancy in the literature might be explained by varying exercise regimens and the levels of hypoxia being used in different studies.\textsuperscript{14–17}

Very few studies have examined the optimal combination of RSIT and hypoxia. An animal study revealed that a higher level of hypoxia resulted in greater positive adaptation on respiratory response.\textsuperscript{22} Consistently, our previous study demonstrated that, during a 5-week intervention of HIIT under a stable hypoxia (FiO2: 0.15), the peripheral oxygen saturation (SpO2) increased gradually, suggesting a progressive attenuation of hypoxic stress.\textsuperscript{17} However, to date there is no clear consensus on the ideal dose of hypoxic training. Physical inactivity is more prevalent among women than among men, and sedentary women are at an increased risk for non-communicable diseases and are susceptible to mental health problems.\textsuperscript{24} The complexities of the menstrual cycle are considered major barriers to the inclusion of women into clinical trials; consequently, the research in sport and exercise medicine was conducted mainly in men.\textsuperscript{25} Additionally, sex-specific differences in blood oxygen saturation\textsuperscript{26} are an important parameter for determining the differential effects of hypoxia in males and females. Thus, the purpose of the present study was to investigate the effect of short-term RSIT under different levels of moderate hypoxia (i.e. a stable protocol at FiO2 0.155, and an incremental protocol at FiO2 0.155–0.135) on CRF and metabolic health in young sedentary women. We hypothesized that the additional stress of hypoxia during RSIT would improve cardiometabolic outcomes, and a higher hypoxic level (i.e. FiO2: 0.155–0.135), used to maintain the hypoxic stress, would result in more health benefits in comparison with exercise in lower hypoxic levels (i.e. FiO2: 0.155) throughout the intervention.

2. Methods

2.1. Participants

Participants were publicly recruited through advertising in local media. The inclusion criteria were (1) neither residence at nor travel to altitudes above 1500 m during the past 6 months; (2) no hypoxic training experience and no participation in any regular exercise (less than 90 min of moderate-intensity exercise or 45 min of strenuous exercise per week); (3) general good health and absence of habitual smoking; (4) presence of regular and normal menstrual cycles between 28 and 34 days in length without severe premenstrual syndrome; and (5) absence of known cardiovascular and other associated diseases.

In total, 72 female volunteers (age: 21.9 ± 2.8 years; BMI: 23.0 ± 3.7 kg m\textsuperscript{-2}; VO\textsubscript{2}peak: 25.9 ± 4.5 mL kg\textsuperscript{-1} min\textsuperscript{-1}) were recruited. Ten participants were excluded due to consumption of medication for the common cold, irregular menstrual periods, history of exposure to altitude and excess physical activity. After the screening process, all participants provided the written informed consent and were randomized into one of the following: a normoxic RSIT group (n = 16), a RSIT group under stable hypoxia simulated at 2500 m (H2500, FiO2: 0.155; n = 15), a RSIT group under incremental hypoxia simulated at 2500–3400 m (H2500–3400, FiO2: 0.155–0.135; n = 15) and a non-exercising control group (C; n = 16). To reduce the influence of hormonal fluctuation on physical performance and blood markers, the pre- and post-tests were completed during each participant’s individual follicular phase of the menstrual cycle. Participants who reported having a regular menstrual cycle were asked to recall their last two periods to calculate the length of their menstrual period and cycle. If the calculated menses date matched the actual menses onset, it was identified as a regular menstrual cycle. Pre- and post-test measurements were performed in the early follicular stage (3 days after the last day of menses).\textsuperscript{27}

The study was approved by the Ethical Committee of the University of Macau for the Use of Human and Animal Subjects in Research and performed according to the Declaration of Helsinki. Before recruitment, a prior power analysis was performed by G*Power Version 3.1 for a repeated-measures ANOVA with four groups and two measures to determine the sample size. When the effect size was set at medium (partial \( \eta^2 = 0.05 \) or \( f = 0.23 \)), and the correlation among the repeated measures and non-sphericity correction were kept at 0.5 and 1, respectively, a sample size of 14 participants in each group was needed to detect a significant difference in VO\textsubscript{2}peak with a statistical power of 80% at an alpha level of 0.5. Similarly, a total sample size of 36 would be adequate to distinguish differences in training parameters in the three exercise intervention groups with four repeated measures a two-way repeated-measures ANOVA when \( \alpha = 0.05 \) and \( 1−\beta = 0.80 \).

2.2. Experimental procedure

This was a randomized controlled trial with pre-test and post-test design, which included pre-intervention measurements, a 4-week training intervention and post-intervention measurements. The pre- and post-intervention measurements consisted of blood sampling, anthropometric assessments and a maximal incremental exercise test. All pre- and post-intervention measurements were taken within 3–5 days before and after the intervention, respectively.

2.3. Training protocol

Participants in the N, H\textsubscript{2500–3400} and H\textsubscript{2500} groups were subjected to a supervised training intervention at the laboratory with controlled room temperature (22 °C) and humidity (50%–60%). Given that altering hypoxia and normoxia on alternate days may reduce the short-term adaptation to hypoxia and thus cause additional benefits in terms of body composition,\textsuperscript{20} the training groups were planned 3 days per week (24 h apart) for 4 weeks within one
menstrual cycle. For each training session, two research assistants supervised the training process and recorded the training data. Participants in the training groups were blinded to their allocation, and the normoxic or hypoxic condition they were assigned to. Upon arriving at the laboratory, participants were fitted with a facemask connected to a modified gas mixing system (Everest Summit II Hypoxic Generator, New York, NY, USA). The normoxic or hypoxic gas mixtures were generated by this system and were delivered to participants through tubes and a breathing mask. In the N group, participants exercised under normoxic conditions at sea level (FiO2: 0.209). In the H2500 group, participants exercised under stable hypoxic condition (FiO2: 0.155, stimulating an altitude of approximately 2500 m). In the H2500–3400 group, participants exercised under an incremental hypoxic condition, the FiO2 was set at 0.155 initially (stimulating an altitude of approximately 2500 m) and was decreased by 0.005 every week until the FiO2 dropped to 0.135 (corresponding to an altitude of 3400 m). The reasons for this gradually incremental hypoxic stress are that, FiO2 0.155–0.135 is widely adopted in experimental and clinical applications, whereas the more severe hypoxic level above might have a deteriorating effect on vascular and metabolic function.20,21 After resting 10 min under the corresponding condition, participants warmed up for 5 min and performed RSIT on a cycle ergometer (Monark 894 E, Sweden), which included 80 repetitions of 6 s maximal cycling exercise interspersed with 9 s recovery, followed by a 5-min cool-down at a free pedalling rate. The initial resistance was 1 kg in all training groups. After successfully completing two consecutive training sessions (i.e. the pedalling cadence of all sprint bouts was maintained above 100 revolutions per minute [rpm]), the resistance was increased by 0.5 kg until it reached a workload equivalent to 5% of each participant's individual body mass. Heart rate (HR) and SpO2 before and after each sprint bout were measured using a pulse oximeter (Polar F4M BLK, Kemele, Finland). Participants could take off the mask after the completion of the cool-down phase.

2.4 Blood profiles

Blood samples were drawn during the early follicular phase of the menstrual cycle. Participants were instructed to refrain from strenuous exercise and caffeine for 48 h before blood sampling and arrived at the laboratory in the morning (7:30 a.m.) after 8 h of fasting. After the subject rested in a supine position for 30 min, 8-ml blood samples were taken from the antecubital vein. The blood samples were left to clot for 1 h and then centrifuged at 3000 rpm for 15 min. After centrifugation, the serum supernatants were aliquoted into 0.5-mL test tubes and stored at −80 °C for later analysis. Fasting blood glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein (LDL-C) were determined using human metabolic hormone kit on Luminex equipment (Luminex, HMHEMAG-34, KingMed Center for Clinical Laboratory Co. Ltd, Guangzhou, China).

2.5 Anthropometric assessments

After blood sampling, anthropometric measures including height (cm), body mass (kg) and body mass index (BMI, kg m⁻²) were accessed. Standing height was measured using a wall-mounted stadiometer (with the participant barefoot) and recorded to the nearest 0.1 cm. Body mass was assessed using a bioelectrical impedance analyser (Tanita MC-180 MA, Tanita Corporation, Tokyo, Japan) in light clothing and recorded to the nearest 0.1 kg. BMI (in kg m⁻²) was calculated by dividing weight (kg) by height squared (m²).

2.6 Maximal incremental exercise test

Before and after the intervention, participants performed a maximal incremental exercise test on an electric-controlled cycle ergometer (Monark 839 E, Vansbro, Sweden) with gas analyser (Meta-Max 3B, Cortex Biophysik Gmbh, Leipzig, Germany), where oxygen consumption (VO₂), carbon dioxide production (VCO₂), and expired minute volume (VE) were recorded continuously. After a 3-min warmup at 50 W, participants started cycling at an initial power output of 25 W, which increased by 15 W every 3 min; the pedalling rate was maintained at 60 ± 5 rpm. The test would be stopped if the participant reached the stage of exhaustion, assessed as meeting two or more of the following criteria: Borg 6–20 rating of perceived exertion (RPE) reached 18; HR reached an exhaustion on age-predicted maximal HR (220 – age); unable to maintain the pedalling rate of 60 rpm; the respiratory exchange ratio (RER) was larger than 1.0 or reached a plateau where the change in VO₂ was less than 150 mL min⁻¹. VO₂peak was determined as the average of 15 s of the highest oxygen uptake values over the final stage,14 and HRpeak was defined as the highest value attained during the test. Peak oxygen pulse (O₂Ppeak ml·beat⁻¹) was calculated as the VO₂peak divided by the HRpeak.

2.7 Physical activity and dietary assessments

Participants were required to maintain their regular physical activity and dietary behaviour strictly throughout the training intervention. Daily physical activity was tracked for 6 weeks (i.e. one week before and after the intervention, and 4 weeks during the intervention) using a previously validated pedometer (Yamaz Digi-Walker SW-200, Japan). To record their daily food and beverage intake, 3-day diet logs (two weekdays and one weekend day) were kept by all participants for 6 weeks using the Chinese Nutrition Action Plan (CNAP) surveys.17,32 The nutrition analysis and management system (version 3.1) of the National Research Institute of Sports Medicine (NRISM) was used to measure macronutrient ratios and total energy intake.

2.8 Statistical analysis

Data analysis was conducted using the PASW software (Release 22.0; IBM, New York, USA). Before the main statistical analyses, Kolmogorov–Smirnov tests were conducted to confirm a normal distribution of all outcome variables. Levene’s test was used to assess homogeneity of variance. A two-way ANOVA (time × condition) with repeated measures was used to examine the main effects (time) and interaction effects (time × group) on the outcome variables. Significant interaction effects were further analysed using the Tukey post hoc test. One-way ANOVA test was computed to detect the changes of training workload, duration, mean power output and percentage of maximal HR during the 4 intervention weeks. Regarding effect size (ES) measures of the main and interaction effects, partial η² was considered small if η² < 0.01 and large if η² > 0.14. Cohen’s d values were also used to access the effect sizes for the difference between variables, which was considered small when d was between 0.20 and 0.49, medium when d was around 0.50–0.79 and large when d > 0.80.33 All data were presented as mean ± standard deviation, and p < 0.05 was considered as significant statistical difference.

3. Results

3.1 Training data

During the intervention, the training workload was gradually
increased from week 1 to week 4 in all training groups. Specifically, the workload was increased from 1.1 ± 0.1 kg to 2.3 ± 0.8 kg in the N group (p < 0.001), from 1.1 ± 0.1 kg to 2.5 ± 0.8 kg in the H2500 group (p < 0.001), and from 1.0 ± 0.1 kg to 1.9 ± 0.7 kg in the H2500–3400 group (p < 0.001, Table 1). Accordingly, mean power output was increased by 0.7 W kg⁻¹, 0.9 W kg⁻¹, and 0.6 W kg⁻¹ in the N, H2500 and H2500–3400 Group, respectively (p < 0.001, Table 1). Participants were maintained at approximately 90% of the maximal HR (HRmax measured as the maximal values from HRpeak recorded at the maximal incremental exercise test before intervention or during training sessions) during the intervention without group differences (p > 0.05), but the %HRmax was the highest in week 1 compared with the following weeks in all groups. Similarly, training impulse (TRIMP) as the indicator of relative training intensity, was reduced in week 3 and week 4 than that of week 1 or week 2 in all training groups (p < 0.001, Table 1). No adverse events were reported during training in either group.

The SpO2 of the normoxia and hypoxia groups, the main effects of the time (p < 0.001, partial η² = 0.402), group (p < 0.001, partial η² = 0.402) and interaction (p < 0.001, partial η² = 0.344) effects were significant. During 4 weeks of training, SpO2 remained constant in the normoxia group, while a significant reduction in SpO2 occurred in the hypoxic groups (p < 0.001, Fig. 1). The reduction of the SpO2 in the H2500–3400 group was highest among all groups (N: ~0.4%; H2500: ~2.5%; H2500–3400: ~4.5%), but a trivial effect (d = 0.046) was observed when comparing it to the H2500 group.

3.2. Habitual physical activity and dietary profiles

There were no significant differences in daily physical activity among the four weeks (Table 2). The three macronutrient compositions in their diet were similar among the four groups, with 51% carbohydrate, 16% protein and 33% fat. Similarly, no significant differences were found in habitual caloric intake among the four groups or between any time points (Table 2).

3.3. Body composition and blood profiles

Participants’ demographic characteristics and physiological outcomes before and after the intervention are provided in Table 3. After the 4-week intervention, there were no changes in body mass and BMI in any group. Moreover, all three training modes failed to trigger any changes in fasting glucose and blood lipids (i.e. TG, TC, HDL-C and LDL-C) (p > 0.05, Table 3).

Table 1

| Training parameters during the intervention. | N | H2500 | H2500–3400 | Time effect | Group effect | Time × group effect |
|---------------------------------------------|---|-------|------------|-------------|--------------|---------------------|
| **Workload (kg)**                            |   |       |            | p partial η²| p partial η² | p partial η²         |
| wk1                                         | 1.1 ±0.1  | 1.1 ±0.1 | 1.0 ±0.1   | <0.001      | 0.707        | 0.223               |
| wk2                                         | 1.6 ±0.3  | 1.5 ±0.3  | 1.5 ±0.3   | <0.001      | 0.367        | 0.866               |
| wk3                                         | 2.0 ±0.6  | 2.0 ±0.6  | 1.8 ±0.6   | <0.001      | 0.007        | 0.760               |
| wk4                                         | 2.3 ±0.8  | 2.5 ±0.8  | 1.9 ±0.8   | <0.001      | 0.017        | 0.077               |
| **Duration (min)**                           |   |       |            |             |              |                     |
| wk1                                         | 16.7 ±4.2 | 16.2 ±4.7 | 14.9 ±4.2  | 0.002       | 0.133        | 0.479               |
| wk2                                         | 17.3 ±3.9 | 17.7 ±2.9 | 15.1 ±3.9  | 0.002       | 0.100        | 0.043               |
| wk3                                         | 14.8 ±4.2 | 16.8 ±4.3 | 12.1 ±5.0  | 0.002       | 0.007        | 0.003               |
| wk4                                         | 12.9 ±5.0 | 14.8 ±5.0 | 12.9 ±4.9  | 0.002       | 0.017        | 0.017               |
| **MPO (W·kg⁻¹)**                             |   |       |            |             |              |                     |
| wk1                                         | 1.9 ±0.2  | 1.9 ±0.2  | 2.0 ±0.4   | <0.001      | 0.367        | 0.866               |
| wk2                                         | 2.3 ±0.4  | 2.4 ±0.4  | 2.2 ±0.6   | <0.001      | 0.007        | 0.003               |
| wk3                                         | 2.5 ±0.7  | 2.6 ±0.7  | 2.6 ±0.9   | <0.001      | 0.202        | 0.949               |
| wk4                                         | 2.6 ±1.0  | 2.8 ±1.0  | 2.6 ±1.0   | <0.001      | 0.017        | 0.077               |
| **% HRmax**                                  |   |       |            |             |              |                     |
| wk1                                         | 91.2 ±3.6 | 90.7 ±5.6 | 93.2 ±4.1  | <0.001      | 0.232        | 0.876               |
| wk2                                         | 89.0 ±3.7 | 90.4 ±3.9 | 89.4 ±4.4  | <0.001      | 0.029        | 0.295               |
| wk3                                         | 89.3 ±4.5 | 88.5 ±5.5 | 89.4 ±3.9  | <0.001      | 0.007        | 0.062               |
| wk4                                         | 89.4 ±3.9 | 88.1 ±4.9 | 87.7 ±7.5  | <0.001      | 0.007        | 0.007               |
| **TRIMP (au)**                               |   |       |            |             |              |                     |
| wk1                                         | 57.3 ±16.6| 53.6 ±16.7| 59.5 ±18.7 | <0.001      | 0.232        | 0.876               |
| wk2                                         | 54.0 ±9.6 | 56.2 ±9.8 | 50.0 ±16.1 | <0.001      | 0.029        | 0.295               |
| wk3                                         | 49.3 ±12.1| 48.9 ±13.5| 40.9 ±16.5 | <0.001      | 0.007        | 0.007               |
| wk4                                         | 40.8 ±12.3| 43.7 ±11.7| 44.4 ±18.3 | <0.001      | 0.029        | 0.295               |

Values are expressed as means ± standard deviation. MPO: mean power output relative to body weight, %HRmax: percentage of maximal heart rate, TRIMP (au): training impulse (arbitrary unit); N: repeated sprint interval training in normoxia, H2500: repeated sprint interval training in normobaric hypoxia simulated at 2500 m, H2500–3400: repeated sprint training in normobaric hypoxia simulated at 2500–3400 m. Significantly different from the corresponding wk1, wk2 and wk3 values in the same group (p < 0.05).
Table 2

| Energy intake (kcal/day⁻¹) | C | N | H2500 | H2500-3400 | Time effect | Group effect | Time × group effect |
|---------------------------|---|---|-------|------------|-------------|--------------|-------------------|
| Pre | 2118 ± 499 | 1837 ± 630 | 1928 ± 575 | 1661 ± 292 | 0.439 | 0.025 | 0.178 | 0.120 | 0.580 | 0.065 |
| wk1 | 2164 ± 423 | 1497 ± 404 | 1719 ± 599 | 1764 ± 460 | | | | | | |
| wk2 | 2055 ± 516 | 1684 ± 403 | 1964 ± 809 | 1817 ± 517 | | | | | | |
| wk3 | 1877 ± 347 | 1347 ± 80 | 1810 ± 580 | 1698 ± 355 | | | | | | |
| wk4 | 2029 ± 771 | 1687 ± 209 | 1918 ± 750 | 1776 ± 490 | | | | | | |
| Post | 2063 ± 518 | 1479 ± 599 | 1602 ± 631 | 1760 ± 559 | | | | | | |

Values are expressed as means ± standard deviation. C: control group; N: normoxic repeated sprint interval training, H2500: hypoxic repeated sprint interval training at 2500 m, H2500-3400: hypoxic repeated sprint interval training at 2500–3000 m.

Table 3

| Changes in blood outcome measures before and after the intervention. | C | N | H2500 | H2500-3400 | Group effect |
|--------------------------|---|---|-------|------------|--------------|
| Age (y) | 22.2 ± 3.8 | 20.9 ± 2.6 | 21.9 ± 2.0 | 21.9 ± 2.2 | | |
| Height (cm) | 162.6 ± 5.5 | 161.7 ± 4.6 | 162.4 ± 5.9 | 160.9 ± 4.8 | | |
| Weight (kg) | 61.0 ± 7.1 | 60.8 ± 6.7 | 59.1 ± 8.3 | 59.4 ± 5.0 | | |
| BMI (kg/m²⁻¹) | 23.0 ± 2.3 | 23.0 ± 2.1 | 22.6 ± 2.1 | 22.7 ± 2.0 | | |
| Glucose (mmol/L⁻¹) | 4.6 ± 0.4 | 4.5 ± 0.4 | 4.6 ± 0.4 | 4.6 ± 0.4 | | |
| TC (mmol/L⁻¹) | 4.9 ± 1.0 | 5.0 ± 1.0 | 4.9 ± 1.0 | 4.9 ± 1.0 | | |
| LDL-C (mmol/L⁻¹) | 2.9 ± 0.9 | 3.0 ± 0.9 | 2.9 ± 0.9 | 2.9 ± 0.9 | | |
| HDL-C (mmol/L⁻¹) | 1.8 ± 0.3 | 1.8 ± 0.3 | 1.7 ± 0.4 | 1.6 ± 0.5 | | |
| Triglyceride (mmol/L⁻¹) | 0.9 ± 0.3 | 0.8 ± 0.4 | 1.0 ± 0.4 | 1.0 ± 0.3 | | |

Observed values are expressed as means ± standard deviation. C: control group; N: normoxic repeated sprint interval training, H2500: hypoxic repeated sprint interval training at 2500 m, H2500-3400: hypoxic repeated sprint interval training at 2500–3000 m.

3.4. CRF

CRF as reflected by VO₂peak was significantly increased (p < 0.01, Table 4) in the two hypoxic groups following 4 weeks of training. Although not statistically significant, there was a 3.6% increase in VO₂peak in the N group (d = 0.21) compared with the C group. After the intervention, the H2500 group showed an 8.2% increase in VO₂peak (from 1.6 ± 0.2 to 1.7 ± 0.3 L·min⁻¹, d = 0.52), while the increment in the H2500-3400 group was 10.9% (from 1.5 ± 0.2 to 1.7 ± 0.2 L·min⁻¹, d = 0.99). The two hypoxic training groups had higher VO₂peak than the N group (p < 0.05). Group differences were found in VO₂peak, RERpeak, HRpeak and O₂Ppeak were (p < 0.001) but not in RPEpeak, VEpeak, V̇Epeak and V̇Epeak/VO₂peak during the maximal incremental exercise test. Participants in the C group had no change in VO₂peak and related parameters (p > 0.05).

4. Discussion

The main findings of this study were that both of the two hypoxia protocols (H2500 and H2500-3400 group) were similarly effective at improving CRF compared with the no training controls in sedentary young women, while the normoxia protocol resulted in no significant increase in VO₂peak after 12 sessions of RSIT in 4 weeks. In terms of metabolic outcomes, no significant alterations were observed in body composition and blood lipids in all groups.

4.1. Effect on CRF

In the present study, VO₂peak and O₂Ppeak did not change following 4 weeks of normoxic RSIT when compared with the no training controls. In our previous study, we observed significant CRF improvement using normoxic RSIT with a frequency of four times/week for 5 weeks. With a reduced training frequency and shorter training duration (12 sessions for 4 weeks), the normoxic RSIT failed to show significant improvement in CRF, suggesting that the training dose of normoxic RSIT in the present study is insufficient to induce CRF improvement. In contrast, VO₂peak was increased by 8.2% in the H2500 group (FiO₂: 0.155) and 10.9% in the H2500-3400 group (FiO₂: 0.155–0.135), despite having a similar ~13% increase in O₂Ppeak. These findings provide further evidence for the additional beneficial effects of hypoxia on CRF, which could even compensate for the reduced training dose. The attainment of aerobic capacity under hypoxia is influenced by the mechanism of compensatory vasodilation that occurred during increased pulmonary ventilation. Based on the absence of changes in CRF after normoxic RSIT and a significant improvement in CRF resulting from adding moderately hypoxic stimulus, it is suggested that CRF improvement was initiated earlier under hypoxic conditions than under normoxia. These findings provide further evidence to support the notion that combining hypoxia with short-term RSIT exhibited additional beneficial effects on CRF in the sedentary population.
The pattern of duration of exposure and levels of hypoxia differentiate mechanisms of respiratory and metabolic response. Moderate hypoxia (>10% inspired O₂) with or without exercise appears to present a safe method to elicit beneficial effects with minimal detrimental effect. However, mechanisms that maintain balance between efficacy and side effects are still unclear. To our surprise, we did not observe a significant difference between the two hypoxic groups. Both hypoxic groups had similar SpO₂, demonstrating that the hypoxic stress was comparable. Nevertheless, during the last training week, the mechanical workload during H₂500–3400 stimulus was lower compared with that during H₂500 stimulus. This was possibly because of the reduced PO₂ in the hypoxic condition that made it more difficult for the H₂500–3400 group to complete the whole training session with the same intensity compared with the H₂500 group; thus, they failed to progressively increase the workload. Although there was no significant difference, the large effect size (ES = 0.99) suggested that the cardiorespiratory strain in the H₂500–3400 group was greater so that the mechanical workload was compromised. It is possible that the mediation between the mechanical workload and cardiorespiratory strain affected the final outcomes. The insignificant cardiorespiratory outcome might be accounted for in part by difference between distinct levels of triggering stimulus in the two hypoxic groups. Therefore, it is emphasized that the hypoxic level plays a crucial role in physiological processes. Perhaps the slight difference indicates that a higher dose (i.e. either a longer duration or more stimulation from hypoxia) is necessary to impose a higher cardiorespiratory load, which could be responsible for the greater beneficial effects of a higher vs. a lower level of hypoxia.

4.2. Effect on cardiometabolic risk factors (body mass and blood lipids)

In the current study, we found that hypoxic RSIT did not change BMI, body weight, triglycerides, TC, LDL-C, HDL-C and glucose levels in all groups. The potential reason for this observation regarding metabolic-related factors may be related to the characteristics of the study subjects. In view of short-term RSIT, body weight, BMI, body fat percentage and waist circumference was less pronounced in normal weight, non-obese and healthy individuals than in overweight individuals. A population-based study reported that sedentary, normal weight subjects may have a lower metabolic impact on lipid profiles compared with overweight people. Some evidence revealed that fat mass was decreased after 4 weeks RSIT under hypoxia compared with normoxic RSIT in obese women, although others reported no difference.

Training stimulus is another potent inducer of metabolic change. It seemed that the hypoxic dose of the present training protocol was insufficient to evoke metabolic alterations such as body composition and blood lipid changes in the short term. More experimental hypoxic exercise protocols are required to provide additional evidence about the effectiveness of hypoxic variation as an extra stimulus. To illuminate the potential strategy for improving body composition, blood glucose and lipid profiles, it is worthy of future research at a range of FiO₂ 0.12–0.15, which has been commonly used without negative physiological and psychological effects.

Intermittent hypoxia alone or combined with exercise has been proposed as a valuable and viable strategy to improve health outcomes in different populations. The present study demonstrated that RSIT, regardless of whether the training was performed in normoxia or hypoxia, had an exceptional completion rate with no adverse events, suggesting that this protocol is a well-tolerated exercise option for sedentary young women. Our results were further supported by the previous findings that exercise...
incorporating short-term intermittent hypoxia ameliorated exercise tolerance by improving stress resistance and oxygen delivery in obese individuals, and elderly men with and without coronary artery disease. Nevertheless, hypoxic RSIT are warranted to further confirm the application in clinical cohorts.

4.3. Strengths and limitations

There are several strengths of our study. First and foremost, the experiment was performed at the same point in the menstrual cycle in all participants to exclude the potential impact of the menstrual cycle on physical performance and blood markers. Moreover, hormonal differences are potentially involved in physiological responses to hypoxic exercise; therefore the menstrual cycle and gender difference should be taken into consideration in future studies. Another strength was that habitual physical activity and diet were controlled throughout the study period. Moreover, our study was strengthened by the randomized control trial design, which was commonly neglected in previous research.

There are a few limitations that should be considered. The 20 min, relatively high-volume RSIT regimen used in this study might be too long and intense to sustain, resulting in diminished working outputs for the subjects. Future studies could adopt modified low-volume RSIT protocols covered in several minutes (e.g. ≤ 10 s all out exercise) to achieve more favourable health outcomes. In addition, the hypoxic response varied from person to person, related to the hypoxic ventilatory response. To offer less SpO2 variation among individuals, future research may consider using fixed SpO2 instead of fixed FiO2. For instance, FiO2 can be individually titrated based on the oxygenation response to maintain a target SpO2. We did not measure haematological parameters (i.e. haemoglobin and haematocrit) in the present study. These biochemical biomarkers need to be examined to facilitate understanding of cardiorespiratory adaptation following different exercise modalities. Finally, considering that studies of hypoxic RSIT longer than 12 weeks significantly improved body composition and metabolic risk factors, further studies could extend the intervention duration, and involve varied hypoxic stimulus to avoid weakening hypoxic effects.

5. Conclusion

The present study indicates that combining hypoxia with RSIT can enhance the improvement of CRF compared with normoxic RSIT alone in a sedentary population. Nevertheless, compared with RSIT under stable hypoxia (FiO2 0.155), gradually incremental hypoxia stress (FiO2 0.155–0.135) in the short term does not have an additive effect on facilitating CRF.

Authors statement

Study conception and design: ZK, JN.
Acquisition of data: ZK, OKL, LL.
Analysis and interpretation of the data: ZK, OKL, SS, LL, QS, HZ, JN.
Drafting the paper: ZK, OKL, SS, JN.
Critical revision: ZK, OKL, SS, LL, QS, HZ, JN.
All authors read and approved the final version of the manuscript.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

The study was supported by a research grant from University of Macau (MYRG2015-00223-FED). The views expressed are those of the authors and not necessarily those of the UM.

References

1. Young DR, Hivert MF, Alhassan S, et al. Sedentary behavior and cardiovascular morbidity and mortality: a science advisory from the American heart association. Circulation. 2016;134(13). https://doi.org/10.1161/ CIR.0000000000000440
2. Kujala UM, Jokelainen J, Oksa H, et al. Increase in physical activity and cardiovascular risk profile change during lifestyle intervention in primary healthcare: 1-year follow-up study among individuals at high risk for type 2 diabetes. BMJ Open. 2011;1(2). https://doi.org/10.1136/bmjopen-2011-000292.
3. Trost S, Owen N, Bauman A, Salis J, Brown W. Correlates of adults’ participation in physical activity: review and update. Med Sci Sports Exerc. 2003;34: 1996–2001. https://doi.org/10.1249/01.MSS.0000088387.76900.32
4. Gist NH, Fedewa MV, Dishman RK, Cureton KJ. Sprint interval training effects on aerobic capacity: a systematic review and meta-analysis. Sports Med. 2014;44(2):259–279. https://doi.org/10.1007/s40279-013-0115-0
5. Weston KS, Wislielf U, Coombes JS. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. Br J Sports Med. 2014;48(16):1227–1234. https://doi.org/10.1136/bjsports-2013-092576
6. Oja P, Kelly P, Pedisic Z, et al. Associations of specific types of sports and exercise with all-cause and cardiovascular-disease mortality: a cohort study of 80 306 British adults. Br J Sports Med. 2017;51(10):812–817. https://doi.org/10.1136/bjsports-2016-096822.
7. Biddle SJH, Batterham AM. High-intensity interval exercise training for public health: a big HIT or shall we HIT it on the head? Int J Behav Nutr Phys Act. 2015;12:1.95. https://doi.org/10.1186/s12966-015-0254-9
8. Wen D, Utesch T, Wu J, et al. Effects of different protocols of high intensity interval training for VO2max improvements in adults: a meta-analysis of randomised controlled trials. J Sci Med Sport. 2019;22(8):941–947. https://doi.org/10.1016/j.jsams.2019.01.013
9. Batacan RB, Duncan MJ, Dalbo VJ, Tucker PS, Fenning AS. Effects of high-intensity interval training on cardiometabolic health: a systematic review and meta-analysis of intervention studies. Br J Sports Med. 2017;51(6):494–503. https://doi.org/10.1136/bjsports-2015-095841
10. Burtscher M, Pachinger O, Ehrenbourg I, et al. Intermittent hypoxia increases exercise tolerance in elderly men with and without coronary artery disease. Int J Cardiol. 2004;96(2):247–254. https://doi.org/10.1016/j.ijcard.2003.07.021
11. Mai K, Klug L, Rakova N, et al. Hypoxia and exercise interactions on skeletal muscle and visceral sensitivity in obese subjects with metabolic syndrome: results of a randomized controlled trial. Int J Obes. 2020;44(5):1119–1128. https://doi.org/10.1038/s41366-019-0504-z
12. De Nardi AT, Tolves T, Lenzi TL, Lu Signori, Silva AMV da. High-intensity interval training versus continuous training on physiological and anthropometric variables in preobese and type 2 diabetes: a meta-analysis. Diabetes Res Clin Pract. 2018;137:149–159. https://doi.org/10.1016/j.diabres.2017.12.017
13. Robbins L, Hunter S, Gaza N, Girard O. Normobaric hypoxic conditioning to maximize weight loss and ameliorate cardio-metabolic health in obese populations: a systematic review. Am J Physiol Regul Integr Comp Physiol. 2017;313(3):R251–R264. https://doi.org/10.1152/ajpregu.00160.2017
14. Haude S, Wiesner S, Engeli S, Luft FC, Jordan J. Influences of normobaric hypoxia training on metabolic risk markers in human subjects. Med Sci Sports Exerc. 2008;40(11):1939–1944. https://doi.org/10.1249/MSS.0b013e31817f1988
15. Shin S, Matsuoka T, So WY. Influences of Short-term Normobaric Hypoxic Training on Metabolic Syndrome-related Markers in Overweight and Normal-Weight Men. Normobaric Hypoxic Training on Metabolic Syndrome. 14. 2018:5.
16. Kong Z, Zang Y, Hu Y. Normobaric hypoxic training causes more weight loss than normoxia training after a 4-week residential camp for obese young adults. Sleep Breath. 2014;18(3):591–597. https://doi.org/10.1007/s11325-013-0922-4.
17. Kong Z, Shi Q, Nie J, et al. High-intensity interval training in normobaric hypoxia improves cardiopulmonary fitness in overweight Chinese young women. Front Physiol. 2017;8. https://doi.org/10.3389/fphys.2017.00175
18. Casey DP, Joyner MJ. Compensatory vasodilatation during hypoxic exercise: mechanisms responsible for matching oxygen supply to demand: hypoxic dependent vasodilatation during exercise. J Physiol. 2012;590(24):6321–6326. https://doi.org/10.1113/jphysiol.2012.242396
19. Zbrowska A, Jastrzębski D, Sadowska-Krepa E, Sikora M, Di Giulio C. Comparison of the effectiveness of high-intensity interval training in hypoxia and normoxia in healthy male volunteers: a pilot study. BioMed Res Int. 2019;2019:1–10. https://doi.org/10.1155/2019/7315714.
20. Quintero P, Milagro FL, Campián J, Martínez JA. Impact of oxygen availability on body weight management. Med Hypotheses. 2010;74(5):901–907. https://doi.org/10.1016/j.mehy.2009.10.022.
capacity during sprint interval training. J Sports Med Phys Fit. 2015;55(10): 1099–1106.

22. Wilkinson KA, Huey K, Dinger B, He L, Fidone S, Powell FL. Chronic hypoxia increases the gain of the hypoxic ventilatory response by a mechanism in the central nervous system. J Appl Physiol. 2010;109(2):424–430. https://doi.org/10.1152/japplphysiol.00311.2009.

23. Millen GP, Debec J, Brocherie F, Malatesta D, Girard O. Therapeutic use of exercising in hypoxia: promises and limitations. Front Physiol. 2016;7. https://doi.org/10.3389/fphys.2016.00224.

24. Segar M, Jayaratne T, Hanlon J, Richardson CR. Fitting fitness into women's lives: effects of a gender-tailored physical activity intervention. Wom Health Issues. 2002;12(2):338–347. https://doi.org/10.1016/S1054-3870(01)00156-1.

25. Bruinvels G, Burden RJ, McGregor AJ, et al. Sport, exercise and the menstrual cycle: where is the research? Br J Sports Med. 2017;51(6):487–488. https://doi.org/10.1136/bjsports-2016-096279.

26. Levental S, Picard E, Mimouni F, et al. Sex-linked difference in blood oxygen saturation. Clin Res J. 2018;12(5):1900–1904. https://doi.org/10.1111/crj.12753.

27. Lei OK, Kong Z, Loprinzi PD, et al. Severe hypoxia does not offset the benefits of exercise on cognitive function in sedentary young women. Int J Environ Res Public Health. 2019;16(6). https://doi.org/10.3390/ijerph16061003.

28. Navarrete-Opazo A, Mitchell GS. Therapeutic potential of intermittent hypoxia: a matter of dose. Am J Physiol Regul Integr Comp Physiol. 2014;307(10): R1811–R1197. https://doi.org/10.1152/ajpregu.00208.2014.

29. Nagahisa H, Mukai K, Ohmura H, Takahashi T, Miyata H. Effect of high-intensity interval training in normobaric hypoxia on thoroughbred skeletal muscle. Oxid Med Cell Longev. 2016;2016:1–10. https://doi.org/10.1155/2016/1535367.

30. Wei J, Cimistroni M. Hypoxic training: clinical benefits on cardiometabolic risk factors. J Sci Med Sport. 2015;18(1):56–61. https://doi.org/10.1016/j.jsams.2013.10.247.

31. Rosstatter H, Kowalchuk J, Whipp B. A test to establish maximum O2 uptake despite no plateau in the O2 uptake response to ramp incremental exercise. J Appl Physiol. 2006;100(3):764–770. https://doi.org/10.1152/japplphysiol.00532.2005.

32. Kong Z, Fan X, Sun S, Song L, Shi Q, Nie J. Comparison of high-intensity interval training and moderate-to-vigorous continuous training for cardiometabolic health and exercise enjoyment in obese young women: a randomized controlled trial. PLoS One. 2016;11(7). e0158589. https://doi.org/10.1371/journal.pone.0158589.

33. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 0 ed. Routledge; 2013. https://doi.org/10.4324/9780203771587.

34. Kong Z, Hu M, Liu Y, et al. Affective and enjoyment responses to short-term high-intensity interval training with low-carbohydrate diet in overweight young women. Nutrients. 2020;12(2):442. https://doi.org/10.3390/nu12020442.

35. Powell FL, Milson WK, Mitchell GS. Time domains of the hypoxic ventilatory response. Respir Physiol. 1998;112(2):123–134. https://doi.org/10.1016/S0034-9807(98)00026-7.

36. Burtscher M, Haiden T, Domej W, et al. Intermittent hypoxia increases exercise tolerance in patients at risk for or with mild COPD. Respir Physiol Neurobiol. 2009;165(1):97–103. https://doi.org/10.1016/j.resp.2008.10.012.

37. Urdampilleta A, González-Muniesa P, Portillo MP, Martínez JA. Usefulness of combining intermittent hypoxia and physical exercise in the treatment of obesity. J Physiol Biochem. 2012;68(2):289–304. https://doi.org/10.1007/s11055-011-0151-3.

38. Gatterer H, Haacke S, Burtscher M, et al. Normobaric Intermittent Hypoxia over 8 Months Does Not Reduce Body Weight and Metabolic Risk Factors—a Randomized, Single Blind, Placebo-Controlled Study in Normobaric Hypoxia and Normobaric Sham Hypoxia. Published Online. 2015:10.

39. Wiesner S. Influences of normobaric hypoxia training on physical fitness and metabolic risk markers in. Overweight to Obese Subjects. 2010;18(1):5.

40. Gao H, Xu J, Zhang L, Lu Y, Gao B, Feng L. Effects of living high-training low and high on body composition and metabolic risk markers in overweight and obese females. Biomed Res Int. 2020;2020:1–9. https://doi.org/10.1155/2020/1279710.

41. Palmer BF, Clegg DJ. Ascent to altitude as a weight loss method: the good and bad of hypoxia inducible factor activation: ascent to Altitude as a Weight Loss Method. Obesity. 2014;22(2):311–317. https://doi.org/10.1002/oby.20499.

42. Campbell J, Phillips SM. The effects of two weeks low-volume self-regulated high-intensity interval training on cardiorespiratory fitness, exercise enjoyment, and intentions to repeat. J Hum Phys Exerc. 2020;16(2). https://doi.org/10.14198/jhse.2021.162.15.

43. Fais R, Rapillard A. Repeated sprint training in hypoxia: case report of performance benefits in a professional cyclist. Front Physiol Living. 2020;2;35. https://doi.org/10.3389/fsphor.2020.00035.

44. Camacho-Cardenosa A, Camacho-Cardenosa M, Burtscher M, et al. High-intensity interval training in normobaric hypoxia leads to greater body fat loss in overweight/obese women than high-intensity interval training in normoxia. Front Physiol. 2018;9. https://doi.org/10.3389/fphys.2018.00060.

45. Little JP, Saltär A, Wilkin GP, Tarnopolsky MA, Gibala MJ. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms: interval training adaptations. J Physiol. 2010;588(6):1011–1022. https://doi.org/10.1113/jphysiol.2009.181743.

46. Albert TJ, Swenson ER. Peripheral chemoreceptor responsiveness and hypoxic pulmonary vasoconstriction in humans. High Alt Med Biol. 2014;15(1):15–20. https://doi.org/10.1089/ham.2013.1072.

47. Mira J, Fioreani M, Savoldelli A, et al. Neuromuscular fatigue of cycling exercise in hypoxia. Med Sci Sports Exerc. 2020;52(9):1888–1899. https://doi.org/10.1249/MSS.0000000000002311.

48. Soo J, Girard O, Ihmsen M, Fairchild T. The use of the SpO2 to FiO2 ratio to individualize the hypoxic dose in sport science, exercise, and health settings. Front Physiol. 2020;11:570472. https://doi.org/10.3389/fphys.2020.570472.

49. Trapp EG, Chisholm DJ, Freund J, Bouchier SH. The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. Int J Obes. 2008;32(4):684–691. https://doi.org/10.1038/ijj.2008.03781.

50. Zhang H, Tong TK, Kong Z, Shi Q, Liu Y, Nie J. Exercise training-induced visceral fat loss in obese women: the role of training intensity and modality. Published online September 4 Scand J Med Sci Sports. 2020:13803. https://doi.org/10.1111/sms.13803. sms.