Journal of Exercise Science & Fitness 20 (2022) 70–76

Contents lists available at ScienceDirect

Journal of Exercise Science & Fitness

journal homepage: www.elsevier.com/locate/jesf

Methods to match high-intensity interval exercise intensity in hypoxia and normoxia – A pilot study

Yanchun Li [a, 1], Jia Li [b, c, 1], Muhammed M. Atakan [b, d], Zhenhuan Wang [b], Yang Hu [a], Mostafa Nazif [b], Navabeh Zarekookandeh [b], Henry Zhihong Ye [e], Jujiao Kuang [b, f], Alessandra Ferri [b], Aaron Petersen [b], Andrew Garnham [b], David J. Bishop [b], Olivier Girard [g], Yaru Huang [h, **, 2], Xu Yan [b, f, i, 2, *]

[a] China Institute of Sport and Health Science, Beijing Sport University, Beijing, 100084, China
[b] Institute for Health and Sport,(iHeS), Victoria University, Melbourne, 3011, Australia
[c] College of Physical Education, Southwest University, Chongqin, China
[d] Division of Exercise Nutrition and Metabolism, Faculty of Sport Sciences, Hacettepe University, Ankara, 06800, Turkey
[e] School of Biological Sciences, Monash University, Melbourne, 3800, Australia
[f] Sarcopenia Research Program, Australia Institute for Musculoskeletal Sciences (AIMSS), Melbourne, 3021, Australia
[g] School of Human Sciences, The University of Western Australia, Perth, 6009, Australia
[h] Department of Physical Education and Art, China Agricultural University, Beijing, 100083, China
[i] Department of Medicine - Western Health, The University of Melbourne, Melbourne, 3021, Australia

ARTICLE INFO

Article history:
Received 1 October 2021
Received in revised form
20 December 2021
Accepted 22 December 2021
Available online 27 December 2021

Keywords:
High-intensity interval exercise
Hypoxia
Exercise prescription
Peak power output
Lactate threshold

ABSTRACT

Objectives: The aim of this study was to compare high-intensity interval exercise (HIIE) sessions prescribed on the basis of a maximal value (peak power output, PPO) and a submaximal value (lactate threshold, LT) derived from graded exercise tests (GXTs) in normoxia and hypoxia. Methods: A total of ten males (aged 18–37) volunteered to participate in this study. The experimental protocol consisted of a familiarization procedure, two GXTs under normoxia (FiO_2 = 0.209) and two GXTs under normobaric hypoxia (FiO2 = 0.140), and three HIIE sessions performed in a random order. The HIIE sessions included one at hypoxia (HY) and two at normoxia (one matched for the absolute intensity in hypoxia, designated as NA, and one matched for the relative intensity in hypoxia, designated as NR). Results: The data demonstrated that there was significant lower peak oxygen uptake ($\dot{V}O_{2peak}$), peak heart rate (HR_{peak}), PPO, and LT derived from GXTs in hypoxia, with higher respiratory exchange ratio (RER), when compared to those from GXTs performed in normoxia ($p < 0.001$). Among the three HIIE sessions, the NA session resulted in lower percentage of HR_{peak} (85.0 ± 7.5% vs 94.4 ± 5.0%; $p = 0.002$) and $\dot{V}O_{2peak}$ (74.1 ± 9.1% vs 88.7 ± 7.7%; $p = 0.005$), when compared to the NR session. HIIE sessions in HY and NR resulted in similar percentage of HR_{peak} and $\dot{V}O_{2peak}$, as well as similar rating of perceived exertion and RER. The blood lactate level increased immediately after all the three HIIE sessions ($p < 0.001$), while higher blood lactate concentrations were observed immediately after the HY ($p = 0.0003$) and NR (p = 0.014) sessions when compared with NA. Conclusion: Combining of PPO and LT derived from GXTs can be used to prescribe exercise intensity of HIIE in hypoxia.

1. Introduction

Prescribing exercise intensity remains a challenging task. A review from 2020 has summarised current practices in determining exercise intensities.[1] Classic approaches of prescribing exercise intensity are based on percentage of maximal anchors, such as maximal ($\dot{V}O_{2max}$) or peak oxygen uptake ($\dot{V}O_{2peak}$), maximum

* Corresponding author. Department of Physical and Art, China Agricultural University, Beijing, 100083, China.
** Corresponding author. Institute for Health and Sport (iHeS), Victoria University, Melbourne, 3011, Australia.
E-mail addresses: xu.yan@vu.edu.au (Y. Huang), cauhuangyr@163.com (X. Yan).
[1] These authors contributed equally to this work and are co-first authors.
[2] These authors contributed equally to this work and are co-corresponding authors.

https://doi.org/10.1016/j.jesf.2021.12.003

(HR_{max}) or peak heart rate (HR_{peak}), and maximum work rate/peak power output (PPO).[1] However, using fixed percentages of the above-mentioned maximal anchors could result in large variability in the physiological responses.[1] Alternatively, submaximal anchors can be used for the purpose of deriving exercise intensity, such as the first and second lactate threshold (LT_1 and LT_2), the maximal lactate steady state (MLSS), the ventilatory threshold (VT), the gas exchange threshold (GET), the respiratory compensation point (RCP), critical speed (CS), and critical power (CP).[2–5] However, current evidence suggests that prescribing exercise intensity relative to the submaximal anchors remains to be validated in different settings and environments.[1]

Hypoxia is defined as reduced oxygen supply to tissues leading to decreased oxygen availability[6] which in turn results in decreased $\dot{V}O_{2peak}$ and PPO derived from incremental tests.[7–9] Training in hypoxic conditions is widely used by athletes who aim to increase physical performance at sea level and to improve exercise tolerance during competitions held at terrestrial altitude.[10] With minimal costs and small disturbance to daily routine, interval hypoxic training is often used by athletes to increase performance.[11] High-intensity interval exercise (HIIE) is comprised of bursts of vigorous activity lasting several minutes, and performed at a workload eliciting either ≥75% of $\dot{V}O_{2max}$,[12] ≥75% maximal power output,[13] ≥90% minimal running speed required to elicit $\dot{V}O_{2max}$,[14,15] or a rating of perceived exertion (RPE) of "hard" to "very hard" (RPE ≥6 or ≥15 on the 0–10 and 6–20 Borg scales, respectively)[16] interspersed with brief rest periods. Performing HIIE under hypoxic conditions has gained popularity lately, due to the possibility to achieve larger performance gains and associated metabolic and cardiorespiratory benefits compared to similar training near sea level.[12]

In general, exercise intensity in hypoxia is prescribed based on PPO,[17–21] maximal aerobic speed[22] HR_{max},[23–25] $\dot{V}O_{2max}$,[26,27] RPE,[28] or LT.[20,29] Different methods of prescribing exercise intensity across studies may limit the possibility to directly compare performance and physiological outcomes between training studies.[1] A lower than expected exercise intensity can also lead to a detraining effect in highly trained individuals. In contrast, prescribing exercise intensity inclusive of maximal, submaximal, and resting values is likely to result in a more homogeneous physiological response.[1] For example, recent studies by our group have suggested that prescription of HIIE in normoxia based on both a maximal value (PPO) and a submaximal value (LT_2) resulted in comparable physiological adaptations, when compared with studies based on only a maximal value or a submaximal value.[30–34] Exercising at similar workloads (same absolute exercise intensity such as pedalling at 100 W), hypoxia will increase physiological and perceptual responses. However, since hypoxia decreases $\dot{V}O_{2max}$, pedalling at a given physiological intensity (for example, 75% $\dot{V}O_{2max}$) represents a reduced mechanical output (power sustained). To our knowledge, however, no study has explored the prescription of exercise intensities for HIIE in hypoxia, based on the combination of a maximal value (i.e., PPO) and a submaximal value (i.e., LT_2), derived from a graded exercise test (GXT). Therefore, the aim of this study was to explore and evaluate the prescription of HIIE intensity based on PPO and LT on physiological parameters in simulated hypoxia, and compare them with two HIIE sessions (matched for absolute and relative intensity in hypoxia) performed in normoxia.

2. Methods

2.1. Participants

A total of ten males (aged 18–37) were recruited from the Victoria University student population and residents of nearby communities. Inclusion criteria were 1) males aged 18–45 years old; 2) a body mass index of 20–30 kg/m^2; 3) no hypertension (resting systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg); 4) free of medications before and during the study, without unstable angina or diagnosed reversible cardiac ischemia, diagnosed uncontrolled cardiac arrhythmia with recurrent episodes or symptoms on exertion, heart failure, symptomatic aortic stenosis; 5) non-smokers; 6) not having been to an altitude greater than 1000 m for more than 24 h in the last three months. A risk factor assessment questionnaire (assessing the medical history, symptoms during or after exercise, family medical history, and exercise participation) was obtained from each participant before enrolling in the study. Informed consent was obtained from the participants before participation. The study was approved by Victoria University Human Research Ethics Committee (Ethics Approval NO. HRE18-214). Calculation of the required sample was based on the assessment of a main effect for difference in gene expression, at a significance level $\alpha = 0.05$ and power 1 - $\beta = 80\%$. Using peroxisome proliferator-activated receptor gamma co-activator 1-α mRNA levels reported as fold changes after an acute exercise in normoxia and hypoxia by Slivka et al.,[35] we performed simulations in R, assuming a random exponential distribution of the fold-change data for the normoxia and hypoxia groups, sample size was estimated as 12 for the current study. Due to the COVID pandemic, we had to terminate the human exercise trial in early 2020, with a final sample size of ten participants.

2.2. Study design

The experimental protocol included a familiarization procedure, two GXTs in normoxia ($FiO_2 = 0.209$) and two GXTs in normobaric hypoxia ($FiO_2 = 0.140$, corresponding to a simulated altitude of ~3200 m, similar to our previous study[36]), and three HIIE sessions performed in a random order at the same time of the day (±2 h). The three HIIE sessions included one in hypoxia (HY), one in normoxia with workload matched to the relative workload performed in hypoxia (NR), and one in normoxia with workload matched to the absolute workload performed in hypoxia (NA). The study duration, inclusive of the familiarization procedure, was about 6 weeks. During the study, participants were asked to maintain their normal daily diet and physical activities. Participants were required to refrain from any strenuous physical activity for 48 h before the familiarization and GXTs and from alcohol and any exercise for 24 h before HIIE sessions. Additionally, participants were advised to restrict eating 2 h before the GXTs and HIIE sessions, and completed a physical activity and dietary intake questionnaire before each test.

2.3. Familiarization and graded exercise tests

At least 1 week before baseline measurements, all participants performed two familiarization tests, including a GXT in normoxia and a HIIE session to become accustomed with all the testing procedures on two occasions, with at least two days between each session. Following the familiarization sessions, participants performed two GXTs in normoxia or two GXTs in hypoxia, in a randomised and counterbalanced order. A fifth GXT (normoxia and/or hypoxia) was conducted if PPO reached for the two GXTs in the same condition (normoxia or hypoxia) differed by more than 10%. All tests were performed, at least 48 h apart. The PPO, LT and $\dot{V}O_{2peak}$ data from the two closest GXTs were averaged and then used to determine the exercise intensity of HIIE. The GXTs were conducted on an electronically braked cycle ergometer (Excalibur Sport, Lode B·V., The Netherland), as previously reported, with some modifications (starting at 25% of each participant's PPO, with an increment of a eleventh of their PPO every 4 min, aiming to

achieve 10 completed 4-min stages for each participant).[1,31] A 30-s break was given after each completed stage during which capillary blood samples were taken to analyse blood lactate (YSI 2300 Stat; Yellow Springs Instruments, Yellow Springs, USA).[30] The blood lactate data were utilized to calculate the lactate threshold (LT, expressed in W) with the modified D-max formula.[37] The test was terminated when the participants reported 20 on the RPE scale and/or when the participants voluntarily stopped cycling. Exhaled gas during the GXTs was collected and analysed using a MOXUS Metabolic Cart (AEI Technologies, Bastrop, TX, United States) or a Cosmed Quark CPET (COSMED, Rome, Italy) based on equipment availability. In our laboratory, the differences in $\dot{V}O_{2peak}$ values between the MOXUS and Cosmed analysers were shown to be non-significant, as evidenced by coefficient of variations (6.4% vs 3.9%) and technical error of measurements (7.4% vs 7.1%). Since the $\dot{V}O_2$ data were collected every 15 s (Cosmed, Rome, Italy) or 30s (Moxus, AEI Technologies Inc., Naperville, IL, USA), the 1-min mean values for $\dot{V}O_2$, $\dot{V}CO_2$, and ventilation ($\dot{V}E$) were obtained. The MOXUS Metabolic Cart was the preferred system to measure the gas composition, due to its capacity to input FiO_2 prior to the test. However, the system was occupied by other researchers and unavailable some of the days, when the Cosmed Quark CPET was used to measure the gas composition. Heart rate was monitored by a Polar heart rate monitor throughout the whole test.[31] For the GXTs in hypoxia, participants were acclimatised to the environmental chamber for 10–15 min before performing the test.

2.4. HIIE sessions

Each HIIE session consisted of 6x4-min exercise bouts, separated by 2 min of rest. For the HY session, the exercise workload was based on the PPO achieved in hypoxia (PPO_H) and the LT achieved in hypoxia (LT_H) and was calculated as 50% PPO_H + 50% LT_H. For the NR session, the exercise workload was based on the PPO achieved in normoxia (PPO_N) and the LT achieved in normoxia (LT_N) and was calculated as 50% PPO_N + 50% LT_N. For NA, the exercise workload was the same as HY, equalling 50% PPO_H + 50% LT_H. For the HIIE in hypoxia, participants stayed in the simulated hypoxia chamber for 75–85 min, which consisted of 30–40 min of acclimatisation to the environment, baseline blood sampling (after approximately 30 min into the chamber), a 5-min warm-up, a 34-min exercise session, and a 5-min post-exercise blood sampling. All participants then rested for 3 h in normoxia and also reported to the lab the next morning. Blood samples were collected before (B), immediately post (P0), 3 h post (P3) and 24 h post (P24) each HIIE session. Participants consumed a controlled diet for 48 h before and 24 h following the HIIE session to avoid any confounding effects of different dietary habits and arrived at the lab fasted in the morning. The energy requirement of each participant was estimated using the Mifflin St-Jeor equation, by including each participant's body mass, height and age.[30] The controlled diet included approximately 53–56% of energy from carbohydrates, 22–24% from fats, and 18–21% from protein.

2.5. Plasma blood lactate

Venous blood samples were collected from the antecubital vein and allocated into 3 mL BD Vacutainer® heparin blood collection tubes (BD, Franklin Lakes, United States), inverted 6–10 times, and centrifuged at 3500 rpm at 4 °C for 10-min. The supernatant plasma was collected and carefully aliquoted into 1.5 mL Eppendorf tubes. Plasma lactate was measured with YSI 2300 Stat (YSI Incorporated, Yellow Springs, OH, USA). The plasma lactate level immediately post-exercise (P0) was normalised in fold-change and percentage to the baseline (B) and the corresponding peak blood lactate reached during the GXTs (Peak), respectively.

2.6. Statistical analyses

All data in text, figures, and tables are presented as mean ± standard deviation (SD). Student's t-tests and two-way analyses of variance (ANOVA) were used to assess differences between samples. Statistical analyses were conducted using the statistical software package GraphPad Prism (V8.0, GraphPad Software, Inc., San Diego, CA, USA), except for the calculation of effect size, which was performed using the R software. Statistical significance was accepted as $p < 0.05$.

3. Results

The characteristics of the participants are presented in Table 1.

All participants showed a lower absolute and relative $\dot{V}O_{2peak}$, PPO, and LT during the GXT in hypoxia compared to the GXT performed in normoxia, with the biggest differences in $\dot{V}O_{2peak}$. Absolute $\dot{V}O_{2peak}$ decreased by 20.2 ± 8.8% ($p < 0.001$; effect size = 0.87; Fig. 1A), while $\dot{V}O_{2peak}$ relative to body mass decreased by 20.2 ± 9.1% in hypoxia when compared with normoxia ($p < 0.001$; effect size = 0.84; Fig. 1B). End-exercise RER was 12.5 ± 10.1% higher in hypoxia than in normoxia ($p < 0.001$; effect size = 0.75) (Fig. 1C). End-exercise HR_{peak} was lower in hypoxia compared to normoxia (181 ± 6 vs. 187 ± 6 beats·min^{-1}; $p < 0.001$; effect size = 0.77; Fig. 1D). PPO decreased by 9.4 ± 2.1% in hypoxia ($p < 0.001$; effect size = 0.33; Fig. 1E) and power at LT was 13.1 ± 3.0% lower in hypoxia than normoxia ($p < 0.001$; effect size = 0.40; Fig. 1F). During the GXTs, there was no difference between the peak blood lactate level between normoxia (8.7 ± 1.9 mmol/L) and hypoxia (8.8 ± 2.0 mmol/L).

The workload was 170.1 ± 50.9 W for NA and HY (Fig. 2A), equivalent to 78.2 ± 2.4% and 85.8 ± 1.9% of PPO in normoxia and hypoxia, respectively (Fig. 2B). The workload for NR was significantly higher (189.3 ± 53.7 W, $p < 0.001$, Fig. 2A), equivalent to 87.4 ± 1.7% of the PPO in normoxia (different from both the NA and HY, Fig. 2B). The percentage of HR_{peak} was higher after the HY (92.4 ± 6.6%, $p = 0.026$) and NR sessions (94.4 ± 5.0%, $p = 0.002$), when compared with NA session ((85.0 ± 7.5%; Fig. 2C). Compared with NA (15.3 ± 2.6), RPE was higher in HY (17.2 ± 2.7; $p = 0.017$) and NR (17.9 ± 3.2, $p = 0.005$), with no difference between HY and NR ($p = 0.15$) (Fig. 2C). The percentage of $\dot{V}O_{2peak}$ was higher after NR when compared with NA ($p = 0.005$), with no difference between NA and HY ($p = 0.29$) (Fig. 2E). No significant difference was observed in RER among the three conditions (Fig. 2F).

There was no difference for the baseline plasma lactate level among the three HIIE sessions (Fig. 3A). Immediately after the HIIE sessions, plasma lactate level increased significantly, by 5.8 ± 1.8, 10.1 ± 3.2, and 8.5 ± 3.1 fold for NA, HY, and NR, respectively (Fig. 3B and E) ($p < 0.01$). The plasma lactate concentration was higher in

Table 1
Participant characteristics in normoxia (n = 10).

Parameter	Mean ± SD
Age (years)	28 ± 5
Body mass (kg)	73.5 ± 9.7
Height (cm)	175 ± 7
BMI (kg·m^{-2})	26.0 ± 3.4
$\dot{V}O_{2peak}$ (mL·min^{-1})	3269 ± 746
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	44.7 ± 7.8
Peak power output (W)	216 ± 56
Peak HR (beat·min^{-1})	187 ± 6

BMI, body mass index; $\dot{V}O_{2peak}$, peak oxygen uptake; HR, heart rate; W, Watts.

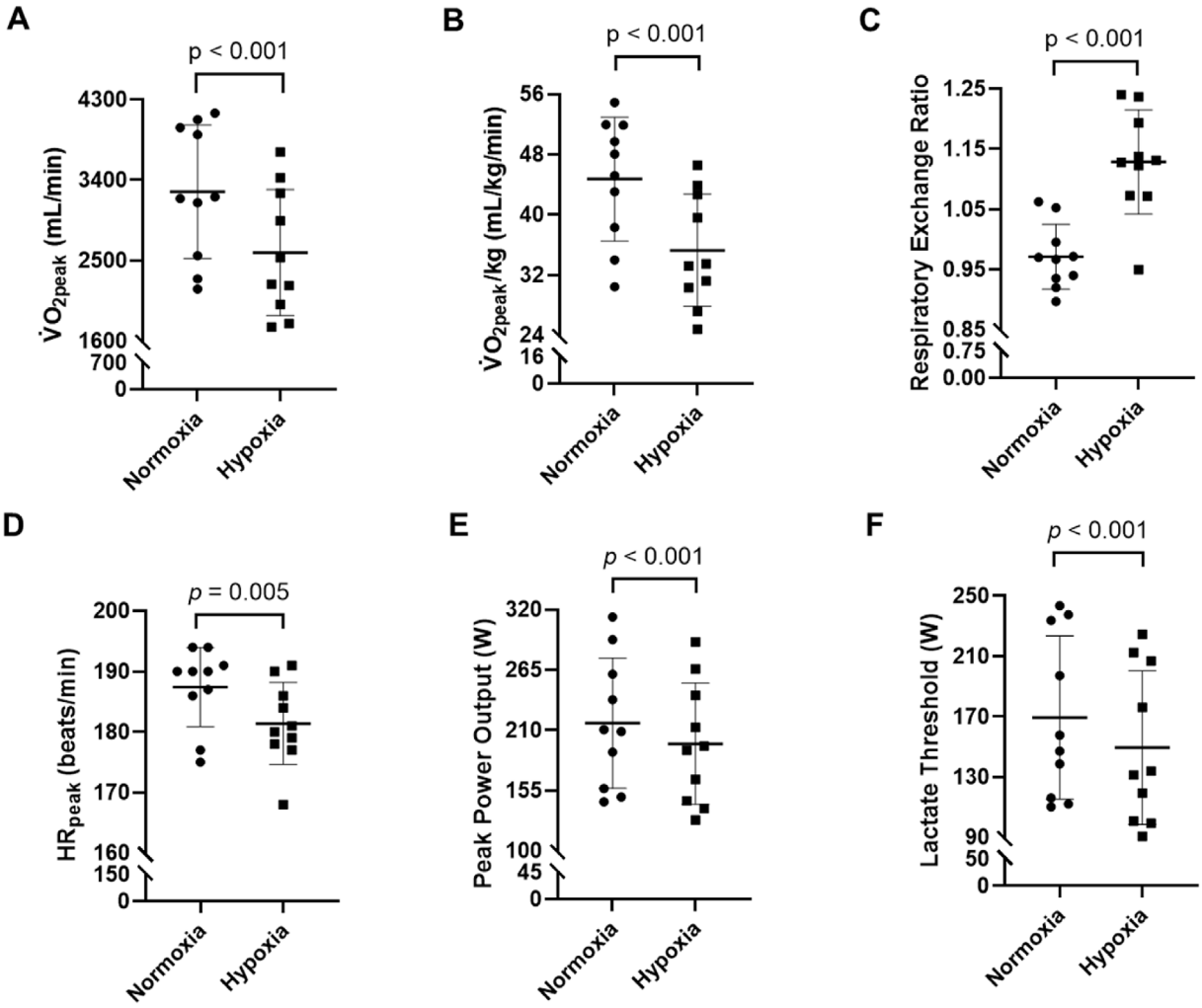

Fig. 1. The effects of hypoxia on physiological data during the graded exercise tests. Dots and squares in the graphs represent individual data of each participant in normoxia and hypoxia. In hypoxia, there was an overall decrease in absolute $\dot{V}O_{2peak}$ (A), $\dot{V}O_{2peak}$/kg (B), heart rate peak (D), peak power output (E), and the lactate threshold (F), with an increase in respiratory exchange ratio (C). $\dot{V}O_{2peak}$, Peak oxygen uptake; W, Watt.

the HY ($p < 0.001$) and NR sessions ($p = 0.005$) than that of NA immediately after HIIE (Fig. 3B). The plasma lactate level returned to near baseline 3 h after the exercise interventions (Fig. 3C). When plasma lactate level at P0 was normalised to baseline values, a significant difference in plasma lactate occurred between NA and HY sessions ($p = 0.01$) (Fig. 3E), but not between NA and NR sessions ($p = 0.11$) (Fig. 3E). Once the plasma lactate from the HIIE sessions was normalised to the corresponding peak blood lactate reached during the GXTs, significant difference between NA and HY sessions ($p = 0.003$) (Fig. 3F) and between NA and NR sessions ($p < 0.001$) (Fig. 3F) were observed.

4. Discussion

In the current study, we prescribed three HIIE sessions based on PPO and LT derived from GXTs, one HIIE in hypoxia (FiO2 = 0.140, HY) and two in normoxia (FiO2 = 0.209, one matched for the absolute intensity in hypoxia, NA; and one matched for the relative intensity in hypoxia,NR). Our study showed that when the workload during the HIIE session was matched to the absolute value in hypoxia, a lower percentage of HR_{peak} and $\dot{V}O_{2peak}$, and a lower RPE were observed. On the contrary, when the workload was matched to the relative workload in hypoxia, the percentage of HR_{peak} and $\dot{V}O_{2peak}$, as well as RPE and RER, were not different. Whilst plasma lactate level increased immediately after all the three HIIE sessions, it was higher after the HY and NR sessions, when compared with that of NA. All the findings suggest that the strategy of matching intensity between normoxia and hypoxia was effective. Interestingly, the percentage of PPO was slightly but significantly higher in the NR than in the HY session. The data suggested if the exercise intensity were prescribed solely based on PPO in normoxia and hypoxia, it would lead to differences in RPE, HR_{peak}%, and $\dot{V}O_2$peak %.

We also observed a decrease in $\dot{V}$O2peak, PPO, LT, and HRpeak, with an increase in RER, from the GXTs in hypoxia, when compared with GXTs in normoxia. The observed decrease of $\dot{V}O_{2peak}$ was in agreement with the literature.[8,38] The 8.8% decrease of PPO from GXTs was comparable to a previous study, with a 5.5% decrease when exposure to simulated hypoxia ($FiO_2 = 0.142$ or ~3000 m) and a 11.1% decrease when exposure to higher simulated hypoxia ($FiO_2 = 0.125$ or ~4000 m).[39] However, the observed decrease in PPO was less than what was reported by Ozcelik et al. (by 20.2%).[8] It is worth noting that the FiO_2 in the Ozcelik study was 0.120, much less than that in the current study. It is expected that the lower the FiO_2, the lower PPO achieved by the participants. Another explanation for the discrepancy is the differences in the GXT protocols,

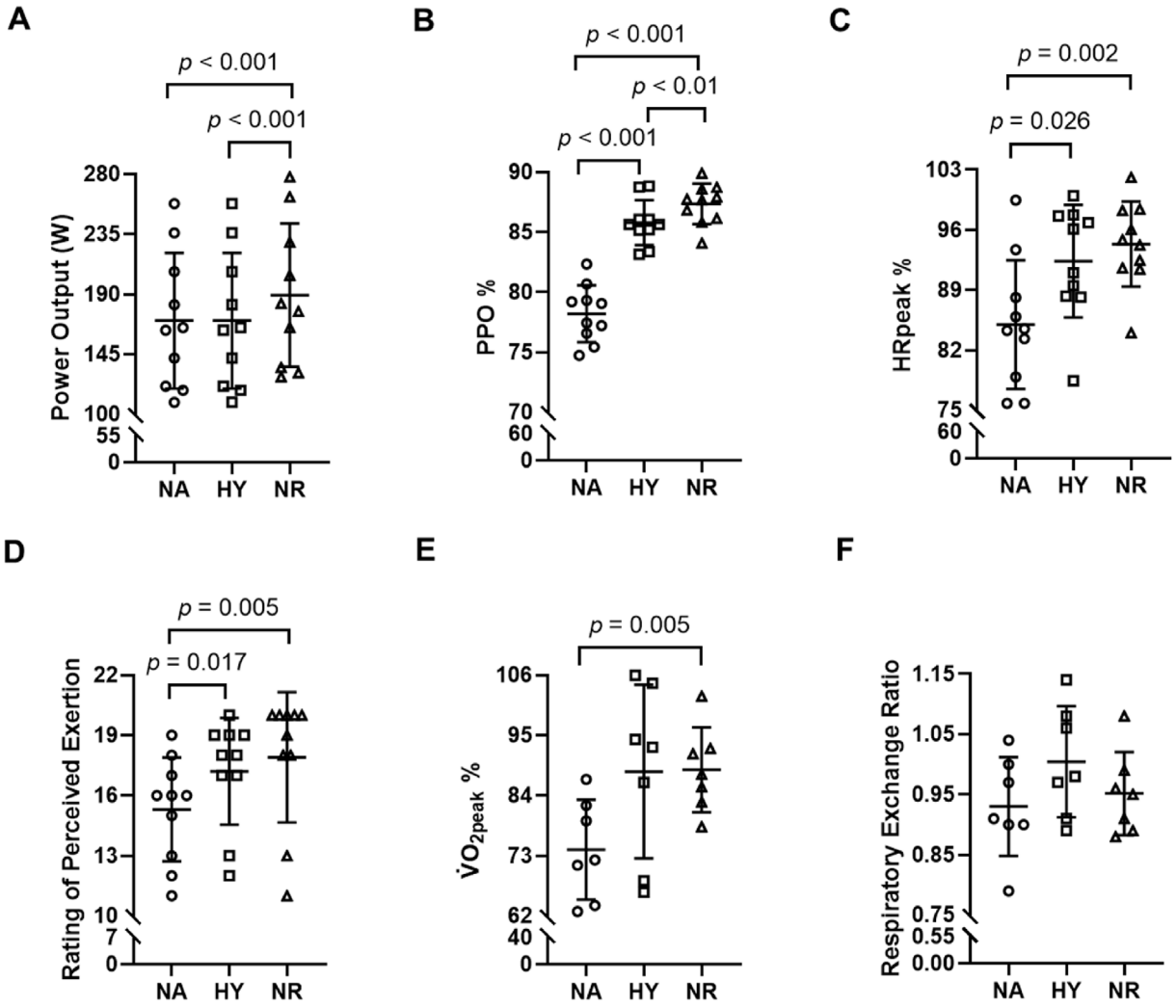

Fig. 2. HIIE in hypoxia compared with HIIE in normoxia with absolute and relative matched intensities. Circles, squares, triangles in the graphs represent individual data of each participant. NA, HIIE at normoxia matched for absolute intensity to that of hypoxia; HY, HIIE in hypoxia; NR, HIIE at normoxia matched for relative intensity to that of hypoxia. The power output of HIIE sessions (A), the percentage of power out to peak power out (PPO) (B), the percentage of heart rate to heart rate peak HRpeak (C), the rating of perceived exertion (D), the percentage of $\dot{V}O_2$ to $\dot{V}O_{2peak}$ of the three HIIE sessions (E), the respiratory exchange ratio reached for the three HIIE sessions (F). Due to equipment availability, full sets of VO2 and respiratory exchange ratio data were only collected in 7 participants.

the current study adopted an increment every 4 min, while the study by OZcelik used an increment every minute. However, the observed decrease of power associated with LT (by 11.8%) was not different from the study by Ozcelik (by 12.5%).[8] An early study reported a larger decrease in LT (expressed as the $\dot{V}O_2$ at the LT, by 16.1%) than our study, but the study included only highly-trained athletes.[40] The lower HR_{peak} and higher RER were both similar to previously reported.[41]

The peak blood lactate values reached after the GXTs in normoxia and hypoxia were not different, which was supported by previous research reporting peak/maximal blood was similar from incremental tests in normoxia and acute hypoxia.[42] Yet the blood lactate level during the GXTs was higher at a given power output, in agreement with a previous study.[43] Since the intensities of all the three HIIE sessions were above the power of the corresponding lactate threshold, it is not surprising to observe an increase in plasma lactate concentration immediately after the exercise. The increase in blood lactate concentration was not different immediately after the HY and NR sessions, yet it was higher than that after the NA session, suggesting the exercise intensities were well matched in the HY and NR sessions. This increase in plasma lactate was transient, as it returned to baseline 3 h after the HIIE sessions.

5. Limitations

One limitation of the current study is the wide range of participants' fitness levels, as illustrated by the PPO (between 145 and 293 W) and $\dot{V}O_{2peak}$ (between 2180 and 3985 mL min^{-1}) values. It has been suggested that fit individuals experienced larger decreases in $\dot{V}O_{2peak}$ when exposed to hypoxia.[40] A more homogenous group of participants may have provided better indications on some of the measured parameters.

Another potential limitation concerns the protocol used for the GXTs, which lasted 4 min for each increment. It has been suggested that GXTs with longer duration resulted in lower PPO when compared with short durations,[44,45] which may indicate the PPO in the current study is underestimated. This would also partially explain the small decrease in PPO observed in the current study. However, GXTs with longer duration seem not to affect the $\dot{V}O_{2peak}$ estimation,[44,45] which could explain the comparable decrease in

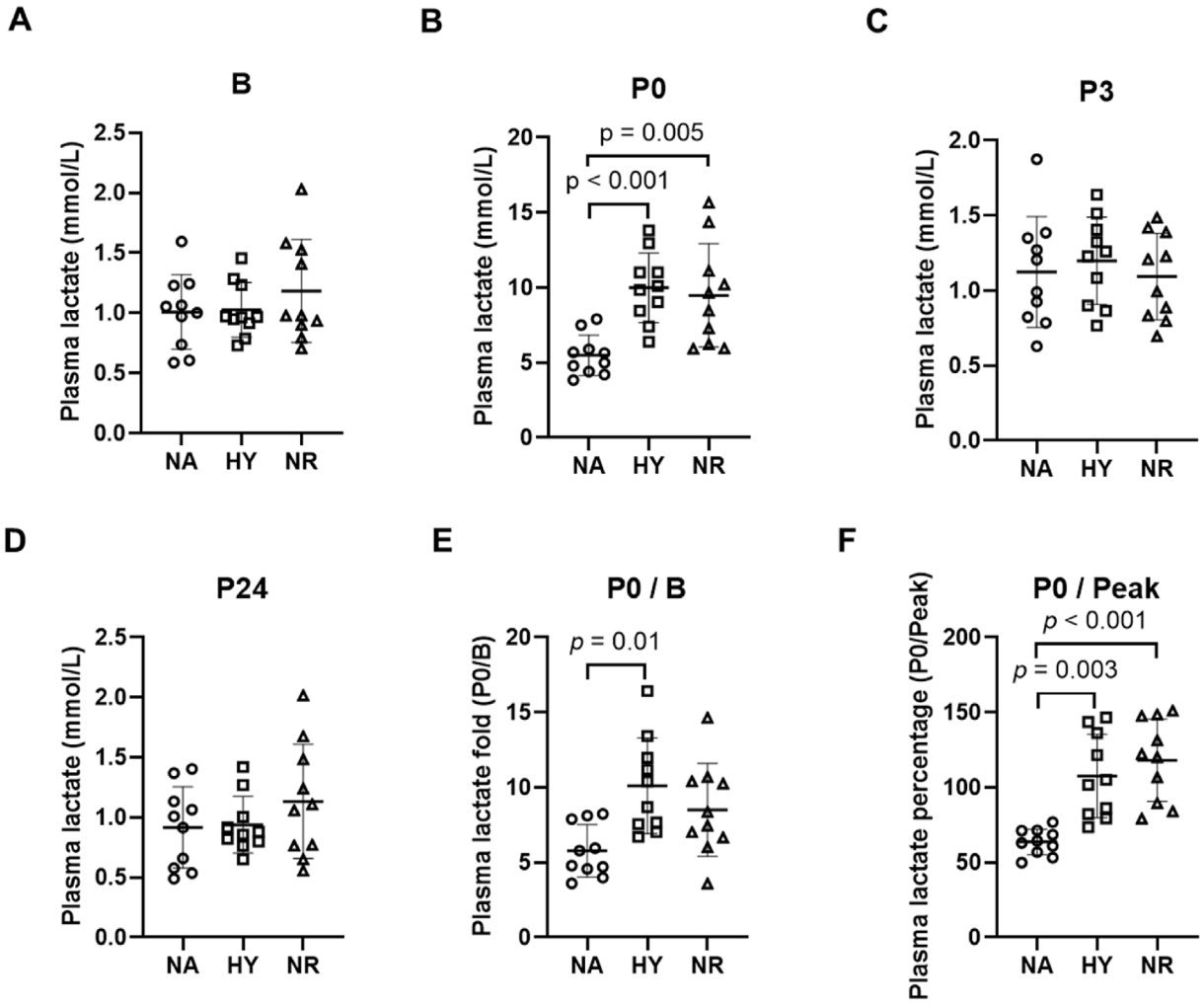

Fig. 3. Plasma lactate level before and after the three HIIE sessions. Circles, squares, triangles in the graphs represent individual data of each participant. NA, HIIE at normoxia matched for absolute intensity to that of hypoxia; HY, HIIE in hypoxia; NR, HIIE at normoxia matched for relative intensity to that of hypoxia. The baseline (B) plasma lactate level (A), plasma lactate level immediately post (P0, B), 3 h post (P3, C), 24 h post (P24, D) each HIIE session. The fold of plasma lactate level immediately post each HIIE session when compared with that of baseline (P0/B, E). The percentage of plasma lactate level immediately post each HIIE session to the peak blood lactate reached during the GXTs (P0/Peak, F).

VO_{2peak} in the current study, when compared with other studies.[41]

Last, but not the least, our study only included heathy males and therefore our results can not be extrapolated to other populations. Future studies are warranted to investigate whether similar results would be obtained in females and other cohorts, including individuals with different diseases.

6. Conclusions and implications

The prescription of HIIE intensity based on a maximal value and a submaximal value in hypoxia warrants further study. Our data indicate that when matched for a relative intensity determined from the PPO and the LT, there was no difference in the percentage of HR_{peak}, $VO2_{peak}$, RPE, or RER. All of these parameters were higher than those after HIIE in normoxia when matched for absolute intensity. We conclude that the combination of PPO and LT can be used to prescribe HIIE intensities in hypoxia, and that HIIE matched for absolute and relative intensity in normoxia will lead to distinct adaptations when compared with HIIE in hypoxia. Practically, the matching exercise intensity in hypoxia with that of normoxia is critical for the physiological adaptive response to HIIE. Future studies comparing systemic and tissue-specific adaptations to exercise in hypoxia with that of normoxia are warranted to refine best practice.

Funding

Y.L., Y·H, D.J.B. and X.Y. are supported by an international collaborative grant from Beijing Sport University (2018GJ005). Y. L. is supported by a grant from National Natural Science Foundation of China (31771317). X.Y. is supported by a seed grant from Australia Institute for Musculoskeletal Sciences. M.M.A. is funded by the Scientific and Technological Research Council of Turkey (TUBITAK) 2214-A research fellowship.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We would like to thank all the volunteers of the study. We dedicate this paper to Professor Yang Hu, who unfortunately lost his battle against cancer in 2020.

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