Biomedicine & Pharmacotherapy 116 (2019) 109003

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Trimetazidine attenuates high-altitude fatigue and cardiorespiratory fitness impairment: A randomized double-blinded placebo-controlled clinical trial

Jie Yang[a,b], Laiping Zhang[a,b], Chuan Liu[a,b], Jihang Zhang[a,b], Shiyong Yu[a,b], Jie Yu[a,b], Shizhu Bian[a,b], Sanjiu Yu[a,b], Chen Zhang[a,b], Lan Huang[a,b,*]

[a] Institute of Cardiovascular Diseases of PLA, the Second Affiliated Hospital, Third Military Medical University (Army Medical University), Chongqing 400037, China
[b] Department of Cardiology, the Second Affiliated Hospital, Third Military Medical University (Army Medical University), Chongqing 400037, China

ARTICLE INFO

Keywords:
Trimetazidine
Cardiorespiratory fitness
High altitude
Fatigue
Cardiac function

ABSTRACT

Trimetazidine (TMZ) has been shown to optimize myocardial energy metabolism and is a common anti-ischemic agent. Our trial (ChiCTR-TRC-13003298) aimed to explore whether TMZ has any preventive effect on high-altitude fatigue (HAF), cardiac function and cardiorespiratory fitness upon acute high-altitude exposure and how it works on HAF. Thirty-nine healthy young subjects were enrolled in a randomized double-blinded placebo-controlled trial and were randomized to take oral TMZ (n = 20) or placebo (n = 19), 20 mg tid, 14 days prior to departure until the end of study. The 2018 Lake Louise Score questionnaire, echocardiography, assessments of physical working capacity, circulating markers of myocardial energy metabolism and fatigue were performed both before departure and arrival at highland. At follow-up, TMZ significantly reduced the incidence of HAF ($p = 0.038$), reversed cardiorespiratory fitness impairment, decreased left ventricular end-systolic volume (LVESV, $p = 0.032$) and enhanced left ventricular ejection fraction (LVEF, $p = 0.015$) at highland. Relative to the placebo group, the TMZ group had significantly lower LDH ($p = 0.025$) and lactate levels before ($p < 0.001$) and after ($p = 0.012$) physical exercise after acute high-altitude exposure. Additionally, improved left ventricular systolic function might have contributed to ameliorating HAF during TMZ treatment (LVEF, OR = 0.859, 95% CI = 0.741-0.996, $p = 0.044$). In conclusion, our results demonstrated that TMZ could prevent HAF, cardiorespiratory fitness impairment and improves left ventricular systolic function during acute high-altitude exposure. This trial provides new insights into the effect of TMZ and novel evidence against HAF and cardiorespiratory fitness impairment at highland.

1. Introduction

People originating from low-altitude areas and ascending to altitudes > 2500 m, may suffer a cluster of nonspecific symptoms, including headache, nausea or vomiting, fatigue, light-headed ness and poor appetite. These symptoms usually occur 6 to 12 h after ascending and last more than 3 days if there is further ascent. The collective term of these symptoms is summarized as acute mountain sickness (AMS), that significantly affects high-altitude physical capacity and threatens high-altitude livings [1,2].

Previous studies mostly focused on the incidence of AMS and high-altitude headache (HAH), the indispensable symptom of AMS diagnosis, little information involved fatigue at high altitudes. Fatigue could appear as isolated symptom or in conjunction with AMS. The incidence of high-altitude fatigue (HAF) ranged from 10 to 50% in unacclimatized population after acute high-altitude exposure [3–6]. Due to high prevalence and susceptibility in individuals with acute high-altitude exposure, HAF extensively interferes with mental health and physical performance, reduces efficiency to respond to stimuli. Ameliorating HAF is beneficial to reducing the incidence of AMS and improving

Abbreviations: AMS, acute mountain sickness; BP, blood pressure; CNS, central nervous system; CI, confidence interval; CO, cardiac output; DBP, diastolic blood pressure; EF, ejection fraction; HAF, high-altitude fatigue; HAH, high-altitude headache; HR, heart rate; GABA, γ-aminobutyric acid; LDH, lactic dehydrogenase; LVEDD, left ventricular end-diastolic dimension; LVEDV, left ventricular end-diastolic volume; LVESD, left ventricular end-systolic dimension; LVESV, left ventricular end-systolic volume; OR, odd ratio; PL, placebo group; PWC170, physical working capacity at 170 beats per minute; SBP, systolic blood pressure; SpO_2, pulse oxygen saturation; SV, stroke volume; TMZ, trimetazidine; tid, three times daily

* Corresponding author at: Department of Cardiology, the Second Affiliated Hospital, Third Military Medical University (Army Medical University), Chongqing 400037, China.
E-mail address: huanglan260s@126.com (L. Huang).

https://doi.org/10.1016/j.biopha.2019.109003
Received 10 March 2019; Received in revised form 15 May 2019; Accepted 15 May 2019

mental state and physical capacity at high altitudes. Despite more than a century of research on AMS, little evidence is available regarding treatment of HAF and the mechanism of HAF development is still unclear.

Fatigue is defined as extreme and persistent mental and/or physical tiredness that is reversible by rest. As no clinically useful blood-borne or cell-based biomarker for fatigue has been identified, its assessment and characterization primarily rely on self-report questionnaires and fatigability testing [7]. Nowadays, the exact mechanism underlying human fatigue is complicated and subject to debate. According to the origins, fatigue is categorized as physiologic and pathologic fatigue [8,9]. Physiologic fatigue includes mental stress, tiredness after work and exercise, boredom, and sleep deprivation. Pathologic fatigue is further subdivided into mental and physical diseases [8,9]. During acute high-altitude exposure, hypobaric hypoxia is the primary challenge for the unacclimatized individuals. Hypobaric hypoxia probably contributes to the initiation and development of HAF. Moderate hypoxia increases respiratory work, inducing hyperventilation and accelerating metabolite accumulation while activating the sympathetic nervous system, which results in the redistribution of cardiac output, further reducing the peripheral oxygen content and aggravating fatigue [10–13]. In severe hypoxic conditions, the central nervous system (CNS) may play a major role in fatigue progression. The lack of oxygen for the CNS leads to the accumulation of inhibitory neurotransmitters, such as serotonin and γ-aminobutyric acid (GABA), which reduces central motor drive [14,15].

Besides, hypoxia as well induces a cascade of cellular energy metabolic responses including a decrease in mitochondrial oxidative flux and reduction in ATP production in cardiomyocytes [16,17]. Sherpa people demonstrate a lower capacity for fatty acid oxidation, along with enhanced efficiency of oxygen utilization, that is natural selection and contributes to their metabolic adaptation [18]. Thus, change of energy metabolism probably is another mechanism associated with HAF. We considered that drugs optimize cardiomyocyte energy metabolism were likely to promote acclimatization and prevent HAF or alleviate performance capacity impairment. Trimetazidine (TMZ) (1-[2,3,4-trimethoxybenzyl] piperazine), a traditional cardioprotective drug, is commonly used against myocardial injury induced by ischemia or reperfusion [19]. It works by selectively inhibiting the last enzyme in the beta oxidation of fatty acids and long-chain 3-ketoacyl-coenzyme A thiolase. The inhibition of fatty acid oxidation causes a transformation of energy metabolism due to elevated levels of glucose oxidation [20], which implies that TMZ may have potential for preventing or treating HAF and reversing the loss of physical performance ability.

In this study, we hypothesized that TMZ treatment potentially decreased the incidence of fatigue and reverse cardiorespiratory fitness impairment during high-altitude exposure. Therefore, we recruited healthy young participants and randomly divided them into a placebo group (PL, intake of placebo) and a TMZ group (intake of TMZ) 2 weeks prior to acute high-altitude exposure. We followed them during their ascent to high altitude and collected related information with the aim of examining possible effect and mechanism of TMZ.

2. Methods

2.1. Study design and procedure

This was a randomized, double-blinded, placebo-controlled study. 39 healthy lowlanders were recruited in two days and screened for the eligibility criteria in Chongqing, China, 14 days before their departure to a high-altitude location. 20 subjects were randomly assigned to the TMZ group, which received TMZ 20 mg three times daily (tid). 19 participants were allocated to the placebo groups (PL), which orally took similar pills made of starch. Randomization and distribution of pills were done by the clinicians who did not involve in the study and the randomization list had not been disclosed to any of the participants

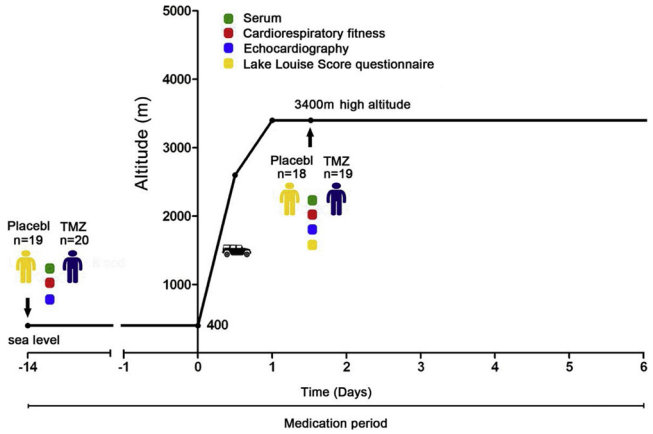

Fig. 1. Ascending process.

or researchers in the study. All the participants were transported to 3400 m (Xinduqiao, China) from 400 m (Chongqing, China) during a 24 -hs motor trip (Fig. 1, Ascent process). We collected individual data using the 2018 Lake Louise Score questionnaire [21], physiological examinations, echocardiography, cardiorespiratory fitness and serum marker testing both at sea level 14 days before departure and after arrival at high altitude for about 12 h. In the end, 18 individuals in the PL completed all the follow-ups, and one participant was lost because he did not undergo high-altitude exposure; 19 individuals in the TMZ group completed all the follow-ups, and one was lost due to non-compliance (Fig. 2, Flow chart). Thus, the data analysis included 18 subjects in the PL and 19 subjects in the TMZ. This clinical trial ended after all the participants received the follow-ups at high altitudes.

2.2. Participants

We recruited 39 volunteers aged from 17 to 24 years at Chongqing city (400 m, above sea level) in this clinical trial. All the participants were males and in good health condition. Participants with any one of the following conditions were excluded: severe organic diseases; a history of TMZ treatment or contraindications of TMZ; high-altitude exposure (> 2500 m, above sea level) history before or living at highland; has been diagnosed with fatigue-related illness; suffering physical activity or working capacity loss; orally taking any other medicine during the study. All subjects underwent a comprehensive medical examination before the expedition, and informed consent was obtained from each participant. After arriving at high altitude, all participants did not carry out physical work and orally take something containing stimulants before the measurement of cardiorespiratory fitness and echocardiography. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the Human Ethics Committee, Xinqiao Hospital, Third Military Medical University. This study was registered at www.chictr.org.cn (ChiCTR-TRC-13003298).

2.3. Physiological data

Blood pressure (BP) and heart rate (HR) were obtained using electronic sphygmomanometers (OMRON HEM-6200; OMRON Healthcare, Ltd.; Kyoto, Japan). Pulse oxygen saturation (SpO_2) was measured with a pocket pulse oximeter (NONIN-9550, Nonin Onyx, America). BP, HR, and SpO_2 were measured three times after the participants had rested in a quiet environment for more than 30 min. The measurements were separated by 3 min intervals.

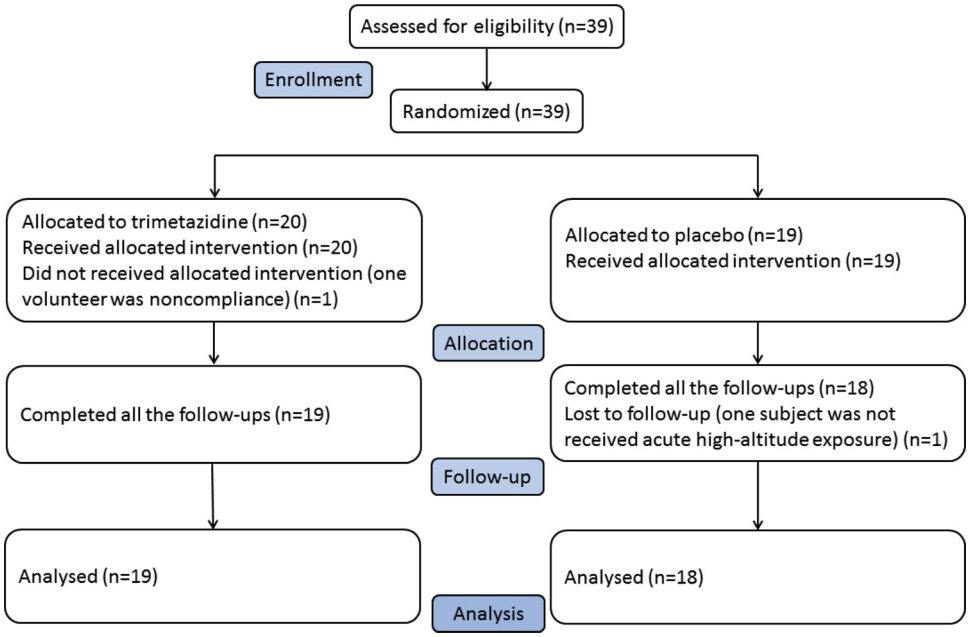

Fig. 2. Depicts a flow chart of the trial design used to complete this study.

2.4. Echocardiography

A color Doppler echocardiograph (CX 50, Philips Ultrasound System, Andover, MA, USA) with a probe of 2 to 4 MHz was used to measure the left ventricular end-systolic dimension (LVESD) and end-diastolic dimension (LVEDD). We obtained the left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) using Teichholz's formula. The cardiac output (CO), stroke volume (SV) and ejection fraction (EF) were calculated in accordance with the data above. To avoid interference from physical activity, data collection was carried out at least 30 min after rest.

2.5. AMS and fatigue score questionnaire

AMS was diagnosed by Lake Louise Acute Mountain Sickness Scoring system, which includes four self-reported symptoms: headache, fatigue, dizziness, and gastrointestinal symptoms [21]. Each symptom was scored on a scale from 0 to 3, with 0 indicating none and 1, 2, and 3 indicating mild, moderate, and severe, respectively. AMS was defined if headache was present and the total score for all symptoms was 3 or more. AMS with a total score of 3 to 4 was defined as mild AMS, whereas severe AMS was indicated by a total score of 5 or more. Fatigue at high altitude was diagnosed according to fatigue symptoms scoring in Lake Louise Acute Mountain Sickness Scoring system. In brief, high-altitude fatigue was scored on a scale from 0 to 3. High-altitude fatigue was defined if fatigue scoring was 1 or more.

2.6. Fitness testing

We used a modified two-step physical working capacity test to evaluate cardiorespiratory fitness, which predicted the workload at an HR of 170 beats per minute (PWC170) [22]. The assessment was based on two 5-min submaximal physical effort events, where the level of effort was such that each event could be concluded with the HR approaching 120–170 beats per minute. The average HR values were recorded at the end of each 5-min period of physical effort, which served to determine the strain output in accordance with the PWC170. The PWC170 was calculated with the formula $PWC170 = W1 + (W2 - W1)[170 - P1/P2 - P1]$, where W1 and W2 are the output of subsequent physical efforts expressed in watts (W), and P1 and P2 are the HR during a particular physical effort event.

2.7. Serum biomarkers

We collected the blood samples at almost same time of day 30 min before exercise and as soon as exercise finished. Blood samples were collected from a vein in an upper extremity without the use of a tourniquet. 5 ml blood sample was collected into a vacutainer tube containing sodium fluoride, immediately placed on ice. After gathered, blood samples were delivered to routinely centrifuge for 5 min at 10,000 rpm to obtain serum. All serum samples were stored and transported to Shanghai in liquid nitrogen. The measurement of serum markers was performed by laboratory personnel of Sangon Biotech (Sangon Biotech Co. Shanghai, China), who were blinded to the sample sources and study design. Serum markers were measured by a colorimetric assay with an enzymatic reaction using an auto-analyzer (Roche/Hitachi Cobas C Systems, USA), according to the manufacturer's protocol.

2.8. Blinding

The starch tablets were similar to the TMZ tablets in shape, size, and color. The independent physician mentioned above repackaged these drugs in medicine boxes for each subject and retained the blinding code. The subjects, researchers, and other physicians were blinded.

2.9. Statistical analysis

All analyses were performed using SPSS 19.0 software. The normally distributed measurement variables are presented as the mean ± standard deviation (SD), while the non-normally distributed variables are expressed as the median (interquartile range). The frequencies of AMS and related symptoms were compared between the two groups using Fischer's exact chi-squared tests. Between-group differences were analyzed with unpaired two-tailed Student's *t*-test for normally distributed data, and within-group differences were analyzed with a paired *t*-test. Data that did not fit a normal distribution were analyzed with the Wilcoxon signed-rank test (within-group) and the Wilcoxon rank sum (between-group). Pearson or Spearman correlation tests were utilized for correlation analyses, as appropriate. The variable was subjected to

Table 1
General characteristics of the study participants.

Variable	Placebo (n = 19)	TMZ	P Value
Age (year)	20.00 ± 2.03	20.06 ± 1.79	0.333
Weight (kg)	64.21 ± 4.38	64.30 ± 4.16	0.948
Height (cm)	171.00 (5.00)	171.00 (5.00)	0.966
Ethnicity			1.000
Han, n (%)	18 (94.74)	18 (90.00)	
Non-Han, n (%)	1 (0.053)	2 (10.00)	
Tobacco history			0.667
Never, n (%)	6(31.60)	5(25.00)	
Occasionally, n (%)	10(52.60)	11(55.00)	
Mild, n (%)	3(15.80)	4(20.00)	
Moderate, n (%)	0(0.00)	0(0.00)	
Severe, n (%)	0(0.00)	0(0.00)	
Alcohol history			1.000
Never, n (%)	17(89.50)	18(90.00)	
Mild, n (%)	2(10.50)	1(5.00)	
Moderate, n (%)	0(0.00)	1(5.00)	
Severe, n (%)	0(0.00)	0(0.00)	
Mountaineering history			1.000
Yes, n (%)	0 (0)	0 (0)	
No, n (%)	19 (100%)	20 (100%)	
Medicine therapy			1.000
Yes, n (%)	0 (0)	0 (0)	
No, n (%)	19 (100%)	20 (100%)	

Data of normal distribution are presented as mean ± SD, abnormal distribution are shown by median (quartile). n, number of subjects. P values for test of placebo and TMZ groups by Fisher's exact test.

an adjusted binary logistic regression model to identify the adjusted independent protective factors for HAF. Cohen's *d* was used to calculate effect size, 0.2, 0.5, and 0.8 were considered small, medium, and large effect sizes, respectively. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Demographic data

Two groups did not differ in terms of the demographic data as shown in Table 1, including age, weight, height, race, as well as tobacco, alcohol and high-altitude exposure history. One subject from TMZ group withdrew from trail as a result of noncompliance, while one subject from PL group lost to follow up due to without high-altitude exposure. Finally, 37 participants (TMZ [n = 19] and PL [n = 18]) completed all the follow-ups (Fig. 2, Flow chart).

3.2. Physiological data at sea level and high altitude

HR was elevated but SpO_2 was reduced after acute exposure at high altitudes compared with sea level in two groups (**Supplemental** Fig. 1**A–B**). However, there was no significant difference in HR or SpO_2 between the PL and TMZ groups. Moreover, systolic blood pressure (SBP) and diastolic blood pressure (DBP) showed no remarkable difference between the PL and TMZ groups at sea level or high altitudes (**Supplemental** Fig. 1**C–D**).

3.3. High-altitude fatigue and AMS incidence

There was no significant difference in the incidence ($P = 0.725$) and the severity ($P = 0.728$) of AMS between the TMZ and the PL groups (Fig. 3**A** and Table 2). Moreover, we compared the primary AMS symptoms, including headache, gastrointestinal symptoms, fatigue and dizziness in the two groups. As shown in Fig. 3**B** and Table 2, TMZ remarkably reduced the occurrence of fatigue (PL: n = 9, TMZ: n = 3, $P = 0.038$, $d = 0.785$) during acute ascending, without pronounced influences on other symptoms. The data imply that TMZ did not exert a significant impact on preventing AMS but effectively decreased the incidence of fatigue during acute high-altitude exposure.

3.4. Cardiorespiratory fitness

As shown in Fig. 3**C** and Table 3, there was no significant difference in the baseline of PWC170 in the two groups, but cardiorespiratory fitness was impaired in the PL participants (748.33 ± 63.33 vs. 797.12 ± 72.75 kg × m/min; $P = 0.020$) after high-altitude exposure compared with the baseline data. In contrast, no significant change was observed in participants in the TMZ group between sea level and high altitude (780.99 ± 40.30 vs. 788.50 ± 61.42 kg × m/min; $P = 0.659$). This indicates that TMZ effectively reversed high altitude-induced cardiorespiratory fitness impairment.

3.5. Myocardial energy metabolism and left ventricular systolic function

TMZ improved the oxygen utilization efficiency of the myocardium in hypoxia. As shown in Table 4, there was no difference in left ventricular systolic function, including LVEDD, LVEF, LVEDV, LVESV, SV and CO, between the PL and TMZ groups before ascending. However, the TMZ group showed significantly increased LVEF ($P = 0.015$) and reduced LVESV ($P = 0.032$) in comparison to the PL group after acute high-altitude exposure with a large effect size ($d_{LVEF} = 0.843$ and $d_{LVESV} = 0.736$, Table 4).

To evaluate the effect of TMZ on myocardial energy metabolism, we measured the level of circulating lactic dehydrogenase (LDH), which represents the anaerobic metabolism level [23,24]. As shown in Table 5, acute high-altitude exposure elevated the level of serum LDH in the PL group, while TMZ significantly inhibited this effect ($P = 0.025$, $d = 0.771$). In addition, we detected serum lactate levels before physical exercise and immediately after physical exercise at sea level and high altitude in both groups. The exercise was carried out according to a modified two-step physical working capacity, which ensured that all the participants performed the equal work at the same time. The data indicated that the concentrations of serum lactate in the TMZ group were remarkably lower than those in the PL group both in the calm condition ($P < 0.001$, $d > 1.339$) and after physical exercise ($P = 0.012$, $d > 0.872$) at high altitudes (Table 5). The results above imply that TMZ encourages glucose aerobic oxidation, especially in cardiomyocytes, to provide energy instead of glycolysis during hypoxia. This reduced the production and accumulation of lactate at rest and after exercise at high altitudes.

3.6. Regression analysis of associated-variables with HAF

To explore the possible mechanism through which TMZ reduces the incidence of HAF and reverses cardiorespiratory fitness, we employed logistic regression analysis. In a univariate regression analysis, LVEF (OR = 0.867, 95%CI = 0.741 to 0.996, $P = 0.044$) was a protective factor against HAF in the TMZ treatment group as shown in Fig. 4. Multivariate regression was not carried out as a result of only LVEF associated with HAF. Furthermore, a correlation analysis indicated that none of the left ventricular systolic function, physiological parameters or serum markers showed a correlation with the PWC170 (**Supplemental** Table 1). We did not include the HR in the correlation analysis because the PWC170 was calculated using a formula that included HR.

4. Discussion

For this trial, we recruited volunteers and divided them randomly into PL and TMZ groups. Both groups underwent acute high-altitude exposure and follow-up examinations. This study provides several novel results: (i) Taking TMZ orally reduced the occurrence of HAF and reversed cardiorespiratory fitness impairment; (ii) TMZ improved LVEF and transformed the energy metabolism to aerobic oxidation at high

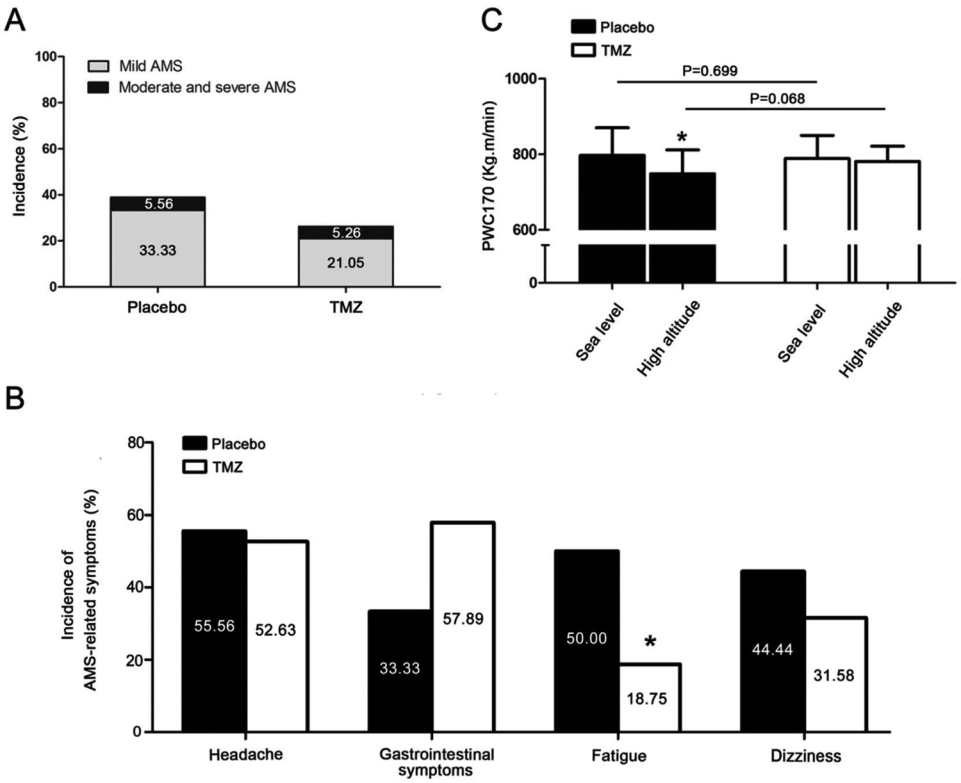

Fig. 3. The incidence of AMS, AMS-related symptoms, and cardiorespiratory fitness during high-altitude exposure. (A) There was no significant difference in the incidence and severity of AMS between TMZ and PL groups. (B) TMZ remarkably ameliorated the occurrence of fatigue at high altitudes without significantly effect on other three symptoms. (C) Acute high-altitude exposure significantly impaired cardiorespiratory fitness in PL (P = 0.020), TMZ reversed this effect (P = 0.659). There was no significant difference between two groups at sea level (P = 0.699) and high altitudes (P = 0.068).

Table 2
The effect of TMZ on AMS and AMS-related symptoms.

High altitude	Placebo (n = 18)	TMZ (n = 19)	P Values	Effect size (*d*)
Non-AMS (n, %)	11	12	0.725	0.195
AMS (n, %)	7	5		
Mild	6 (33.33)	4 (21.05)	0.708	0.213
Moderate and Severe	1 (5.56)	1 (5.26)	1.000	0.018
AMS-related symptoms (n, %)				
Headache	10 (55.56)	10 (52.63)	1.000	0.058
Gastrointestinal symptoms	6 (33.33)	11 (57.89)	0.191	0.508
Fatigue	9 (50.00)	3 (18.75)	0.038	0.785
Dizziness	8 (44.44)	6 (31.58)	0.508	0.267

The incidence and severity of AMS showed no significant difference between the PL and the TMZ groups. However, in the AMS-related symptoms, the number of fatigue patients in TMZ was 3 in comparison to 9 in PL group (P = 0.038, d = 0.785), which showed significant deviation. No prominent difference was observed in other three symptoms. Cohen's d was used to calculate effect size and the values of d represented the effect size. The occurrence of AMS and related symptoms are presented as number (%). P values for test of placebo and TMZ groups by Fisher's exact test.

Table 4
Effect of TMZ on cardiac function at high altitudes.

Sea level	Placebo (n = 18)	TMZ (n = 19)	P Value	Effect size (*d*)
LVEDD (cm)	4.50 (0.53)	4.50 (0.50)	0.226	–
LVEF (%)	62.25 ± 8.26	63.10 ± 9.45	0.775	0.096
LVEDV (ml)	88.71 ± 23.35	89.89 ± 25.51	0.884	0.048
LVESV (ml)	33.96 ± 12.53	32.79 ± 10.45	0.760	0.102
SV (ml)	54.75 ± 14.39	57.10 ± 18.80	0.672	0.140
CO (L/min)	3.40 ± 0.91	3.49 ± 1.15	0.808	0.087
High altitude				
LVEDD (cm)	4.36 ± 0.59	4.29 ± 0.48	0.709	0.056
LVEF (%)	61.88 ± 6.21	68.79 ± 9.70	0.015	0.843
LVEDV (ml)	85.28 ± 21.04	85.65 ± 18.77	0.955	0.019
LVESV (ml)	32.06 ± 8.14	26.25 ± 7.66	0.032	0.736
SV (ml)	53.22 ± 15.80	59.40 ± 17.52	0.268	0.370
CO (L/min)	4.28 ± 1.09	4.73 ± 1.71	0.355	0.312

There showed no difference in LVEDD, LVEF, LVEDV, LVESV, SV and CO, between the PL and TMZ groups at sea level. TMZ group showed prominent increased LVEF (P = 0.015) and reduced LVESV (P = 0.032) in comparison to PL group after acute high-altitude exposure. Cohen's d was used to calculate effect size and the values of $d$represented the effect size. Data of normal distribution are presented as mean ± SD, abnormal distribution are shown by median (quartile).

Table 3
TMZ reversed cardiorespiratory fitness impairment at high altitudes.

	Placebo (n = 18)		TMZ (n = 19)	
	Sea level	High altitude	Sea level	High altitude
Cardiorespiratory fitness (PWC170, kg × m/min)	797.12 ± 72.75	748.33 ± 63.33	788.50 ± 61.42	780.99 ± 40.30
P values and Effect size (*d*)	0.020, 0.717		0.659, 0.145	

Acute high-altitude exposure reduced the cardiorespiratory fitness from 797.12 ± 72.75 to 748.33 ± 63.33 kg × m/min in PL group (P = 0.020), but in TMZ group this effect was reversed with 788.50 ± 61.42 kg × m/min at sea level and 780.99 ± 40.30 kg × m/min at high altitudes. Cohen's d was used to calculate effect size and the values of d represented the effect size. Data are mean ± SD. P values presented as test between sea level and high altitude by paired sample t-test.

Table 5
The circulating level of LDH and lactate before and after exercise.

	Sea level				High altitude			
	Before exercise		After exercise		Before exercise		After exercise	
	Placebo	TMZ	Placebo	TMZ	Placebo	TMZ	Placebo	TMZ
Lactate (mM)	3.15 ± 1.14	3.47 ± 1.52	5.09 ± 2.08	4.40 ± 1.44	2.92 ± 0.92	1.95 ± 0.47	4.94 ± 1.93	3.59 ± 1.07
P and effect size (*d*)	0.477,0.237		0.247, 0.388		< 0.001,1.339		0.012, 0.872	
	Sea level				**High altitude**			
	Placebo		**TMZ**		**Placebo**		**TMZ**	
LDH (U/L)	159.00 ± 46.37		166.83 ± 46.21		188.74 ± 38.47		157.94 ± 41.32	
P and effect size (*d*)	0.610, 0.169				0.025, 0.771			

PL and TMZ groups showed no significant difference in circulating LDH (P = 0.610) and lactate level before and after exercise at sea level (P = 0.477, P = 0.247). High-altitude exposure increased the level of LDH in PL group, while TMZ reduced this effect. Meanwhile, TMZ group showed lower lactate level both before and after exercise (P < 0.001, P = 0.012) related to PL group. Cohen's *d* was used to calculate effect size and the values of *d*represented the effect size. Data are represented as mean ± SD.

altitudes. Left ventricular systolic function improvement might contribute to the alleviation of HAF.

Acute high-altitude exposure stimulates a series of neurohumoral and hemodynamic responses to enable the body to face the challenge and adapt to the new environment [25]. However, this will induce a series of uncomfortable symptoms, including headache, fatigue, nausea and sleep disturbance. Most of these symptoms could be categorized as AMS. Fatigue is one of major diagnostic symptoms to AMS. Previous reports revealed that the incidences of fatigue at high altitude ranged from 10 to 50% in different populations [3–6]. Similarly, in this study, the incidences of fatigue in two groups were 50.0% and 18.75%, respectively. Interestingly, we found that the incidence of fatigue was higher than that of AMS in PL group, which implied that HAF might be an independent symptom. Individuals who suffered HAF may not be AMS patients and their symptoms and treatments may be ignored at high altitudes.

Although, many researches have proved that some pharmacotherapies, such as acetazolamide and dexamethasone were effective in AMS prevention, hardly any information involving prevention or treatment for HAF has been reported before. In this trial, we found that TMZ treatment reduced the incidence of HAF, but hardly had effect on AMS as well as other AMS-related symptoms, including headache and dizziness. However, the incidence of gastrointestinal symptoms appeared to be a little higher (p = 0.191) in TMZ group compared with that in PL group. Previous study has indicated that the most frequently reported adverse events in TMZ recipients were gastrointestinal disorders, although the incidence of these events was very low [26]. Besides, we employed to orally take TMZ 20 mg tid 2 weeks prior to acute high-altitude exposure as this dose of TMZ was widely used and acceptable in treatment of angina, heart failure and myocardial infarction. Thus, we considered that high incidence of gastrointestinal symptoms might be associated with TMZ treatment, this may account for its little effect on preventing AMS. Although, TMZ seems to increased gastrointestinal disorders slightly, its benefits in fatigue prevention at high altitudes were far greater.

All participants were oral intake TMZ in our trial, skeletal muscle and other tissue might be affected by circulating TMZ. However, glucose oxidation is the major metabolic pattern in skeletal muscle, while free fatty acids β-oxidation only takes place during long-time and low-intensity exercise [27,28]. On the contrary, free fatty acids β-oxidation accounts for 60% of cardiac energy utlization in most of the time. Thus, cardiomyocytes are the main target of TMZ. Furthermore, we as well accordingly measured the left- and right-hand grips, but hardly any difference was observed between the two groups (**Supplemental**

Variable	Regression Analysis		
	OR	95% CI	P value
Age	0.943	0.432-2.059	0.883
HR	0.943	0.795-1.119	0.503
SpO2	0.679	0.306-1.509	0.342
SBP	0.943	0.787-1.129	0.521
DBP	1.018	0.866-1.197	0.826
LVEF	0.859	0.741-0.996	0.044
LVESV	1.127	0.963-1.319	0.284
LVEDV	0.950	0.881-1.024	0.177
SV	0.895	0.788-1.016	0.086
CO	0.220	0.040-1.201	0.080
LDH	1.008	0.985-1.031	0.234
Lactate	0.718	0.098-5.287	0.391
Serotonin	0.978	0.945-1.012	0.431
GABA	1.020	0.983-1.060	0.143

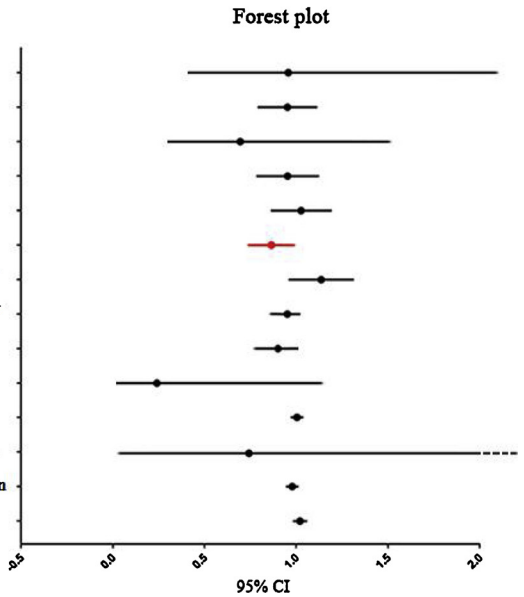

Fig. 4. Regression analysis of variables with HAF. In a univariate analysis, LVEF was a protective factor (OR = 0.867, 95%CI = 0.741 to 0.996, P = 0.044) against HAF in TMZ group.

Table 2). Therefore, we considered that cardiomyocytes are more sensitive to TMZ related to skeletal muscle cell as their difference in energy metabolism pattern. In addition, TMZ has been indicated to increase glucose uptake in all dissected regions of brain and protect cells in cerebral tissue [29,30]. However, the impact of TMZ on brain may be partly due to its effect on the heart. Therefore, in our study, we considered that TMZ served as a protective role at high altitudes probably through energy metabolism pattern shifting in heart.

Our trial is the first to reveal the effect of TMZ on reducing the incidence of HAF. The development of fatigue is regulated by various factors, such as CO, glycogen stores and circulating metabolites, but the endpoint of increased fatigue is in the CNS [31]. Serotonin and GABA are the primary modulators of fatigue in the CNS [32]. Release of inhibitory neurotransmitters, such as serotonin and GABA in the CNS, is related to the development of fatigue [7,15,33–35]. Therefore, we accordingly measured the level of serous serotonin and GABA. No significant difference was observed between the two groups at sea level or at high altitudes (**Supplemental** Fig. 2). We considered that circulating level of inhibitory neurotransmitters might not represent the concentrations in the CNS as a result of the blood-brain barrier. Due to ethical issue and sterile limitation, it is difficult for us to obtain cerebrospinal fluid to get further analysis to evaluate fatigue.

In our study, we found that SV and CO in TMZ group were slightly higher in comparison to placebo group, and LVEF was closely associated with fatigue. Due to LVEF is calculated by LVEDV and LVESV, LVEF increment in TMZ group was attributed to reduction of LVESV. TMZ has been confirmed to improve LVEF and preservation of phosphocreatine/adenosine triphosphate ratio [36]. Lower myocardial contractility contributes to cardiac output insufficient, tissue hypoperfusion and increment of anaerobic metabolism, that promote fatigue. Therefore, we considered that TMZ prevented high-altitude fatigue through optimizing myocardial energy metabolism pattern and improving myocardial contractility, however, further research on the mechanism of TMZ in cardiomyocytes during acute hypoxia exposure need to be carried out in near future.

Hypobaric hypoxia causes a rapid decrease in high-energy phosphate metabolism in the human cardiac left ventricle, which may lead to left ventricle diastolic and systolic dysfunction [37,38]. Previous studies have demonstrated that CO and HR were elevated upon the initial ascent to high altitude [39–41]. After a relatively short period of acclimatization (3–5 days), CO gradually recovered to that at sea level, but left ventricle diastolic filling and diastolic function were still impaired, which might be associated with hypoxia-induced tachycardia and pulmonary vasoconstriction [42]. In our study, CO and HR increased significantly with end diastolic volume reduction in the two groups following acute high-altitude exposure. Intriguingly, TMZ treatment remarkably improved LVEF, which was attributed to the decrease in left ventricular end systolic volume. This implies that TMZ treatment enhanced left ventricular systolic function at high altitudes. Our regression analysis further pointed out that this effect might be associated with fatigue prevention at high altitudes.

High-altitude exposure is associated with decreased aerobic exercise capacity and impaired cardiac performance [22]. The hypobaric hypoxia environment is the leading cause of cardiorespiratory fitness impairment. The current treatment focuses on reducing pulmonary arterial pressure and/or improving CO using sildenafil and endothelin receptor antagonism combined with ephedrine or theophylline [43–45]. However, most of these therapeutic strategies have only been used in animal models. In addition, ephedrine and theophylline are not recommended for long-term use because they have obvious side effects or are unsuitable for individuals with hypertension. This study revealed that TMZ was beneficial for attenuating cardiorespiratory fitness impairment at high altitudes. However, there was not a significantly reduction of cardiorespiratory fitness in PL group compared with that in TMZ group. We considered that a longer time of TMZ intaking might make this effect more pronounced.

Although we demonstrated in this randomized, double-blinded, placebo-controlled clinical trial, that taking TMZ orally was beneficial to alleviate HAF and cardiorespiratory fitness impairment, there were several limitations for our study. (i) Individuals we recruited in this trial were Chinese young men and sample size was small; (ii) Our study focused on TMZ and lacked a comparison between TMZ and other prevention strategies. Therefore, a further larger scale comparison research including different sex, age and race should be carried out to confirm the preventive effect of TMZ. This study provides novel evidence and possible intervention that has positive effect against HAF and cardiorespiratory fitness impairment.

Conflicts of interest

The authors declare that there are no conflicts of interest. I would like to declare on behalf of my co-authors that the work described is original research that has not been published previously. I confirm to have permission to reprint any figures or tables that are initially printed elsewhere.

Acknowledgements

This trial was supported by the National Natural Science Foundation of China (Grant No. 81730054), the Ministry of Health of P.R. China (Grant No. 201002012), National Natural Science Foundation of China (Grant No. 81800396) and the Chongqing Post-doctoral Innovation Foundation (Grant No. CQBX201706).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2019.109003.

References

[1] J. Jin, Acute mountain sickness, JAMA. 18 (2017) 1840, https://doi.org/10.1001/jama.2017.16077.
[2] F. Macholz, M. Sareban, M.M. Berger, Diagnosing acute mountain sickness, JAMA. 14 (2018) 1509, https://doi.org/10.1001/jama.2018.0220.
[3] F.Y. Cheng, M.J. Jeng, Y.C. Lin, S.H. Wang, S.H. Wu, W.C. Li, K.F. Huang, T.F. Chiu, Incidence and severity of acute mountain sickness and associated symptoms in children trekking on Xue Mountain, Taiwan, PLoS One 8 (2017) e183207, , https://doi.org/10.1371/journal.pone.0183207.
[4] P. Koirala, S.E. Wolpin, J.T. Peterson, High altitude illness: knowledge, practice, and attitudes of porters in Nepal, Wilderness Environ. Med. 4 (2018) 431–436, https://doi.org/10.1016/j.wem.2018.06.002.
[5] J.Q. Dong, J.H. Zhang, J. Qin, Q.N. Li, W. Huang, X.B. Gao, J. Yu, G.Z. Chen, X.G. Tang, L. Huang, Anxiety correlates with somatic symptoms and sleep status at high altitudes, Physiol. Behav. (2013) 23–31, https://doi.org/10.1016/j.physbeh.2013.02.001.
[6] S.Z. Bian, J. Jin, J.H. Zhang, Q.N. Li, J. Yu, Chen J.F. Yu SY, Qin J. Yu XJ, L. Huang, Principal component analysis and risk factors for acute mountain sickness upon acute exposure at 3700 m, PLoS One 11 (2015) e142375, , https://doi.org/10.1371/journal.pone.0142375.
[7] I.K. Penner, F. Paul, Fatigue as a symptom or comorbidity of neurological diseases, Nat. Rev. Neurol. 11 (2017) 662–675, https://doi.org/10.1038/nrneurol.2017.117.
[8] A.J. Dittner, S.C. Wessely, R.G. Brown, The assessment of fatigue: a practical guide for clinicians and researchers, J. Psychosom. Res. 2 (2004) 157–170, https://doi.org/10.1016/S0022-3999(03)00371-4.
[9] J. Finsterer, S.Z. Mahjoub, Fatigue in healthy and diseased individuals, Am. J. Hosp. Palliat. Care 5 (2014) 562–575, https://doi.org/10.1177/1049909113494748.
[10] I. Vogiatzis, D. Athanasopoulos, R. Boushel, J.A. Guenette, M. Koskolou, M. Vasilopoulou, H. Wagner, C. Roussos, P.D. Wagner, S. Zakynthinos, Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists, J. Physiol. (Paris) 22 (2008) 5575–5587, https://doi.org/10.1113/jphysiol.2008.162768.
[11] Morgan B.J. St CC, T.J. Wetter, J.A. Dempsey, Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans, J. Physiol. (Paris) (2000) 493–504.
[12] J.A. Dempsey, L. Romer, J. Rodman, J. Miller, C. Smith, Consequences of exercise-induced respiratory muscle work, Respir Physiol, Neurobiol. 2–3 (2006) 242–250, https://doi.org/10.1016/j.resp.2005.12.015.
[13] C.A. Harms, T.J. Wetter, S.R. McClaran, D.F. Pegelow, G.A. Nickele, W.B. Nelson, P. Hanson, J.A. Dempsey, Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise, J. Appl, Physiol 2 (1998) (1985) 609–618, https://doi.org/10.1152/jappl.1998.85.2.609.

[14] E. Blomstrand, Amino acids and central fatigue, Amino Acids 1 (2001) 25–34.
[15] J.L. Fan, B. Kayser, Fatigue and exhaustion in hypoxia: the role of cerebral oxygenation, high alt, Med. Biol. 2 (2016) 72–84, https://doi.org/10.1089/ham.2016.0034.
[16] A.E. Consolini, M.I. Ragone, P. Bonazzola, G.A. Colareda, Mitochondrial bioenergetics during ischemia and reperfusion, Adv. Exp. Med. Biol. (2017) 141–167, https://doi.org/10.1007/978-3-319-55330-6_8.
[17] M.S. Nanadikar, L.A. Vergel, S. Borowik, A. Hillemann, A. Zieseniss, V.V. Belousov, I. Bogeski, P. Rehling, J. Dudek, D.M. Katschinski, O2 affects mitochondrial functionality ex vivo, Redox Biol. (2019) 101152, https://doi.org/10.1016/j.redox.2019.101152.
[18] J.A. Horscroft, A.O. Kotwica, V. Laner, J.A. West, P.J. Hennis, D. Levett, D.J. Howard, B.O. Fernandez, S.L. Burgess, Z. Ament, E.T. Gilbert-Kawai, A. Vercueil, B.D. Landis, K. Mitchell, M.G. Mythen, C. Branco, R.S. Johnson, M. Feelisch, H.E. Montgomery, J.L. Griffin, M. Grocott, E. Gnaiger, D.S. Martin, A.J. Murray, Metabolic basis to Sherpa altitude adaptation, Proc. Natl. Acad. Sci U S A. 24 (2017) 6382–6387, https://doi.org/10.1073/pnas.1700527114.
[19] L. Bonello, P. Sbragia, N. Amabile, O. Com, S.V. Pierre, S. Levy, F. Paganelli, Protective effect of an acute oral loading dose of trimetazidine on myocardial injury following percutaneous coronary intervention, Heart. 6 (2007) 703–707, https://doi.org/10.1136/hrt.2006.107524.
[20] G. Fragasso, A. Salerno, G. Lattuada, A. Cuko, G. Calori, A. Scollo, F. Ragogna, F. Arioli, G. Bassanelli, R. Spoladore, L. Luzi, A. Margonato, G. Perseghin, Effect of partial inhibition of fatty acid oxidation by trimetazidine on whole body energy metabolism in patients with chronic heart failure, Heart. 18 (2011) 1495–1500, https://doi.org/10.1136/hrt.2011.226332.
[21] R.C. Roach, P.H. Hackett, O. Oelz, P. Bartsch, A.M. Luks, M.J. MacInnis, J.K. Baillie, The 2018 Lake Louise Acute Mountain Sickness Score, High Alt. Med. Biol. 1 (2018) 4–6, https://doi.org/10.1089/ham.2017.0164.
[22] T. Yang, X. Li, J. Qin, S. Li, J. Yu, J. Zhang, S. Yu, X. Wu, L. Huang, High altitude-induced borderline pulmonary hypertension impaired cardiorespiratory fitness in healthy young men, Int. J. Cardiol. (2015) 382–388, https://doi.org/10.1016/j.ijcard.2014.12.044.
[23] D. Wei, L. Wei, X. Li, Y. Wang, L. Wei, Effect of hypoxia on Ldh-c expression in somatic cells of Plateau Pika, Int. J. Environ. Res. Public Health 8 (2016), https://doi.org/10.3390/ijerph13080773.
[24] A.K. Mohammed, S. Jovanovic, Q. Du, A. Sukhodub, A. Jovanovic, Mild hypoxia in vivo regulates cardioprotective SUR2A: a role for Akt and LDH, Biochim. Biophys. Acta 5 (2015) 709–719, https://doi.org/10.1016/j.bbadis.2015.01.001.
[25] R.T. Meehan, D.C. Zavala, The pathophysiology of acute high-altitude illness, Am. J. Med. 3 (1982) 395–403.
[26] K.J. McClellan, G.L. Plosker, Trimetazidine. A review of its use in stable angina pectoris and other coronary conditions, Drugs. 1 (1999) 143–157, https://doi.org/10.2165/00003495-199958010-00016.
[27] E. Ferraro, A.M. Giammarioli, S. Caldarola, P. Lista, A. Feraco, A. Tinari, A.M. Salvatore, W. Malorni, L. Berghella, G. Rosano, The metabolic modulator trimetazidine triggers autophagy and counteracts stress-induced atrophy in skeletal muscle myotubes, FEBS J. 280 (2013) 5094–5108, https://doi.org/10.1111/febs.12484.
[28] C.M. Stary, S. Kohin, M. Samaja, R.A. Howlett, M.C. Hogan, Trimetazidine reduces basal cytosolic Ca2+ concentration during hypoxia in single Xenopus skeletal myocytes, Exp. Physiol. 88 (2003) 415–421.
[29] P. Nowak, T. Zagził, J. Konecki, G. Szczerbak, R. Szkilnik, J. Niwiski, J. Gorzałek, R.M. Kostrzewa, R. Brus, Trimetazidine increases glucose [3H] uptake in rat brain, Pharmacol. Rep. 58 (2006) 559–561.
[30] A. Oguzhanoglu, T. Kurt, R. Ortac, B. Turman, Somatosensory evoked potential and histopathological investigations in ischaemia-induced brain damage: effects of trimetazidine in rats, Int. J. Neurosci. 113 (2003) 447–454.
[31] T.D. Noakes, Fatigue is a brain-derived emotion that regulates the exercise behavior to ensure the protection of whole body homeostasis, Front. Physiol. (2012) 82, https://doi.org/10.3389/fphys.2012.00082.
[32] H.K. Struder, H. Weicker, Physiology and pathophysiology of the serotonergic system and its implications on mental and physical performance. Part II, Int. J. Sports Med. 7 (2001) 482–497, https://doi.org/10.1055/s-2001-17606.
[33] L. Cordeiro, P. Rabelo, M.M. Moraes, F. Teixeira-Coelho, C.C. Coimbra, S.P. Wanner, D.D. Soares, Physical exercise-induced fatigue: the role of serotonergic and dopaminergic systems, Braz. J. Med. Biol. Res. 12 (2017) e6432, https://doi.org/10.1590/1414-431X20176432.
[34] T. Yamamoto, H. Azechi, M. Board, Essential role of excessive tryptophan and its neurometabolites in fatigue, Can. J. Neurol. Sci. 1 (2012) 40–47.
[35] T. Kanehira, Y. Nakamura, K. Nakamura, K. Horie, N. Horie, K. Furugori, Y. Sauchi, H. Yokogoshi, Relieving occupational fatigue by consumption of a beverage containing gamma-amino butyric acid, J. Nutr, Sci. Vitaminol (Tokyo). 1 (2011) 9–15.
[36] G. Fragasso, G. Perseghin, F. De Cobelli, A. Esposito, A. Palloshi, G. Lattuada, P. Scifo, G. Calori, A. Del Maschio, A. Margonato, Effects of metabolic modulation by trimetazidine on left ventricular function and phosphocreatine/adenosine triphosphate ratio in patients with heart failure, Eur. Heart J. 27 (2006) 942–27948, https://doi.org/10.1093/eurheartj/ehi816.
[37] C.J. Holloway, H.E. Montgomery, A.J. Murray, L.E. Cochlin, I. Codreanu, N. Hopwood, A.W. Johnson, O.J. Rider, D.Z. Levett, D.J. Tyler, J.M. Francis, S. Neubauer, M.P. Grocott, K. Clarke, Cardiac response to hypobaric hypoxia: persistent changes in cardiac mass, function, and energy metabolism after a trek to Mt. Everest Base Camp, FASEB J. 2 (2011) 792–796, https://doi.org/10.1096/fj.10-172999.
[38] C. Holloway, L. Cochlin, I. Codreanu, E. Bloch, M. Fatemian, C. Szmigielski, H. Atherton, L. Heather, J. Francis, S. Neubauer, P. Robbins, H. Montgomery, K. Clarke, Normobaric hypoxia impairs human cardiac energetics, FASEB J. 9 (2011) 3130–3135, https://doi.org/10.1096/fj.11-183426.
[39] M. Rao, J. Li, J. Qin, J. Zhang, X. Gao, S. Yu, J. Yu, G. Chen, B. Xu, H. Li, R. Rao, L. Huang, J. Jin, Left ventricular function during acute high-altitude exposure in a large group of healthy young Chinese men, PLoS One 1 (2015) e116936, , https://doi.org/10.1371/journal.pone.0116936.
[40] Y. Allemann, M. Rotter, D. Hutter, E. Lipp, C. Sartori, U. Scherrer, C. Seiler, Impact of acute hypoxic pulmonary hypertension on LV diastolic function in healthy mountaineers at high altitude, Am. J. Physiol. Heart Circ. Physiol. 3 (2004) H856–H862, https://doi.org/10.1152/ajpheart.00518.2003.
[41] S. Huez, K. Retailleau, P. Unger, A. Pavelescu, J.L. Vachiery, G. Derumeaux, R. Naeije, Right and left ventricular adaptation to hypoxia: a tissue Doppler imaging study, Am. J. Physiol. Heart Circ. Physiol. 4 (2005) H1391–H1398, https://doi.org/10.1152/ajpheart.00332.2005.
[42] M. Stembridge, P.N. Ainslie, R. Shave, Mechanisms underlying reductions in stroke volume at rest and during exercise at high altitude, Eur. J. Sport Sci. 5 (2016) 577–584, https://doi.org/10.1080/17461391.2015.1071876.
[43] J.P. Richalet, P. Gratadour, P. Robach, I. Pham, M. Dechaux, A. Joncquiert-Latarjet, P. Mollard, J. Brugniaux, J. Cornolo, Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension, Am. J. Respir. Crit. Care Med. 3 (2005) 275–281, https://doi.org/10.1164/rccm.200406-804OC.
[44] D.R. Radiloff, Y. Zhao, A. Boico, C. Wu, S. Shan, G. Palmer, K. Hamilton, D. Irwin, G. Hanna, C.A. Piantadosi, T. Schroeder, The combination of theophylline and endothelin receptor antagonism improves exercise performance of rats under simulated high altitude, J. Appl. Physiol. 8 (2012) (1985) 1243–1252, https://doi.org/10.1152/japplphysiol.01622.2011.
[45] D. Radiloff, Y. Zhao, A. Boico, G. Blueschke, G. Palmer, A. Fontanella, M. Dewhirst, C.A. Piantadosi, R. Noveck, D. Irwin, K. Hamilton, B. Klitzman, T. Schroeder, Antihypotensive treatment and endothelin blockade synergistically antagonize exercise fatigue in rats under simulated high altitude, PLoS One 6 (2014) e99309, , https://doi.org/10.1371/journal.pone.0099309.