Received: 6 August 2023 | Revised: 18 February 2024 | Accepted: 28 March 2024

DOI: 10.1002/ejsc.12110

ORIGINAL PAPER

Official ECSS Journal Open Access WILEY

Low- and moderate-intensity aerobic exercise improves the physiological acclimatization of lowlanders on the Tibetan plateau

Rui Su[1,2,3] | Chenxiao Han[1] | Guiquan Chen[4] | Hao Li[1] | Wanying Liu[1] | Chengzhi Wang[1] | Wenrui Zhang[1] | Yuming Zhang[1] | Delong Zhang[1,5,6] | Hailin Ma[1,3]

[1]Tibet Autonomous Region Key Laboratory of High Altitudes Brain Science and Environmental Acclimation, Tibet University, Lhasa, China

[2]School of Psychological and Cognitive Sciences and Beijing Key Laboratory of Behavior and Mental Health, Peking University, Beijing, China

[3]Academy of Plateau Science and Sustainability, People's Government of Qinghai Province/Beijing Normal University, Beijing, Qinghai, China

[4]Department of Acupuncture and Rehabilitation, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou, Sichuan Province, China

[5]Key Laboratory of Brain, Cognition and Education Sciences, Ministry of Education, Beijing, China

[6]School of Psychology, Center for Studies of Psychological Application, and Guangdong Key Laboratory of Mental Health and Cognitive Science, South China Normal University, Guangzhou, China

Correspondence
Hailin Ma, 10 Zangda East Road, Chengguan District, Lhasa 510631, China.
Email: David_ma79@163.com

Funding information
National Natural Science Foundation Regional innovation development joint fund project of China, Grant/Award Number: U23A20476; Science and Technology Major Project of Tibetan Autonomous Region of China, Grant/Award Number: XZ202201ZD0001G; National Natural Science Foundation of China, Grant/Award Number: 32260212; Scientific Development funds for Local Region from the Chinese Government in 2023, Grant/Award Number: XZ202301YD0032C

Abstract

This study investigates whether exercise as a strategy for improving physical fitness at sea level also offers comparable benefits in the unique context of high altitudes (HA), considering the physiological challenges of hypoxic conditions. Overall, 121 lowlanders who had lived on the Tibetan Plateau for >2 years and were still living at HA during the measurements were randomly classified into four groups. Each individual of the low-intensity (LI), moderate-intensity (MI), and high-intensity (HI) groups performed 20 sessions of aerobic exercise at HA (3680 m) over 4 weeks, while the control group (CG) did not undergo any intervention. Physiological responses before and after the intervention were observed. The LI and MI groups experienced significant improvement in cardiopulmonary fitness (0.27 and 0.35 L/min increases in peak oxygen uptake [$\dot{V}O_{2peak}$], both $p < 0.05$) after exercise intervention, while the hematocrit (HCT) remained unchanged ($p > 0.05$). However, HI exercise was less efficient for cardiopulmonary fitness of lowlanders (0.02 L/min decrease in $\dot{V}O_{2peak}$, $p > 0.05$), whereas both the HCT (1.74 %, $p < 0.001$) and glomerular filtration rate (18.41 mL/min, $p < 0.001$) increased with HI intervention. Therefore, LI and MI aerobic exercise, rather than HI, can help lowlanders in Tibet become more acclimated to the HA by increasing cardiopulmonary function and counteracting erythrocytosis.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Authors. European Journal of Sport Science published by Wiley-VCH GmbH on behalf of European College of Sport Science.

KEYWORDS
cardiopulmonary function, erythrocyte, exercise intensity, high altitude

Highlights

- Low- and moderate-intensity exercise at HA can improve oxygen transport of lowlanders that may help them to become more acclimated to high altitude.
- High-intensity exercise performed by lowlanders may result in erythrocytosis without improving oxygen transport.
- The relationship between physiological system changes induced by exercise performed at HA is modulated by exercise intensity.

1 | INTRODUCTION

It has been documented that around 400 million people live at altitudes above 1500 m, and more than 100 million lowlanders travel to high altitudes (HA) annually (Mallet et al., 2021). With increasing altitude, reductions in barometric pressure, and a fraction of inspired oxygen (FIO_2), a subsequent decrease in ambient partial pressure of oxygen (PaO_2), associated with a decline in arterial oxygen saturation (SaO_2) will challenge the oxygen (O_2) transport chain and O_2 supply. Due to the limited O_2 delivery, individuals who are acutely exposed to HA hypoxia environments may be at a risk for acute mountain sickness (AMS). This condition presents symptoms, including headache, anorexia, sleep difficulty, or a combination of these symptoms (Bärtsch & Swenson, 2013). However, for chronic HA exposure lowlanders (people who originally lived near sea level but moved to HA for months or years), a series of physiological acclimatization responses is required to support tissue oxygenation.

This HA acclimatization in lowlanders is a well-coordinated set of physiological responses to ensure O_2 availability through adjustment in the respiratory system, cardiovascular system, and hematologic system (Goldfarb-Rumyantzev & Alper, 2014; Luks & Hackett, 2022). Ventilatory response is the initial major physiological response to HA exposure, which increases minute ventilation ($\dot{V}_E$) and partially attenuates reductions in PaO_2 (West, 2006). A subsequent increase in cardiac output (CO), associated with an increase in heart rate (HR), alters the distribution of blood flow, and enhances O_2 extraction from capillary blood to improve tissue oxygenation (Harold et al., 2012; Naeije, 2010). After chronic HA exposure, CO in lowlanders returns to normal levels with a slight increase in HR and a decrease in stroke volume (SV) (Hartley et al., 1967; Naeije, 2010). When CO remains stable and cannot contribute to an increased O_2 extraction, acclimatization to HA hypoxia in most lowlanders involves an increase in hematocrit (HCT) level to maintain arterial O_2 content (Gassmann et al., 2019). However, despite the increase in HCT that can contribute to acclimatization, lowlanders living at HA still have some degree of compromised adaptation to chronic hypoxic stress. This is because high HCT levels are associated with increased blood viscosity and increased risk of cardiac events, which results in a reduction in fitness and maladjustment (Vargas & Spielvogel, 2006). Meanwhile, the O_2 supply in acclimated lowlanders also remains insufficient, as indicated by reduced maximal oxygen uptake ($\dot{V}O_{2max}$) (Calbet et al., 2003). Although most of the HA residents are free of disease, they live in a state of chronic tissue hypoxia, and often have severe hypoxemia (West, 2017). How to ensure that HA lowlanders, such as the military and mountaineers, remain unaffected by unavoidable hypoxia stress is still unclear.

Exercise significantly improves aerobic capacity under normobaric normoxic conditions (Crowley et al., 2022) and enhances the release of oxygen and the deformability of red blood cells, both of which contribute to the improvement of tissue O_2 supply (Mairbäurl, 2013). Given that limited O_2 supply is a most prominent challenge after HA exposure, it is reasonable to predict that exercise may improve the level of HA acclimatization level by increasing tissue oxygenation. However, the relevant findings from HA hypoxia condition research remain less consistent. Previous studies have revealed that high-intensity intermittent exercise at HA may worsen pulmonary interstitial edema (Edsell et al., 2014); however, the findings have been challenged by recent evidence. For example, a review study concluded that dual stimulation from hypoxia and exercise can induce HA acclimatization (Zhang & Chen, 2018). Another study reported that submaximal exercise performed under conditions of light or moderate hypoxia induced greater metabolic and cardiac responses (Park et al., 2022). Exercise intensity is one of the most important factors regarding the physiological responses to HA exercise, which may contribute to these contradictory findings. The intensity of exercise at HA plays a major role in the subsequent physiological acclimatization (e.g., respiratory, cardiovascular, and hematologic changes) (Mazzeo, 2008). However, most available reports attempting to classify exercise intensity are inaccurate as they do not consider the effects of HA exposure on HR and $\dot{V}O_{2max}$ (Mourot, 2018). Given that chronic HA exposure lowlanders are not fully adapted to the hypoxia environment, excessively high exercise intensity may have detrimental effects. Hence, a comprehensive investigation is needed to determine the optimal exercise intensity to promote HA physiological acclimatization.

To systematically study the effects of exercise with different intensity on the physiological acclimatization of HA lowlanders, the present study implemented exercise interventions with low-,

medium-, and high-intensity performed by young lowlanders who had lived at HA for more than 2 years. This was followed by measuring the cardiovascular function, O_2 transport of red blood cells, renal function (Palubiski et al., 2020), and immune function (Mishra & Ganju, 2010; Pham et al., 2021), given the reports of their association with HA acclimatization. Based on accumulated evidence, hypotheses are made as follows: (H1) LI aerobic exercise does not cause changes in physiological performance; (H2) MI aerobic exercise can improve O_2 transport capacity but not alter the blood viscosity; (H3) HI aerobic exercise can also improve O_2 transport capacity, but increase the blood viscosity, renal, and immune response.

2 | MATERIALS AND METHODS

2.1 | Participants

A total of 160 male students from Tibet University were recruited. All participants were expected to meet the following inclusion criteria: Healthy Han Chinese immigrants (lowlanders) living in Lhasa, on the Tibetan Plateau (3680 m) for >2 years, and their birthplace altitude was <1500 m; had not visited HA areas before attending university; no addiction to cigarette or alcohol; no respiratory or motor system disease, and were fit for exercise according to the Physical Activity Readiness Questionnaire (Shephard, 1988). Thirty-nine subjects were excluded from data analysis for reasons including scheduling conflicts ($n = 27$) and missing data ($n = 12$). Thus, 121 participants were included in the final data analysis. Their daily activity was assessed using the International Physical Activity Questionnaire (IPAQ). Further, all participants were asked to maintain their habitual lifestyle during the study.

The ethics committee of Tibet University approved this study (protocol code: XZTU2021ZRG-06), and participants provided written informed consent. Data measurement and exercise interventions for all participants were conducted at Lhasa, Tibet.

2.2 | Experimental procedure

Before the exercise intervention, blood sampling collection, self-reported questionnaire on demographic data, basic physiological parameters (including HR, percutaneous arterial oxygen saturation [SpO2], body temperature, and blood pressure), and cardiopulmonary exercise testing (CPET) were carried out. Participants were randomly categorized into four groups: low-intensity (LI; $n = 34$), moderate-intensity (MI; $n = 30$), and high-intensity (HI; $n = 25$) exercise groups and the control group (CG; $n = 32$), which did not involve any intervention. The HR range of each exercise intensity was classified according to individual heart rate reserve (HRR). HRR was calculated by subtracting resting HR ($HR_{resting}$) from peak HR (HR_{peak}). This is as follows: LI interval = [0.3 HRR + $HR_{resting}$, 0.39 HRR + $HR_{resting}$]; MI interval = [0.4 HRR + $HR_{resting}$, 0.59 HRR + $HR_{resting}$]; HI interval = [0.6 HRR + $HR_{resting}$, 0.89 HRR + $HR_{resting}$] (Garber et al., 2011).

Participants in the intervention groups were instructed to perform 20 exercise sessions within 30 days. At each exercise intervention, the HR of participants was monitored using a polar heart rate meter (Polar OH1, Polar Electro Oy). We asked participants to reach their target HR within the first 5 min of exercise, and if their HR was not within the target range, they were reminded to adjust their speed. A single intervention ended when participants exercised at their target HR for 20 min, with the total duration not exceeding 30 min. Exercise was conducted on a motorized treadmill at the Exercise and Physiology Laboratory of Tibet University in August and September 2021. Participants completed the experimental posttest within 72 h after the last intervention to replicate all measures conducted before the start of the experiment.

2.3 | Blood tests

Participants were asked to refrain from drinking alcohol and vigorous exercising before blood collection. Fasting blood samples were collected via standard venipuncture techniques approach in a sitting position from 7:00 a.m. to 9:00 a.m. at Fokind Medical Care hospital in Lhasa, Tibet. Venous blood samples were drawn for plasma analysis of the erythrocyte index (red blood cell count [RBC], hemoglobin [HGB], hematocrit [HCT], mean corpuscular volume [MCV], mean corpuscular hemoglobin concentration [MCHC], standard deviation of red cell distribution width [RDW-SD], coefficient of the variation of red cell volume distribution width [RDW-CV]), immune function (total protein [TP], globulin [GLB], albumin [ALB], and albumin/globulin [A/G]), renal function (creatinine [CREA], uric acid [UA], urea and glomerular filtration rate [GFR]). The GFR was calculated using the chronic kidney disease epidemiology collaboration equation (Levey et al., 2009). Blood samples were analyzed in a standardized environment within 12 h after collection at Fokind Medical Care.

2.4 | Electrocardiography measures

Assessment of $HR_{resting}$, CO, and stroke volume (SV) was done using the Biopac MP150 hardware system (MP150, BIOPAC® Systems, Inc.). Specifically, the electrocardiogram (ECG) signal was recorded using an ECG100 C module for calculating $HR_{resting}$, the ECG lead numbers were 3, and the sampling frequency was 1 kHz. The impedance cardiography signal was recorded using an EBI100 C module to assess the SV and CO. The EBI100 C connected pairs of EL500 electrodes were attached to the neck and torso, and the amplifier, high-pass filter, low-pass filter, and frequency were set at 5 Ω/V, DC, 10 Hz, and 5 kHz, respectively. During the experiment, participants were asked to sit comfortably and quietly for 10 min. Raw ECG was used to measure the $HR_{resting}$ of participants, while SV

and CO were exported using the noninvasive cardiac output analysis employing AcqKnowledge software (v 4.2, Biopac Systems Inc.).

2.5 | Cardiopulmonary exercise testing

Cardiopulmonary function was assessed using CPET. After a 1-min rest on the power bicycle (EC3000e, Ergoline GmbH), participants then pedaled, maintaining a speed of 55–65 revolutions/min. The resistance of the power bicycle was increased by 30 W/min, starting from 0. Exhaustion was considered to have been reached when the participant could not maintain speed and the respiratory exchange ratio was exceeding 1.1 or when neither $\dot{V}O_2$ nor HR increased further with increasing speed/power (Lach et al., 2021). The $\dot{V}O_2$ measured at this point was considered the true peak oxygen uptake ($\dot{V}O_{2peak}$) of the participants in the HA environment followed by obtaining the HR_{peak}, $\dot{V}_E$, and work rate (WR) corresponding to this point.

2.6 | Statistical analysis

The Kolmogorov–Smirnov normality test was used to verify the normality of the observations. Based on the test outcome, the observations that did not satisfy the normality criterion were subjected to logarithmic transformation for subsequent analysis. One-way analysis of variance (ANOVA) was used to compare baseline data between groups. The remaining data were analyzed using 2 (time: pretest and posttest) × 4 (group: CG, LI, MI, and HI) repeated measures ANOVA, where the group was a between-participants factor and time was a within-participants factor. Bonferroni post hoc test was used to detected significant differences between groups and times. The criterion for significance was 0.05, and all *p*-values were two-tailed. Effect sizes were calculated using Cohen's method; low correlation range was 0.0–0.39, moderate 0.4–0.69, and high ≥0.7 (Cohen, 1988). Data are presented as means (SD) unless otherwise indicated.

The changes in the observations were calculated by subtracting the pretest value from the posttest value for correlation and mediation analyses. Pearson's correlation was applied to estimate the relationships between the changes in physiological responses. Structural equation modeling was performed to further assess the mediation effect between physiological functions. We used the bootstrapping methods recommended by Preacher and Hayes for testing mediation hypotheses using a resampling procedure of 5000 bootstrap samples (Preacher & Hayes, 2004). The ANOVA test and correlation analysis were calculated using IBM SPSS statistics 23 (IBM Corp.) software. Further mediation analysis was performed using the Mplus statistical package (version 8, Muthe'n & Muthe'n). Data visualization was done with RStudio (Version 2022.07.2, RStudio).

3 | RESULTS

Table 1 presents the fundamental demographic information of the four groups. The participants in each group were matched in age, height, weight, body mass index, SpO_2, $HR_{resting}$, and IPAQ (both $p > 0.077$). In addition, the baseline characteristics showed no significant differences across the four groups (both $p > 0.05$).

3.1 | Cardiopulmonary function

The changes in cardiopulmonary function before and after the exercise intervention were analyzed. The time × group interactions for $\dot{V}O_{2peak}$ [$F(3, 117) = 11.561$, $p < 0.001$, $\eta^2 = 0.229$] were significant. Figure 1A illustrates that the LI and MI exercises induced an increase in $\dot{V}O_{2peak}$ (both $p < 0.001$), whereas there was no significant change in HI group ($p = 0.680$). Although CG also exhibited a significant increase in $\dot{V}O_{2peak}$, the improvement in $\dot{V}O_{2peak}$ was almost twofold higher in LI and MI compared with CG (Table 2). Further, CO was significantly elevated in the LI group ($p = 0.001$), while $HR_{resting}$ remained unchanged in this group (Figure 1A, Table 2). This suggested that the improvement in SV (12.64 mL/beat) could contribute to the increased CO during low intensity exercise. Furthermore, the LI and MI exercise had a significant increase in $\dot{V}_E$ ($p = 0.002$ and $p < 0.001$), while it was unchanged in the HI group (Table 2). The results suggested that exercise intensity is important for the improvement in cardiorespiratory fitness in HA lowlanders.

TABLE 1 Baseline characteristics for the control group and three intervention groups.

Variables	Control group ($n = 32$)	Low-intensity group ($n = 34$)	Moderate-intensity group ($n = 30$)	High-intensity group ($n = 25$)
Age (yrs)	21.00 (0.98)	21.26 (1.05)	21.50 (1.31)	20.88 (1.13)
Height (m)	1.73 (0.07)	1.74 (0.06)	1.77 (0.05)	1.75 (0.05)
Weight (kg)	66.6 (15.5)	64.0 (6.9)	67.3 (10.3)	63.4 (9.4)
BMI (kg/m^2)	22.23 (4.49)	21.20 (2.08)	21.55 (2.80)	20.79 (3.06)
SpO_2 (mmHg)	91.3 (2.3)	89.6 (4.0)	90.3 (3.6)	91.2 (2.4)
$HR_{resting}$ (bpm)	81 (11)	82 (12)	80 (15)	88 (9)
IPAQ	2036 (1321)	2602 (1752)	3250 (3993)	1982 (1323)

Abbreviations: BMI, body mass index; $HR_{resting}$, resting heart rate; IPAQ, international physical activity questionnaire; SaO_2, arterial oxygen saturation.

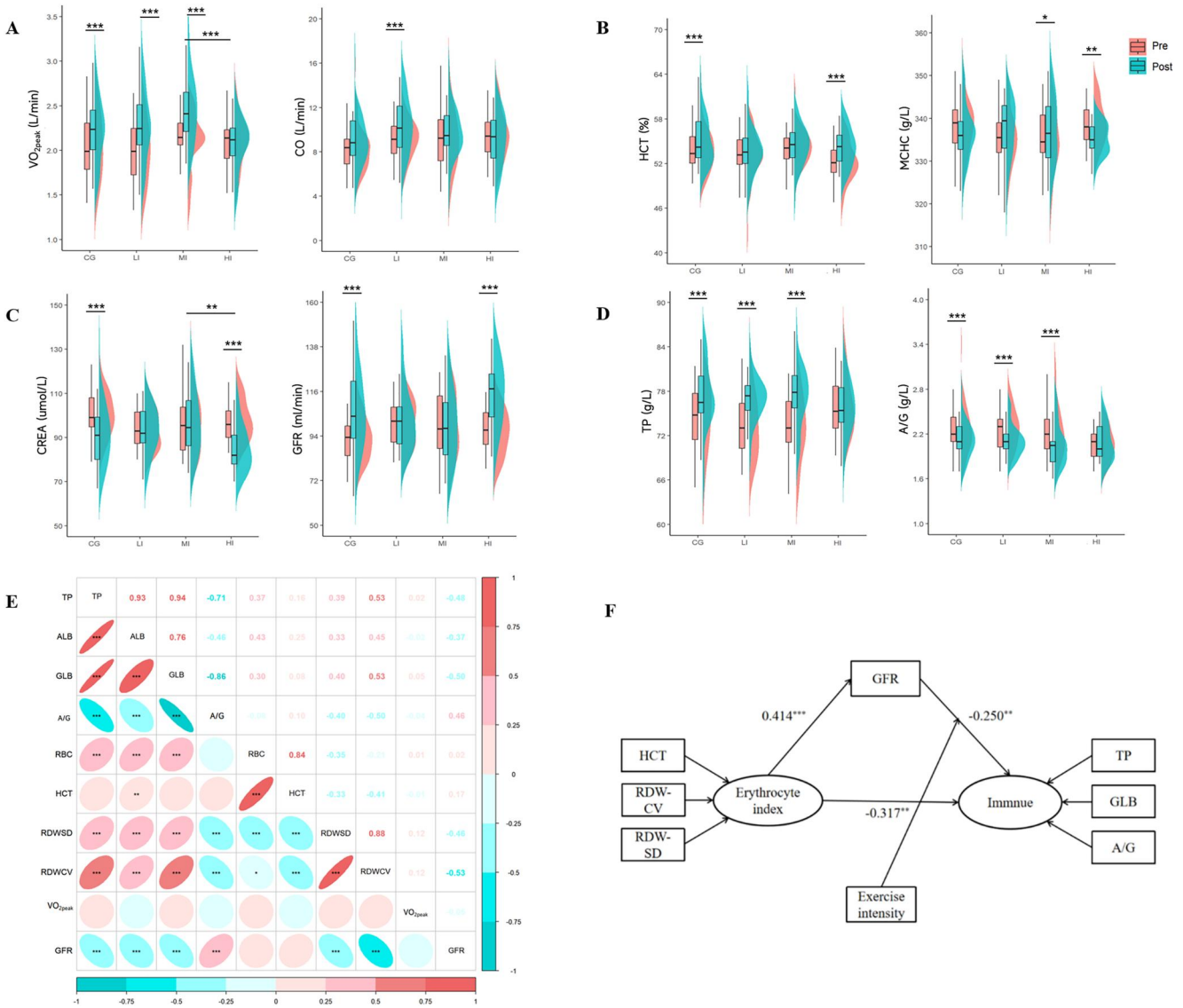

FIGURE 1 The changes and correlations among cardiopulmonary function, erythrocyte index, renal function, and immune function after exercise intervention. (A) Changes in cardiopulmonary function before and after intervention among four groups. (B) Changes in erythrocyte index before and after intervention among four groups. (C) Changes in renal function before and after intervention among four groups. (D) Changes in immune function before and after intervention among four groups. (E) The relationship between each system. The colors represent the relative direction. The numbers above the diagonal are Pearson's correlation coefficients. (F) Two pathways by which erythrocyte function affects immune function. $^{*}p < .05$, $^{**}p < .005$, $^{***}p < .001$. A/G, albumin/ globulin; ALB, albumin; CO, cardiac output; CREA, creatinine; GFR, glomerular filtration rate; GLB, globulin; HCT, hematocrit; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood count; RDW-CV, coefficient of variation of red cell volume distribution width; RDW-SD, standard deviation of red cell distribution width; TP, total protein; $\dot{V}O_{2peak}$, peak oxygen uptake.

3.2 | Erythrocyte index

The exercise intervention significantly affected the blood O_2 transport of lowlanders. For HCT, the time × group interaction [F (3, 117) = 2.714, $p = 0.048$, $\eta^2 = 0.065$] was significant. A total of 1.74% increase in HCT was observed during the 4-week period in the HI group ($p < 0.001$), whereas HCT appeared to be less affected in the LI ($p = 0.165$) and MI ($p = 0.657$) groups (Figure 1B, Table 2). In contrast, MCHC, an indicator of the average oxygen-carrying capacity of the red blood cells, significantly increased in the MI group ($p = 0.038$) and deceased in the HI group ($p = 0.009$). Interestingly, we also found an increased HCT in CG compared with baseline ($p = 0.007$), whereas RBC and HGB remained unchanged during the 4-week period in both control and exercise groups (Table 2).

3.3 | Renal function

The renal function outcomes are displayed in Table 3. There were no significant changes in urea and UA levels in any of the four groups.

TABLE 2 Cardiopulmonary function and erythrocyte index before and after the 4-week exercise.

	Control group		Low-intensity group		Moderate-intensity group		High-intensity group		Interaction effect	
Variables	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	*p*	η^2
Cardiopulmonary function										
$\dot{V}O_{2peak}$ (L/min)										
Pretest	2.05 (0.38)	0.162[a]	2.00 (0.36)	0.271[a]	2.11 (0.32)	0.350[a]	2.09 (0.28)	−0.020[d]	<0.001	0.229
Posttest	2.22 (0.36)		2.27 (0.40)		2.46 (0.38)		2.07 (0.29)			
HR_{peak} (bpm)										
Pretest	177 (12)	−0.563	173 (13)	1.529	174 (15)	−1.167	180 (8)	−8.680[a]	0.004	0.109
Posttest	177 (9)		175 (12)		173 (12)		171 (12)			
$\dot{V}_E$ (L/min)										
Pretest	119.14 (28.80)	5.466	110.71 (23.55)	12.327[a]	116.04 (26.12)	16.020[a]	115.06 (17.29)	−4.140[d]	0.005	0.105
Posttest	124.61 (26.95)		123.03 (28.38)		132.06 (26.67)		110.92 (20.65)			
WR (W)										
Pretest	199 (30)	9.375	204 (33)	6.471	209 (33)	6.000	200 (22)	2.600	0.682	0.013
Posttest	208 (26)		210 (29)		215 (27)		203 (23)			
CO (L/min)										
Pretest	8.36 (2.29)	0.700	9.09 (1.68)	1.249[a]	9.18 (2.67)	0.545	9.52 (2.14)	−0.390	0.041	0.068
Posttest	9.06 (2.30)		10.34 (2.46)		9.72 (2.21)		9.13 (2.32)			
SV (mL/beat)										
Pretest	103.42 (26.63)	7.039	112.73 (24.41)	12.640	116.70 (29.85)	6.205	108.56 (22.72)	7.485	0.603	0.016
Posttest	110.46 (26.40)		125.37 (25.06)		122.90 (32.68)		116.05 (26.40)			
LCWI (kg.m)										
Pretest	4.83 (1.40)	0.392	5.02 (1.32)	0.712[a,b]	5.11 (1.49)	0.271	5.44 (1.13)	−0.176	0.049	0.065
Posttest	5.22 (1.46)		5.90 (1.33)		5.38 (1.23)		5.26 (1.24)			
$HR_{resting}$ (bpm)										
Pretest	81 (11)	3.456	82 (12)	0.131	80 (15)	−0.159	88 (9)	−9.549[a]	0.001	0.139
Posttest	85 (12)		82 (10)		80 (11)		78 (12)			
$\dot{V}O_2$/HR (mL/min/bpm)										
Pretest	14.16 (2.78)	0.406	13.65 (2.55)	1.971[a]	14.23 (2.25)	2.500[a]	13.56 (2.20)	1.120[a]	0.009	0.093
Posttest	14.56 (2.40)		15.62 (3.14)		16.73 (3.75)		14.68 (2.79)			
$\dot{V}E/\dot{V}\ CO_2$										
Pretest	47.17 (6.53)	1.056	43.32 (7.63)	3.521[a]	42.44 (6.11)	5.430[a]	46.44 (4.09)	1.308	0.038	0.069
Posttest	48.23 (7.64)		46.84 (5.85)		47.87 (5.47)		47.75 (5.17)			
Erythrocyte index										
RBC (10^12/L)										
Pretest	5.85 (0.41)	0.115	5.87 (0.30)	0.083	5.92 (0.42)	0.042	5.66 (0.36)	0.111	0.662	0.013
Posttest	5.96 (0.41)		5.96 (0.39)		5.96 (0.40)		5.77 (0.34)			
HGB (g/L)										
Pretest	182.03 (9.11)	3.000	178.17 (11.93)	2.618	181.47 (9.23)	2.000	177.40 (8.14)	4.080	0.746	0.010
Posttest	185.03 (9.50)		180.79 (10.34)		183.47 (8.99)		181.48 (8.45)			

(Continues)

TABLE 2 (Continued)

	Control group		Low-intensity group		Moderate-intensity group		High-intensity group		Interaction effect	
Variables	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	p	η^2
HCT (%)										
Pretest	53.92 (3.01)	1.059[a]	53.13 (3.33)	0.521	54.18 (2.57)	0.177	52.40 (2.56)	1.740[a]	0.048	0.065
Posttest	54.98 (3.10)		53.65 (2.99)		54.35 (2.73)		54.14 (2.50)			
MCHC (g/L)										
Pretest	337.91 (6.23)	−1.281	335.32 (6.72)	1.882	335.03 (7.47)	2.567[a]	338.72 (4.97)	−3.560[a]	0.002	0.116
Posttest	336.63 (6.37)		337.21 (7.09)		337.60 (7.69)		335.16 (3.68)			
MCV (fL)										
Pretest	92.33 (3.56)	0.034	90.58 (5.97)	−0.259	91.82 (6.10)	−0.353	92.67 (3.44)	1.236[a,b]	0.005	0.105
Posttest	92.36 (3.40)		90.32 (6.11)		91.47 (6.79)		93.91 (3.67)			
RDW-SD (fL)										
Pretest	43.64 (2.61)	−0.297	43.10 (2.44)	0.750[a]	43.18 (3.50)	0.647[a]	44.36 (1.76)	−0.664	0.009	0.093
Posttest	43.34 (1.93)		43.85 (2.15)		43.82 (3.10)		43.69 (1.92)		0.006	0.101
RDW-CV (%)										
Pretest	13.26 (0.67)	−0.063	13.37 (0.82)	0.274[a,b]	13.18 (0.77)	0.217[a]	13.56 (0.72)	−0.484[a,c]	<0.001	0.212
Posttest	13.20 (0.67)		13.65 (0.83)		13.40 (0.62)		13.08 (0.55)			

Abbreviations: CO, cardiac output; Delta (Δ), the changes calculated by subtracting the pretest value from the posttest value; HCT, hematocrit; HGB, hemoglobin; HR_{max}, peak heart rate; $HR_{resting}$, resting heart rate; LCWI, left ventricular stroke work index; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell count; RDW-CV, coefficient of variation of red cell volume distribution width; RDW-SD, standard deviation of red cell distribution width; SV, stroke volume; $\dot{V}_E$, ventilation; $\dot{V}O_{2peak}$, peak oxygen uptake; $\dot{V}E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $\dot{V}O_2/HR$, maximal oxygen pulse; WR, work rate.

[a]$p < 0.05$ for comparing changes in with-in group from pretest to posttest.

[b]$p < 0.05$ for the changes in posttest compared with control group.

[c]$p < 0.05$ for the changes in posttest compared with low-intensity group.

[d]$p < 0.05$ for the changes in posttest compared with moderate-intensity group.

However, the significant time × group interactions for CREA [F (3, 117) = 9.405, $p < 0.001$, and $\eta^2 = 0.194$], and GFR [F (3, 117) = 9.672, $p < 0.001$, and $\eta^2 = 0.199$] were found. The CG and HI groups experienced significant decrease in CREA (both $p < 0.001$) and increases in GFR (both $p < 0.001$), whereas there were no changes in the LI and MI groups after 4 weeks of exercise (Figure 1C, Table 3). Meanwhile, the CREA was reduced to a lesser extent in the HI group than in the MI group ($p = 0.005$).

3.4 | Immune function

As shown in Table 3, the levels of TP, ALB, and GLB increased to a similar extent in CG and LI and MI groups at posttest compared to pretest (both $p < 0.001$), whereas the A/G was decreased in CG and LI and MI groups (both $p < 0.001$). Those results suggested a slight increase in the immune responses during the 4-week period in the above groups. In contrast, the immune function remained unaltered in the HI group after the 4-week exercise interventions (Figure 1D, Table 3).

3.5 | A moderated mediation model

The relationship between changes in physiological functions after exercise performed in an HA environment and the role of different exercise intensities were explored by calculating the changes in physiological responses by subtracting the pretest from the posttest value using a structural equation model. Before the structural equation analysis, Pearson's correlation coefficients were calculated to estimate the relationship between changes in physiological functions. The relationships between multiple datasets are shown in Figure 1E.

According to the model (Figure 1F), the kidney function mediated the relationship between erythrocyte indices and immune function ($\beta = -0.104$, 95% confidence interval [CI_{95}] [−0.203, −0.040]). The direct effect of erythrocyte indices on immune function was significant ($\beta = -0.317$, CI_{95} [−0.537, −0.159]). The moderating effect of exercise intensity was also significant. When exercise intensity was low, the indirect effect of erythrocyte indices on immune function was significant ($\beta = 0.182$, CI_{95} [0.047, 0.357]); however, when exercise intensity was moderate ($\beta = 0.020$, CI_{95} [−0.066, 0.124]) or

TABLE 3 Change of the renal and immune function after the 4-week aerobic exercise.

	Control group		Low-intensity group		Moderate-intensity group		High-intensity group		Interaction effect	
Variables	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	Mean (SD)	Delta (Δ)	*p*	η^2
Renal function										
CREA (mg/dL)										
Pretest	100.16 (9.93)	−8.469[a]	94.09 (9.00)	−0.912	96.27 (13.45)	0.367	97.68 (9.22)	−12.720[a,c]	0.000	0.194
Posttest	91.69 (14.12)		93.18 (10.45)		96.63 (14.37)		84.96 (10.30)			
GFR (mL/min)										
Pretest	93.33 (11.63)	12.247[a]	100.31 (11.41)	1.776	98.62 (15.90)	−0.013	96.13 (11.12)	18.415[a,b,c]	0.000	0.199
Posttest	105.57 (19.24)		102.09 (14.92)		98.61 (17.71)		114.54 (16.22)			
Urea (mg/dL)										
Pretest	5.13 (1.38)	−0.103	4.80 (1.04)	−0.053	5.09 (1.03)	0.253[b]	5.03 (1.34)	0.468[a,b]	0.109	0.050
Posttest	5.02 (1.34)		4.75 (0.93)		5.34 (1.12)		5.50 (1.08)			
UA (mg/dL)										
Pretest	467.91 (93.68)	−3.594	474.85 (84.10)	−16.588	440.07 (80.88)	4.300	456.80 (86.29)	20.360	0.202	0.039
Posttest	464.31 (85.99)		458.26 (94.26)		444.37 (84.79)		477.16 (68.12)			
The immune function										
TP (g/L)										
Pretest	74.27 (4.84)	2.766[a]	73.29 (4.20)	3.721[a]	73.24 (4.43)	3.940[a]	75.90 (3.90)	−0.096	0.002	0.119
Posttest	77.03 (4.77)		77.01 (2.87)		77.18 (4.04)		75.81 (3.47)			
ALB (g/L)										
Pretest	51.15 (2.17)	0.919[a]	50.65 (1.55)	1.394[a]	50.13 (2.07)	1.517[a]	50.97 (1.72)	0.096	0.097	0.052
Posttest	52.07 (2.34)		52.04 (1.43)		51.64 (2.48)		51.07 (1.71)			
GLB (g/L)										
Pretest	23.12 (3.51)	1.847[a]	22.64 (3.16)	2.326[a]	23.11 (3.47)	2.423[a]	24.93 (2.85)	−0.192	0.000	0.177
Posttest	24.96 (3.15)		24.97 (2.28)		25.54 (3.10)		24.74 (2.71)			
A/G										
Pretest	2.26 (0.35)	−0.147[a]	2.27 (0.26)	−0.171[a]	2.21 (0.34)	−0.157[a]	2.07 (0.22)	0.016	0.000	0.185
Posttest	2.12 (0.24)		2.10 (0.21)		2.06 (0.29)		2.08 (0.24)			

Abbreviations: A/G, albumin/globulin; ALB, albumin; CREA, creatinine; Delta (Δ), the changes calculated by subtracting the pretest value from the posttest value; GFR, glomerular filtration rate; GLB, globulin; TP, total protein; UA, uric acid.

[a] $p < 0.05$ for comparing changes in with-in group from pretest to posttest.

[b] $p < 0.05$ for the changes in posttest compared with low-intensity group.

[c] $p < 0.05$ for the changes in posttest compared with moderate-intensity group.

high ($\beta = 0.009$, CI_{95} [−0.250, 0.046]), the indirect effect was not significant. Model fit was assessed using a comparative fit index (CFI = 0.879) and the Tucker-–Lewis index (TLI = 0.853).

4 | DISCUSSION

Our study examined the effect of long-term aerobic exercise with different intensities on the physiological responses of acclimated lowlanders. We found that LI and MI exercises are effective stimuli for the improvement in O_2 transport and the prevention of erythrocytosis in acclimatized lowlanders. However, HI exercise is less effective than LI and MI exercise as results in erythrocytosis, and induces an overloaded renal adaption to HA with no improvement in O_2 transport. These results have essentially validated the previously formulated hypotheses.

Given that hypobaric hypoxia is unavoidable and sustained at HA, maintaining aerobic metabolism and adjusting physiological acclimatization is important at HA (McClelland & Scott, 2019). The indigenous people living on the Tibetan plateau are a prime example

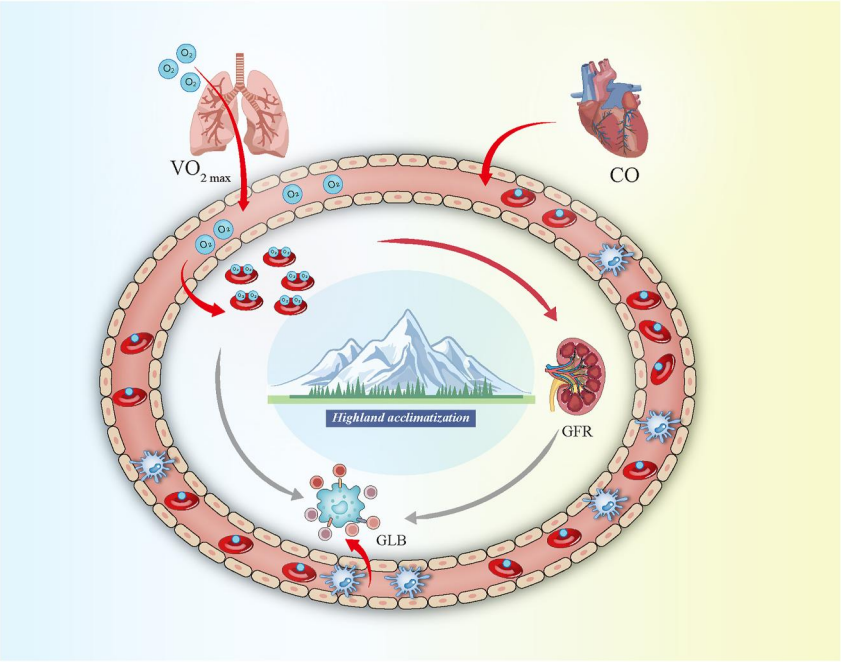

FIGURE 2 Changes in physiological acclimatization of lowlanders after low-moderate intensity exercise. $\dot{V}O_{2max}$ and CO increased after the exercise intervention, and the erythrocyte index positively affected renal function, as indicated by the red arrow. The erythrocyte index and renal function negatively affected immune function, indicated by the gray arrows. ALB, albumin; CO, cardiac output; GFR, glomerular filtration rate; GLB, globulin; TP, total protein; $\dot{V}O_{2max}$, maximal oxygen uptake.

of successful adaptation to HA (Wu, 2001). Studies have shown that Tibetan natives, compared to lowlanders at HA, exhibit greater exercise capacity and lower HGB concentrations (Simonson et al., 2015). Lowlanders exposed to HA obtain higher O_2 uptake mainly by increasing HGB; however, excessive erythrocytosis is associated with risk for cardiovascular disease and is considered maladaptive (Julian & Moore, 2019). Our study showed that LI and MI exercises improve O_2 transport without erythrocytosis. Participants in the LI and MI groups experienced an increase in $\dot{V}O_{2peak}$ after the intervention, consistent with a previous study finding that exercise training in highlanders can successfully improve aerobic capacity and exercise workload (Macarlupu et al., 2021). Additionally, we found that LI exercise increased CO, as has been reported previously (Nystoriak & Bhatnagar, 2018). Increased CO level is the initial cardiovascular response to altitude and can increase aerobic capacity; lowlanders chronically exposed to HA are known to return to the normal baseline CO (Naeije, 2010). This observation provides new insights into the simultaneous increase of CO and $\dot{V}O_{2peak}$ in an HA environment, both of which can increase the diffusional transport of O_2.

Further analysis showed that LI and MI exercises did not produce a significant increase in the erythrocyte index after the intervention, which differs from the results of the "Living Low-Training High" (LLTH) model. The success of this model is attributable to the erythropoietic effects of hypoxia (Wilber et al., 2007). Some studies have confirmed that the erythrocytes of lowlanders during prolonged exposure to HA tend to stabilize as the duration of altitude exposure extends (Luks & Hackett, 2022). Therefore, the LI exercise does not disrupt homeostasis and does not lead to erythrocytosis. It is sufficient to change the body's respiratory function (Crowley et al., 2022) and improve O_2 uptake. In particular, we found an increase in immune response after LI and MI exercise. The results are consistent with previous studies reporting an increase in oxidative stress and the subsequent inflammatory response in LI exercise at chronic HA exposure (Quindry et al., 2016). This may serve a protective role by setting an immune response in motion (Pham et al., 2021). Thus, the upregulated immune response in our study could be considered as an adaptive regulation of inflammation to promote O_2 supply (Harris et al., 2014).

However, in the present study, lowlanders experienced some adverse effects when performing HI exercise at HA, such as erythrocytosis without any improvement in aerobic capacity. Unlike the LLTH study that was performed at moderate altitudes, our study was performed at 3680 m, and showed that the increase in RBC induced by HA exercise did not lead to an increase in O_2 transport capacity. This may be because exercise is a stressor, and the effect of stress on organisms is dose-dependent (Salminen & Kaarniranta, 2010), suggesting that a higher intensity of stress overwhelms the organism and can evoke detrimental effects (Gems & Partridge, 2008) and disrupt physiological homeostasis. Moreover, an elevated RBC level increases HCT level and blood viscosity; therefore, the O_2 uptake of the participants did not improve by exercise (Crawford et al., 2017). In addition,

we found that different from LI and MI of exercises, GFR increased as HCT increased after the HI exercise. However, previous studies showed that the increase in HCT was accompanied by an increase in blood viscosity at chronic exposure to HA, which may lead to a decreased GFR (Palubiski et al., 2020). In our study, given the physiological challenge of both hypoxia and HI exercise, renal adaption to HA could be overloaded, potentially resulting in an elevated GFR.

Notably, during the current experiment, some changes occurred in the CG, including slight increases in $\dot{V}O_{2peak}$, HCT, GFR, and TP, which may have been caused by a decrease in FIO_2 and oxygen concentration in atmosphere. Shi et al. (2021) found that oxygen concentration on the Tibetan plateau is positively related to air temperature. Our study was conducted over a month from August to September, when the air temperature gradually decreased from 18°C to 13°C and the oxygen concentration decreased from 20.282% to 20.176%. Thus, the increase of $\dot{V}O_{2peak}$ in CG is associated with increases in blood viscosity and renal function responses, which may be a potential compensation mechanism to resist the adverse impacts of decrease in O_2 supply (Luks, 2015; Palubiski et al., 2020; Simonson et al., 2015). This mechanism is commonly employed by the lowlanders who experience chronic exposure to HA to ensure enough O_2 supply (West, 2017). However, for LI and MI groups, the increase in $\dot{V}O_{2peak}$ did not lead to physiological compensation, even when accounting for the challenge of a decline in oxygen concentration.

Finally, our study demonstrated that GFR mediates the effect of erythrocytes on the immune function, which was strengthened by lower intensity activity. Considering that human erythrocytes have an important function in the innate immune system (Hotz et al., 2018), the direct relationship between the erythrocyte index and immunity may be expected. Additionally, as mentioned above, the blood viscosity of lowlanders was increased at chronic HA exposure due to the increase in HCT, which was accompanied by an adaptative response in renal plasma flow and caused the change in GFR (Palubiski et al., 2020). The kidneys further regulate fluid and acid–base homeostasis to ensure physiological adaptation to HA, which may be related to individual response to inflammation (Goldfarb-Rumyantzev & Alper, 2014), since resistance to hypoxia stress is associated with anti-inflammation responses. By this means, renal function mediates the relationship between erythrocytes and immunity. The intensity and duration of exercise stress have a dose-dependent effect on the induction of in vivo immunity in humans (Diment et al., 2015). The change in renal function during LI exercise positively affects the change in immune function, which is a normal physiological regulation of the body. However, intense physical activity at HA exacerbates the extent of the oxidative challenge (Dosek et al., 2007). Therefore, this regulatory response gradually diminishes with an increase in exercise intensity. This finding provides further evidence that an intensity threshold exists for exercise performed at HA and that excessively high intensities of exercise can have negative effects.

While this study fills the gap in understanding the physiological response after HA exercise with different intensities, it also unavoidably possesses certain limitations. First, the participants were homogeneous and the experiment was conducted at a fixed altitude (3680 m), challenging the generalizability of the results. Second, the measurements used to evaluate the individual systems and mechanistic explanation were not comprehensive; for example, only immune proteins in the blood were used to assess immune function. Future studies combining enzyme-linked immunosorbent assay (ELISA) need to be conducted to explore the molecular mechanism of exercise effect on HA acclimatization. Third, although previous studies found similar HR_{peak} values (Basset & Boulay, 2003; Zhou et al., 1997) or slightly higher values (Price et al., 2022) in treadmill testing compared with cycle ergometers, conducting an HR_{peak} testing through cycle ergometers, but using treadmill exercise for intervention limits the accuracy of the exercise prescription of this study. The uniformity in measurement and intervention is expected to be improved in the future.

In conclusion, for lowlanders at HA, the benefits of exercise cannot be generalized. This study demonstrates that LI and MI exercises improve O_2 uptake capacity while avoiding an excessive increase in red blood cells and inflammation (Figure 2); thus, further improving HA acclimatization among lowlanders on the Tibetan plateau. However, HI exercise does not improve O_2 uptake capacity and increases blood viscosity. Therefore, subsequent studies should consider exercise mode, duration, and altitude to identify more effective exercise approaches to promote HA acclimatization.

AUTHOR CONTRIBUTIONS

Rui Su: Conceptualization, methodology, writing—reviewing and Editing. **Chenxiao Han:** Methodology, visualization, formal analysis. **Guiquan Chen**: Intervention, resources. **Wanying Liu** and **Chengzhi Wang**: Data curation. **Yuming Zhang** and **Hao Li:** Investigation, validation. **Delong Zhang:** Reviewing and Editing. **Hailin Ma:** Supervision, project administration, funding acquisition. All the authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation Regional innovation development joint fund project of China (U23A20476); Science and Technology Major Project of Tibetan Autonomous Region of China (XZ202201ZD0001G); National Natural Science Foundation of China (32260212); and Scientific Development funds for Local Region from the Chinese Government in 2023 (XZ202301YD0032C).

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

ORCID

Rui Su https://orcid.org/0000-0001-8104-9145

REFERENCES

Bärtsch, Peter, and Erik R. Swenson. 2013. "Clinical Practice: Acute High-Altitude Illnesses." *New England Journal of Medicine* 368(24): 2294–302. https://doi.org/10.1056/NEJMcp1214870.

Basset, F. A., and M. R. Boulay. 2003. "Treadmill and Cycle Ergometer Tests Are Interchangeable to Monitor Triathletes Annual Training." *Journal of Sports Science and Medicine* 2(3): 110–6. https://www.jssm.org/jssm-02-110.xml%3EFulltext.

Calbet, J. A. L., R. Boushel, G. Rådegran, H. Søndergaard, P. D. Wagner, and B. Saltin. 2003. "Why Is VO_{2max} after Altitude Acclimatization Still Reduced Despite Normalization of Arterial O_2 Content?" *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 284(2): R304–16. https://doi.org/10.1152/ajpregu.00156.2002.

Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. (2nd ed.). Hillsdale, NJ: Lawence Erlbaum. https://doi.org/10.4324/9780203771587.

Crawford, Jacob E., Ricardo Amaru, Jihyun Song, Colleen G. Julian, Fernando Racimo, Jade Yu Cheng, Xiuqing Guo, et al. 2017. "Natural Selection on Genes Related to Cardiovascular Health in High-Altitude Adapted Andeans." *The American Journal of Human Genetics* 101(5): 752–67. https://doi.org/10.1016/j.ajhg.2017.09.023.

Crowley, Emmet, Cormac Powell, Brian P. Carson, and Robert W Davies. 2022. "The Effect of Exercise Training Intensity on VO_{2max} in Healthy Adults: an Overview of Systematic Reviews and Meta-Analyses." *Translational Sports Medicine* 2022: 1–10. https://doi.org/10.1155/2022/9310710.

Diment, Bethany C., Matthew B. Fortes, Jason P. Edwards, Helen G. Hanstock, Mark D. Ward, Huw M. Dunstall, Peter S. Friedmann, and Neil P. Walsh. 2015. "Exercise Intensity and Duration Effects on In Vivo Immunity." *Medicine & Science in Sports & Exercise* 47(7): 1390–8. https://doi.org/10.1249/MSS.0000000000000562.

Dosek, Agoston, Hideko Ohno, Zoltan Acs, Albert W. Taylor, and Zsolt Radak. 2007. "High Altitude and Oxidative Stress." *Respiratory Physiology & Neurobiology* 158(2–3): 128–31. https://doi.org/10.1016/j.resp.2007.03.013.

Edsell, Mark E., Yashvi H. Wimalasena, William L. Malein, Kimberly M. Ashdown, Carla A. Gallagher, Chris H. Imray, Alex D. Wright, Stephen D. Myers, and B. M. R. E. Society. 2014. "High-intensity Intermittent Exercise Increases Pulmonary Interstitial Edema at Altitude but Not at Simulated Altitude." *Wilderness and Environmental Medicine* 25(4): 409–15. https://doi.org/10.1016/j.wem.2014.06.016.

Garber, Carol Ewing, Bryan Blissmer, Michael R. Deschenes, Barry A. Franklin, Michael J. Lamonte, I.-Min Lee, David C. Nieman, David P. Swain. and Amer Coll Sports Med. 2011. "Quantity and Quality of Exercise for Developing and Maintaining Cardiorespiratory, Musculoskeletal, and Neuromotor Fitness in Apparently Healthy Adults: Guidance for Prescribing Exercise." *Medicine & Science in Sports & Exercise* 43(7): 1334–59. https://doi.org/10.1249/MSS.0b013e318213fefb.

Gassmann, Max, Heimo Mairbäurl, Leonid Livshits, Svenja Seide, Matthes Hackbusch, Monika Malczyk, Simone Kraut, et al. 2019. "The Increase in Hemoglobin Concentration with Altitude Varies Among Human Populations." *Annals of the New York Academy of Sciences* 1450(1): 204–20. https://doi.org/10.1111/nyas.14136.

Gems, David, and Linda Partridge. 2008. "Stress–response Hormesis and Aging: "That Which Does Not Kill Us Makes Us Stronger"." *Cell Metabolism* 7(3): 200–3. https://doi.org/10.1016/j.cmet.2008.01.001.

Goldfarb-Rumyantzev, A. S., and S. L. Alper. 2014. "Short-term Responses of the Kidney to High Altitude in Mountain Climbers." *Nephrology Dialysis Transplantation* 29(3): 497–506. https://doi.org/10.1093/ndt/gft051.

Hartley, L. H., J. K. Alexander, M. Modelski, and R. F. Grover. 1967. "Subnormal Cardiac Output at Rest and during Exercise in Residents at 3,100 M Altitude." *Journal of Applied Physiology* 23(6): 839–48. https://doi.org/10.1152/jappl.1967.23.6.839.

Hotz, Meghan J., Danielle Qing, Michael G. S. Shashaty, Peggy Zhang, Hilary Faust, Neal Sondheimer, Stefano Rivella, G. Scott Worthen, and Nilam S. Mangalmurti. 2018. "Red Blood Cells Homeostatically Bind Mitochondrial DNA through TLR9 to Maintain Quiescence and to Prevent Lung Injury." *American Journal of Respiratory and Critical Care Medicine* 197(4): 470–80. https://doi.org/10.1164/rccm.201706-1161OC.

Julian, Colleen G., and Lorna G. Moore. 2019. "Human Genetic Adaptation to High Altitude: Evidence from the Andes." *Genes* 10(2): 150. https://doi.org/10.3390/genes10020150.

Lach, Jacek, Szczepan Wiecha, Daniel Śliż, Szymon Price, Mateusz Zaborski, Igor Cieśliński, Marek Postuła, Beat Knechtle, and Artur Mamcarz. 2021. "HR Max Prediction Based on Age, Body Composition, Fitness Level, Testing Modality and Sex in Physically Active Population." *Frontiers in Physiology* 12: 695950. https://doi.org/10.3389/fphys.2021.695950.

Laughlin, M. Harold, Michael J. Davis, Niels H. Secher, Johannes J. van Lieshout, Arturo A. Arce-Esquivel, Grant H. Simmons, Shawn B. Bender, et al. 2012. "Peripheral Circulation." *Comprehensive Physiology* 2(1): 321–447. https://doi.org/10.1002/cphy.c100048.

Levey, Andrew S., Lesley A. Stevens, Christopher H. Schmid, Yaping Lucy Zhang, Alejandro F. Castro, Harold I. Feldman, John W. Kusek, et al. 2009. "A New Equation to Estimate Glomerular Filtration Rate." *Annals of Internal Medicine* 150(9): 604–12. https://doi.org/10.7326/0003-4819-150-9-200905050-00006.

Luks, Andrew M. 2015. "Physiology in Medicine: A Physiologic Approach to Prevention and Treatment of Acute High-Altitude Illnesses." *Journal of Applied Physiology* 118(5): 509–19. https://doi.org/10.1152/japplphysiol.00955.2014.

Luks, Andrew M., and Peter H. Hackett. 2022. "Medical Conditions and High-Altitude Travel." *New England Journal of Medicine* 386(4): 364–73. https://doi.org/10.1056/NEJMra2104829.

Macarlupú, José Luis, Gustavo Vizcardo-Galindo, Rómulo Figueroa-Mujíca, Nicolas Voituron, J.-Paul Richalet, and Francisco C. Villafuerte. 2021. "Sub-maximal Aerobic Exercise Training Reduces Haematocrit and Ameliorates Symptoms in Andean Highlanders with Chronic Mountain Sickness." *Experimental Physiology* 106(11): 2198–209. https://doi.org/10.1113/EP089975.

Mairbäurl, Heimo. 2013. "Red Blood Cells in Sports: Effects of Exercise and Training on Oxygen Supply by Red Blood Cells." *Frontiers in Physiology* 4: 332. https://doi.org/10.3389/fphys.2013.00332.

Mallet, Robert T., Johannes Burtscher, J.-Paul Richalet, Gregoire P. Millet, and Martin Burtscher. 2021. "Impact of High Altitude on Cardiovascular Health: Current Perspectives." *Vascular Health and Risk Management* 17: 317–35. https://doi.org/10.2147/VHRM.S294121.

Mazzeo, Robert S. 2008. "Physiological Responses to Exercise at Altitude: an Update. Sports Medicine." *Sports Medicine* 38(1): 1–8. https://doi.org/10.2165/00007256-200838010-00001.

McClelland, Grant B., and Graham R. Scott. 2019. "Evolved Mechanisms of Aerobic Performance and Hypoxia Resistance in High-Altitude Natives." *Annual Review of Physiology* 81(1): 561–83. https://doi.org/10.1146/annurev-physiol-021317-121527.

Mishra, K. P., and Lilly Ganju. 2010. "Influence of High Altitude Exposure on the Immune System: A Review." *Immunological Investigations* 39(3): 219–34. https://doi.org/10.3109/08820131003681144.

Mourot, Laurent. 2018. "Limitation of Maximal Heart Rate in Hypoxia: Mechanisms and Clinical Importance." *Frontiers in Physiology* 9: 972. https://doi.org/10.3389/fphys.2018.00972.

Naeije, Robert. 2010. "Physiological Adaptation of the Cardiovascular System to High Altitude." *Progress in Cardiovascular Diseases* 52(6): 456–66. https://doi.org/10.1016/j.pcad.2010.03.004.

Nystoriak, Matthew A., and Aruni Bhatnagar. 2018. "Cardiovascular Effects and Benefits of Exercise." *Frontiers in Cardiovascular Medicine* 5: 135. https://doi.org/10.3389/fcvm.2018.00135.

Palubiski, Lisa M., Ken D. O'Halloran, and Julie O'Neill. 2020. "Renal Physiological Adaptation to High Altitude: A Systematic Review." *Frontiers in Physiology* 11: 756. https://doi.org/10.3389/fphys.2020.00756.

Park, H.-Young, J.-Weon Kim, and S.-Seok Nam. 2022. "Metabolic, Cardiac, and Hemorheological Responses to Submaximal Exercise under Light and Moderate Hypobaric Hypoxia in Healthy Men." *Biology* 11(1): 144. https://doi.org/10.3390/biology11010144.

Pham, Kathy, Keval Parikh, and Erica C. Heinrich. 2021. "Hypoxia and Inflammation: Insights from High-Altitude Physiology." *Frontiers in Physiology* 12: 676782. https://doi.org/10.3389/fphys.2021.676782.

Preacher, Kristopher J., and Andrew F. Hayes. 2004. "SPSS and SAS Procedures for Estimating Indirect Effects in Simple Mediation Models." *Behavior Research Methods* 36(4): 717–31. https://doi.org/10.3758/BF03206553.

Price, Szymon, Szczepan Wiecha, Igor Cieśliński, Daniel Śliż, Przemysław Seweryn Kasiak, Jacek Lach, Grzegorz Gruba, Tomasz Kowalski, and Artur Mamcarz. 2022. "Differences between Treadmill and Cycle Ergometer Cardiopulmonary Exercise Testing Results in Triathletes and Their Association with Body Composition and Body Mass Index." *International Journal of Environmental Research and Public Health* 19(6): 3557. https://doi.org/10.3390/ijerph19063557.

Quindry, John, Charles Dumke, Dustin Slivka, and Brent Ruby. 2016. "Impact of Extreme Exercise at High Altitude on Oxidative Stress in Humans." *The Journal of Physiology* 594(18): 5093–104. https://doi.org/10.1113/JP270651.

Salminen, Antero, and Kai Kaarniranta. 2010. "ER Stress and Hormetic Regulation of the Aging Process." *Ageing Research Reviews* 9(3): 211–7. https://doi.org/10.1016/j.arr.2010.04.003.

Shephard, Roy J. 1988. "PAR-Q, Canadian Home Fitness Test and Exercise Screening Alternatives." *Sports Medicine* 5(3): 185–95. https://doi.org/10.2165/00007256-198805030-00005.

Shi, Peijun, Yanqiang Chen, Gangfeng Zhang, Haiping Tang, Zhi Chen, Deyong Yu, Jing Yang, et al. 2021. "Factors Contributing to Spatial-Temporal Variations of Observed Oxygen Concentration over the Qinghai-Tibetan Plateau." *Scientific Reports* 11(1): 17338. https://doi.org/10.1038/s41598-021-96741-6.

Simonson, T. S., G. Wei, H. E. Wagner, T. Wuren, G. Qin, M. Yan, P. D. Wagner, and R. L. Ge. 2015. "Low Haemoglobin Concentration in Tibetan Males Is Associated with Greater High-Altitude Exercise Capacity." *The Journal of Physiology* 593(14): 3207–18. https://doi.org/10.1113/JP270518.

Vargas P, Enrique, and Hilde Spielvogel. 2006. "Chronic Mountain Sickness, Optimal Hemoglobin, and Heart Disease." *High Altitude Medicine & Biology* 7(2): 138–49. https://doi.org/10.1089/ham.2006.7.138.

Walmsley, Sarah, Alison Harris, A. A. Roger Thompson, and MoiraK. B. Whyte. 2014. "HIF-Mediated Innate Immune Responses: Cell Signaling and Therapeutic Implications." *Hypoxia* 2: 47–58. https://doi.org/10.2147/HP.S50269.

West, J. B. 2006. "Human Responses to Extreme Altitudes." *Integrative and Comparative Biology* 46(1): 25–34. https://doi.org/10.1093/icb/icj005.

West, John B. 2017. "Physiological Effects of Chronic Hypoxia." *New England Journal of Medicine* 376(20): 1965–71. https://doi.org/10.1056/NEJMra1612008.

Wilber, Randall L., James Stray-Gundersen, and Benjamin D. Levine. 2007. "Effect of Hypoxic "Dose" on Physiological Responses and Sea-Level Performance." *Medicine & Science in Sports & Exercise* 39(9): 1590–9. https://doi.org/10.1249/mss.0b013e3180de49bd.

Wu, Tianyi. 2001. "The Qinghai-Tibetan Plateau: How High Do Tibetans Live?" *High Altitude Medicine & Biology* 2(4): 489–99. https://doi.org/10.1089/152702901753397054.

Zhang, Ying, and Ning Chen. 2018. "Autophagy Is a Promoter for Aerobic Exercise Performance during High Altitude Training." *Oxidative Medicine and Cellular Longevity* 2018: 3617508–11. https://doi.org/10.1155/2018/3617508.

Zhou, S., S. J. Robson, M. J. King, and A. J. Davie. 1997. "Correlations between Short-Course Triathlon Performance and Physiological Variables Determined in Laboratory Cycle and Treadmill Tests." *The Journal of Sports Medicine and Physical Fitness* 37(2): 122. https://doi.org/10.1016/S1050-6411(97)88884-0.