Effects of a single bout of exercise on human hemocytes and serum interleukin 3, erythropoietin, and soluble transferrin receptor in a hot and humid environment

Yuhu Lv[1,2], Lin Cheng[3], Xiqian Zhang[1,2], Fenglin Peng[3], Yu Yuan[4], Xiquan Weng[4] and Wen-Tao Lin[5]

[1] College of Physical Education, Guangdong University of Education, GuangZhou, Guangdong, China
[2] Research Center for Adolescent Sports and Health Promotion of Guangdong Province, GuangZhou, Guangdong, China
[3] College of Physical Education and Health, Guangxi Normal University, Guilin, Guangxi Zhuang Autonomous Region, China
[4] Guangzhou Sport University, Guangzhou, Gaungdong, China
[5] College of Sports Science, Zhuhai College of Science and Technology, Zhuhai, Guangdong, China

ABSTRACT

Background: Exercise in humid and hot environments (HHEs) may result in decreased perception, motor performance, and memory owing to endogenous heat production and exogenous load. However, whether a single bout of exercise (SBOE) intensity affects the magnitude of changes in the levels of hemocytes remains controversial. In this article, we aimed to investigate the effects of a SBOE of varying intensities on blood cells in HHE.

Methods: Thirty-two volunteers were randomly divided into a quiet control group (QC), 55% VO_2max intensity exercise group (HHE55%), 70% VO_2max intensity exercise group (HHE70%), and 85% VO_2max intensity exercise group (HHE85%). The participants in the exercise groups were assigned to perform an SBOE on the treadmill under HHE conditions for 30 min, whereas participants in the QC remained still under HHE conditions for 30 min (temperature: 28–32 °C, relative humidity: 85–95%).

Results: The net body mass (NBM), perfusion index (PI), mean corpuscular volume (MCV), platelet (PLT), and plateletcrit (PCT) values were affected significantly by the exercise intensity ($P < 0.01$) the hemoglobin (HGB) and neutrophil count (NE) were affected significantly by exercise intensity ($P < 0.05$). After an SBOE, compared with that before exercise, the sublingual temperature (ST) of all groups, the NBM and MCV of all exercise groups, the PI of the HHE55% and HHE70% groups, the HGB, hematocrit (HCT), and NE of the HHE70% group, the red blood cell count (RBC), PLT, and PCT of the HHE70% and HHE85% groups, and the white blood cell count (WBC) of HHE85% changed very significantly ($P < 0.01$). The PCT of QC, blood oxygen saturation (SaO_2), and soluble transferrin receptor (sTfR) levels in the HHE55% group, the lymphocyte count (LY) in the HHE70% group, and the HGB and HCT in the HHE85% group changed significantly ($P < 0.05$).

Conclusion: Low- and moderate-intensity SBOE in HHE could increase the serum EPO and serum sTfR levels and decrease the serum IL-3 levels. Conversely, a

Submitted 9 July 2021
Accepted 6 November 2024
Published 29 November 2024

Corresponding authors
Xiquan Weng, wengxq@gzsport.edu.cn
Wen-Tao Lin, gtwtlin@163.com

Academic editor
Philip Reno

Additional Information and Declarations can be found on page 14

DOI **10.7717/peerj.18603**

How to cite this article Lv Y, Cheng L, Zhang X, Peng F, Yuan Y, Weng X, Lin W-T. 2024. Effects of a single bout of exercise on human hemocytes and serum interleukin 3, erythropoietin, and soluble transferrin receptor in a hot and humid environment. **PeerJ 12:e18603 DOI 10.7717/peerj.18603**

high-intensity load could increase the risk of inflammation. Therefore, low-intensity exercise may be more appropriate for an SBOE in HHE.

Subjects Molecular Biology, Anatomy and Physiology, Hematology, Kinesiology
Keywords Single bout of exercise, Hot and humid environment, Intensity, Hemocytes, Serum hematopoietic factors

INTRODUCTION

Workers, firefighters, athletes, military personnel, and people living in specialized regions frequently face hot and humid conditions (*Wijekulasuriya et al., 2022*; *de Korte et al., 2023*; *Chan et al., 2016*; *Wright et al., 2013*; *Ashworth, Cotter & Kilding, 2021*; *Choudhary & Udayraj, 2023*). When the relative humidity (RH) reaches 70%, the body becomes more prone to fatigue (*Tsutsumi et al., 2007*). Humans in hot and humid environments (HHEs) are exposed to health risks and thermal discomfort that severely affect their physical, physiological, and mental workload (*Golbabaei et al., 2022*). Heat stress may lead to perceptual, motor, and memory loss owing to endogenous heat production and exogenous loading, which consequently reduces the quality of work, training, and exercise performance (*de Korte et al., 2023*; *Lee et al., 2015*; *Nybo, 2008*; *De Jonge et al., 2012*; *Than Tran et al., 2015*; *Randall, Ross & Maxwell, 2015*; *Cleary, Toy & Lopez, 2014*). Heat and humidity can also cause sleep deprivation, thus leading to liver damage and fatigue, which affects the ability of individuals to exercise (*Li et al., 2014*). To improve the adaptability of different populations in HHEs, researchers have conducted studies on multiple strategies, including but not limited to the use of fans (*Rubin, 2019*), determining the appropriate time for acclimatization (*Neal et al., 2016*; *Garrett, Rehrer & Patterson, 2011*), designing cooling apparel (*McFarlin, Henning & Venable, 2017*; *McFarlin et al., 2016*), exploring the mechanisms underlying heat stress generation (*Crandall & Gonzalez-Alonso, 2010*; *Poirier et al., 2013*), and determining the influence of thermal environment and air quality on thermal comfort (*Yu, Matzarakis & Lin, 2020*). A study has shown that even short-term heat acclimatization for 4 days facilitates effective perceptual adaptations without compromising immune status before an ultra-endurance race under heat stress (*Willmott et al., 2017*).

Appropriate exercise has various functions, including but not limited to memory enhancement, cognitive improvement, and inflammation reduction (*De Miguel et al., 2021*). Exercise may also have positive effects on vulnerable patient groups. A study showed that breast cancer treatment can lead to prolonged immunosuppression that leaves patients vulnerable to infection. However, exercise may be a strategy for at-risk groups, such as patients with cancer, to improve resistance to infectious disease (*Bartlett et al., 2021*). A single bout of exercise (SBOE) can affect blood circulation by stimulating the movement of hematopoietic bone marrow stem cells and aging immune cells from peripheral tissues into the circulatory system and stimulating immune cells both during effort and recovery (*Sellami et al., 2018*; *Navalta et al., 2010*; *Simpson et al., 2010*; *Sardeli, Mori & Lord, 2022*; *Mathot et al., 2021*; *Dinh et al., 2017*). Acute exercise can cause stress

and inflammation, increase red blood cell volume and platelet compensation, affect the function of circulating hematopoietic progenitor cells, and alter the classification of white blood cells (*Stelzer et al., 2015*). The "open window" theory suggests that the immune system is temporarily suppressed following an acute bout of endurance exercise (*Kakanis et al., 2010*; *Peake et al., 2017*). One study showed that an SBOE drastically augments the number of cytomegalovirus and Epstein-Barr virus-specific T-cells manufactured over an 8-day period (*Spielmann et al., 2016*), whereas another study showed that NK cell activity is enhanced during moderate as well as severe acute exercise, whereas immunodepression is observed after severe exercise (*Pedersen & Ullum, 1994*). These findings suggest that lymphocyte redeployment after acute exercise may be an evolutionary conservative immune mechanism that improves our ability to resist infection. However, whether the intensity of exercise during acute exercise affects the magnitude of changes in immune cell levels remains controversial (*Dinh et al., 2017*; *Timmerman et al., 2008*). Only a limited number of studies have been conducted on the outcomes of an SBOE of different intensities under HHEs. In this study, we hypothesized that the blood cell count and levels of interleukin 3 (IL-3), erythropoietin (EPO), and soluble transferrin receptor (sTfR) in participants would alter in response to an SBOE under HHE conditions and would be affected by the exercise intensity. We determined the net body mass (NBM), sublingual temperature (ST), blood oxygen saturation (SaO_2), perfusion index (PI), blood cell count, and hematopoietic factors of participants pre- and post-exercise and explored the effects of an SBOE in an HHE on the body. Our results preliminarily verified that the exercise intensity of SBOE can affect human blood cells, hematopoietic factors, and immune functions, which may provide a reference for further research.

MATERIALS AND METHODS

Participants

Thirty-two young men were randomly divided into a quiet control group (QC), 55% VO_2max intensity exercise group (HHE55%), 70% VO_2max intensity exercise group (HHE70%), and 85% VO_2max intensity exercise group (HHE85%), with eight participants in each group. Participants in the exercise groups were assigned to perform an SBOE in an HHE for 30 min, whereas participants in the QC remained still in an HHE for 30 min (temperature: 28–32 °C, relative humidity: 85–95%). The participants included in the study had no history of hematopoietic, liver, kidney, and endocrine diseases and passed an exercise health screening. Participants took a regular diet and did not consume supplements during the study. All participants were volunteers who signed an informed consent form before the commencement of the experiment. The human study protocols were approved by the Guangzhou Sport University Human Ethics Committee (No. 2018LCLL-10). Baseline participant characteristics are shown in Table 1.

Exercise training program

We used air conditioning (GREE, KFR-35GW/(35556) FNDe-3) and humidifiers (Midea, S35U-L, and S20U-M) to create an HHE (approximate temperature of 28 to 32 °C and approximate humidity of 85% to 95%) in a chamber. Participants from the HHE55%,

Table 1 Baseline participant characteristics.

	N	Age, years	Height, cm	Net body mass, kg	VO_2max, mL/kg/min
QC	8	21 ± 1	173 ± 5	64.93 ± 4.79	48.70 ± 5.16
HHE55%	8	21 ± 1	172 ± 8	63.38 ± 4.57	53.44 ± 4.46
HHE70%	8	21 ± 1	175 ± 4	66.50 ± 4.57	51.34 ± 5.26
HHE85%	8	21 ± 1	175 ± 4	66.63 ± 5.34	48.33 ± 5.28

HHE70%, and HHE85% groups were assigned to perform an SBOE on the treadmill in the artificial HHE for 30 min, whereas participants from the QC group remained still in the HHE for 30 min. All participants were free to drink water (*i.e.*, there was no restriction on the amount of water intake) during the test.

Measurements

In the morning before exercise and the morning after exercise, participants entered the laboratory and sat quietly for 10 min, and NBM, ST, SaO_2, and PI were measured. Following this, 2 mL of EDTA-k_2 anticoagulant and 5 mL of non-anticoagulant from venous blood were collected from the elbow area for routine blood examination and serum separation, respectively. Immediately after the mixing of the 2 mL of EDTA-k_2 anticoagulant of venous blood was completed using an oscillator, a routine blood test was performed using the XS-800i Sysmex (Sysmex Co., Ltd., Kobe, Japan). The red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), platelets (PLT), plateletcrit (PCT), mean platelet volume (MPV), white blood cell count (WBC), lymphocyte count (LY), neutrophil count (NE), monocyte count (MO), eosinophil count (EO), and basophil count (BA) were determined. SaO_2 and PI were measured using a Prince-100H pulse oxygen saturation meter (Heal Force Co., Ltd., Shanghai, China). After 5 mL (non-anticoagulant) of venous blood was collected, centrifugation was performed immediately at 3,000 rpm for 10 min in a bench-top centrifuge. The serum was added to a tube and stored in the refrigerator at −20 °C for testing. Enzyme-linked immunosorbent assay (ELISA) was performed to measure serum hematopoietic factors using a TECAN Infinite M200PRO multifunctional microplate reader (Männedorf, Switzerland). All serum hematopoietic factors were measured in accordance with the manufacturer's instructions. First, blank control, standard, and test samples were added to the ELISA plate using a single-channel pipette, and the samples were added within 10 min. In addition, all tests were performed using the same batch of ELISA reagent under the same tester to reduce intra- and inter-assay variations. Human serum IL-3 levels were measured using an IL-3 ELISA Kit (Blue Gene Biotech Co., Ltd., Shanghai, China), with the approximate standard range being 0 to 1,000 pg/mL. Human serum EPO was measured using the EPO ELISA Kit (Blue Gene Biotech Co., Ltd., Shanghai, China), the approximate standard range and sensitivity were 0 to 250 and less than 1.6 mIU/mL, respectively. Human serum sTfR was assayed using an sTfR ELISA Kit (Blue Gene Biotech

Co., Ltd., Shanghai, China). The assay range was between 0 and 100 ng/mL, and the sensitivity in this assay was less than 0.39 ng/mL.

Statistical analysis

The distribution normality of the variables was determined using a 1-sample Kolmogorov–Smirnov test and the homogeneity of variances was determined using Levene's test in IBM SPSS Statistics 26 (IBM, Armonk, NY, USA). Normally distributed variables are presented as mean ± standard deviation (SD) and 95% confidence interval for the mean. A single-sample *t*-test was used to determine baseline participant characteristics and independent-sample *t*-test baseline participant characteristics between different groups. A paired sample *t*-test was used to analyze the pre-exercise and post-exercise indicators in the same group. One-way ANOVA was used to analyze comparisons among groups. The Bonferroni method was used to analyze the data variance homogeneity without significance. Other data with significance were analyzed using the Kruskal–Wallis test. An independent-sample *t*-test was used to analyze data that had been analyzed for significance using the Kruskal–Wallis test. Statistical significance was set at $P < 0.05$.

RESULTS

Effects of SBOE on NBM, ST, SaO_2, and PI

The results of one-way ANOVA showed that the NBM ($P < 0.01$) and PI ($P < 0.01$) were affected significantly by the exercise intensity, whereas ST ($P > 0.05$) was not affected. Results of the Kruskal–Wallis test showed that SaO_2 ($P > 0.05$) was not affected by exercise intensity. Bonferroni's *post hoc* comparison showed that the NBM (HHE55%, $P < 0.01$; HHE70%, $P < 0.05$; HHE85%, $P < 0.01$) and PI (HHE55%, $P < 0.01$; HHE70%, $P < 0.01$; HHE85%, $P < 0.05$) values in the test groups were significantly different from those in the QC group. However, the NBM and PI values from the HHE55%, HHE70%, and HHE85% groups showed no significant differences. The results of a paired sample *t*-test showed that, compared with the pre-exercise values, the NBM in the QC, HHE55%, HHE70%, and HHE85% groups decreased by 0.07 kg, 0.94 kg ($P < 0.01$), 0.88 kg ($P < 0.01$), and 1.07 kg ($P < 0.01$), respectively, after an SBOE in an HHE. The ST values in the QC, HHE55%, HHE70%, and HHE85% groups increased by 0.52 °C ($P < 0.01$), 0.49 °C ($P < 0.01$), 0.89 °C ($P < 0.01$), and 0.92 °C ($P < 0.01$), respectively. The SaO_2 in the QC group increased by 0.38%, whereas the values in the HHE55%, HHE70%, and HHE85% groups decreased by 0.62% ($P < 0.05$), 0.13%, and 0.50%, respectively. The PI in the QC, HHE55%, HHE70%, and HHE85% groups increased by 0.05%, 6.56% ($P < 0.01$), 5.23% ($P < 0.01$), and 3.72%, respectively. These data are presented in Table 2.

Effects of SBOE on blood cells

One-way ANOVA showed that MCV ($P < 0.01$), PLT ($P < 0.01$), and PCT ($P < 0.01$) were affected significantly by exercise intensity, whereas the RBC, WBC, LY, MO, and EO were unaffected ($P > 0.05$). Results of the Kruskal–Wallis test showed that the HGB and NE were affected by the exercise intensity ($P < 0.05$), whereas the HCT, MPV, and BA values were unaffected by the exercise intensity ($P > 0.05$). Bonferroni's *post hoc* comparison

Table 2 Values of NBM, ST, SaO_2, and PI pre- and post-exercise.

	Intervention	QC (N = 8)	HHE55 (N = 8)	HHE70% (N = 8)	HHE85% (N = 8)
NBM, kg	Pre-Ex	64.88 ± 4.74	63.38 ± 4.52	66.19 ± 4.34	66.63 ± 5.59
	Post-Ex	64.81 ± 4.72	62.44 ± 4.47[b, d]	65.31 ± 4.04[b, c]	65.56 ± 5.45[b, d]
ST, °C	Pre-Ex	36.43 ± 0.30	36.67 ± 0.32	36.90 ± 0.39	36.81 ± 0.35
	Post-Ex	36.95 ± 0.08[b]	37.16 ± 0.30[b]	37.79 ± 0.51[b]	37.73 ± 0.64[b]
SaO_2, %	Pre-Ex	98.50 ± 1.41	99.00 ± 0.00	97.88 ± 1.36	98.38 ± 1.41
	Post-Ex	98.88 ± 0.35	98.38 ± 0.52[a]	97.75 ± 1.58	97.88 ± 1.81
PI, %	Pre-Ex	1.73 ± 0.53	1.60 ± 0.56	3.56 ± 1.46	2.26 ± 1.57
	Post-Ex	1.78 ± 0.51	8.16 ± 2.94[b, d]	8.79 ± 3.21[b, d]	5.98 ± 5.02[c]

Notes:
Data are presented as mean ± standard deviation (SD).
Abbreviations: Pre-Ex, pre-exercise; Post-Ex, post-exercise; NBM, net body mass; ST, sublingual temperature; SaO_2, blood oxygen saturation; PI, perfusion index.
[a] $P < 0.05$.
[b] $P < 0.01$, compared with Pre-Ex.
[c] $P < 0.05$.
[d] $P < 0.01$, compared with QC.

showed that the MCV values in the HHE55% ($P < 0.01$), HHE70% ($P < 0.01$), and HHE85% ($P < 0.05$) groups were significantly different from those in the QC groups. However, the values in the other groups did not show significant differences. The PLT (HHE70%, $P < 0.01$; HHE85%, $P < 0.01$) in two test groups was significantly different from that in the QC group, but there were no significant differences between the values in the QC group and the two remaining groups. The PCT values (HHE70%, $P < 0.05$; HHE85%, $P < 0.01$) in two test groups were significantly different from that in the QC group. The PCT value in the HHE55% (HHE70%, $P < 0.05$; HHE85%, $P < 0.05$) was significantly different from the values in two test groups. However, no significant difference was observed between the values in the QC and HHE55% groups. The independent-sample t-test showed that the HGB values (HHE70%, $P < 0.01$; HHE85%, $P < 0.05$) in two test groups were significantly different from that in the QC group, but there were no significant differences between the values in the two remaining groups. NE values in the remaining two groups showed no significant differences. The paired sample t-test showed that, compared with the pre-exercise values, after an SBOE in an HHE, the RBC in the QC, HHE55%, HHE70%, and HHE85% groups increased by 0.04×10^{12}/L, 0.16×10^{12}/L, 0.25×10^{12}/L, and 0.14×10^{12}/L, respectively. The changes in the HHE70% and HHE85% group were significant ($P < 0.01$). The HGB values decreased by 0.87 g/L in the QC group, whereas the HGB values in the HHE55%, HHE70%, and HHE85% groups increased by 5.25 g/L, 7.13 g/L, and 3.50 g/L, respectively. The changes in the HHE70% ($P < 0.01$) and HHE85% ($P < 0.05$) groups were significant. The HCT in the QC, HHE55%, HHE70%, and HHE85% groups increased by 0.36%, 0.62%, 1.67%, and 0.80%, respectively. The values in the HHE70% ($P < 0.01$) and HHE85% ($P < 0.05$) groups increased significantly. The MCV in the QC groups increased by 0.03 fL, whereas the MCV decreased by 1.37 fL, 1.09 fL, and 0.92 fL in the HHE55%, HHE70%, and HHE85% groups ($P < 0.01$), respectively, with the change being significant. The PLT in the QC, HHE55%, HHE70%, and HHE85% groups increased by 1.50×10^9/L, 14.75×10^9/L, 34.50×10^9/L, and $34.75 \times$

10^9/L, respectively. The changes in the HHE70% and HHE85% ($P < 0.01$) were significant. The PCT in the QC, HHE55%, HHE70%, and HHE85% groups increased by 0.01%, 0.02%, 0.04%, and 0.04%, respectively. The changes in the QC ($P < 0.05$), HHE70% ($P < 0.01$), and HHE85% ($P < 0.01$) groups were significant. The WBC in the QC, HHE55%, HHE70%, and HHE85% groups increased by 0.51 × 10^9/L, 0.08 × 10^9/L, 0.50 × 10^9/L, and 0.91 × 10^9/L, respectively. Changes in the HHE85% group were significant ($P < 0.01$). The LY in the QC, HHE55%, HHE70%, and HHE85% groups increased by 0.52 × 10^9/L, 0.23 × 10^9/L, 0.43 × 10^9/L, and 0.04 × 10^9/L, respectively. The change in the HHE70% group was significant ($P < 0.05$). The NE values in the QC and HHE55% groups decreased by 0.10 × 10^9/L and 0.21 × 10^9/L, respectively, whereas those in the HHE70% and HHE85% groups increased by 0.01 × 10^9/L and 0.92 × 10^9/L, respectively. Only the change in the HHE85% group ($P < 0.01$) was significant. The MO, EO, and BA values in the QC, HHE55%, HHE70%, and HHE85% groups showed no significant change ($P > 0.05$). These data are presented in Table 3.

Serum IL-3, EPO, and sTfR

The results of the paired sample *t*-test showed that the IL-3 levels in all groups decreased compared with the pre-exercise values, but no significant difference was observed ($P > 0.05$) (Fig. 1A). The results of one-way ANOVA showed that the IL-3 levels were not affected by the exercise intensity. Bonferroni's *post hoc* comparison showed that there were no significant differences between any pair of groups ($P > 0.05$) (Fig. 1B).

The results of the paired sample *t*-test showed that the EPO in the QC, HHE55%, and HHE70% groups increased compared with the pre-exercise values, whereas that in the HHE85% decreased marginally; however, there were no significant differences ($P > 0.05$) (Fig. 2A). One-way ANOVA results showed that EPO was not affected by exercise intensity. Bonferroni's *post hoc* comparison showed that there were no significant differences between the values in any pair of groups ($P > 0.05$) (Fig. 2B).

Results of the paired sample *t*-test showed that the sTfR levels in all groups increased from the pre-exercise levels, and the level in the HHE55% group increased significantly ($P < 0.05$) (Fig. 3A). One-way ANOVA showed that sTfR was not affected by exercise intensity. Bonferroni's *post hoc* comparison showed that there were no significant differences between any pair of groups ($P > 0.05$) (Fig. 3B).

DISCUSSION

Effects of SBOE on NBM, ST, SaO_2, and PI

Exercise in hot and/or humid environments can lead to exertional heat stress and exertional rhabdomyolysis and reduce perception and athletic ability owing to endogenous heat production and exogenous load, which directly reduces the quality of work, exercise training, and exercise performance (*Skwarczynski et al., 2010*; *Melikov & Kaczmarczyk, 2012*; *Guy et al., 2015*; *Periard, Racinais & Sawka, 2015*; *Willmott et al., 2019*; *McCubbin et al., 2020*; *Carneiro et al., 2021*). Prolonged heat stress can disrupt the thermoregulatory, cardiovascular, and gastrointestinal systems, leading to severe concerns for an athlete's health and performance (*McCubbin et al., 2020*). Some major sporting events are held in

Table 3 Values of blood cells pre- and post-exercise.

	Intervention	QC (N = 8)	HHE55% (N = 8)	HHE70% (N = 8)	HHE85% (N = 8)
RBC, 10^{12}/L	Pre-Ex	5.06 ± 0.31	5.44 ± 0.62	4.86 ± 0.35	5.03 ± 0.34
	Post-Ex	5.10 ± 0.28	5.60 ± 0.63	5.11 ± 0.39[b]	5.17 ± 0.32[b]
HGB, g/L	Pre-Ex	151.25 ± 10.79	150.75 ± 14.22	138.50 ± 10.64	146.00 ± 8.60
	Post-Ex	150.38 ± 9.12	156.00 ± 17.76	145.63 ± 11.61[b, d]	149.50 ± 10.86[a, c]
HCT, %	Pre-Ex	45.10 ± 2.40	45.71 ± 4.00	42.73 ± 2.58	44.90 ± 2.18
	Post-Ex	45.46 ± 2.11	46.33 ± 4.23	44.40 ± 2.81[b]	45.70 ± 2.17[a]
MCV, fL	Pre-Ex	89.20 ± 2.53	84.93 ± 11.32	88.44 ± 9.16	89.36 ± 2.84
	Post-Ex	89.23 ± 2.48	83.56 ± 11.13[b, d]	87.35 ± 9.14[b, d]	88.44 ± 2.82[b, c]
PLT, 10^9/L	Pre-Ex	215.88 ± 53.09	254.75 ± 70.22	217.13 ± 39.84	232.13 ± 36.59
	Post-Ex	217.38 ± 54.67	269.50 ± 56.69	251.63 ± 51.62[b, d]	266.88 ± 45.39[b, d]
PCT, %	Pre-Ex	0.24 ± 0.046	0.28 ± 0.075	0.25 ± 0.046	0.25 ± 0.033
	Post-Ex	0.25 ± 0.053[a]	0.30 ± 0.049	0.29 ± 0.056[b, c, e]	0.29 ± 0.039[b, d, e]
WBC, 10^9/L	Pre-Ex	8.43 ± 3.57	9.03 ± 1.31	8.15 ± 2.20	6.21 ± 1.22
	Post-Ex	8.94 ± 3.04	9.11 ± 1.62	8.65 ± 1.95	7.12 ± 1.38[b]
LY, 10^9/L	Pre-Ex	1.84 ± 0.96	2.31 ± 0.43	2.21 ± 0.38	2.41 ± 0.68
	Post-Ex	2.36 ± 0.44	2.54 ± 0.53	2.64 ± 0.44[a]	2.45 ± 0.62
NE, 10^9/L	Pre-Ex	6.09 ± 3.60	6.10 ± 1.34	5.20 ± 1.99	3.16 ± 0.84
	Post-Ex	5.99 ± 3.15	5.89 ± 1.39	5.21 ± 1.59	4.08 ± 1.35[b]
MO, 10^9/L	Pre-Ex	0.43 ± 0.191	0.54 ± 0.106	0.56 ± 0.130	0.46 ± 0.092
	Post-Ex	0.49 ± 0.173	0.59 ± 0.164	0.61 ± 0.173	0.48 ± 0.089
EO, 10^9/L	Pre-Ex	0.09 ± 0.079	0.09 ± 0.053	0.17 ± 0.160	0.10 ± 0.058
	Post-Ex	0.09 ± 0.069	0.08 ± 0.060	0.18 ± 0.162	0.10 ± 0.068
BA, 10^9/L	Pre-Ex	0.02 ± 0.011	0.02 ± 0.013	0.03 ± 0.019	0.02 ± 0.009
	Post-Ex	0.03 ± 0.021	0.03 ± 0.014	0.03 ± 0.019	0.03 ± 0.017

Notes:
Data are presented as mean ± standard deviation (SD).
Abbreviations: Pre-Ex, pre-exercise; Post-Ex, post-exercis; WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; PLT, platelets count; HCT, hematocrit; MCV, mean red blood cell volume; PCT, plateletcrit; MPV, mean platelet volume; LY, lymphocyte count; NE, neutrophil granulocyte; MO, number of monocytes; EO, number of eosinophils; BA, number of basophils.
[a] $P < 0.05$.
[b] $P < 0.01$, compared with pre-exercise vakyes.
[c] $P < 0.05$.
[d] $P < 0.01$, compared with QC.
[e] $P < 0.05$, compared with HHE55%.

hot and/or humid conditions, which can be challenging for several athletes (*Gerrett et al., 2019*). Nutritional strategies before, during, and after exercise and heat acclimation are suitable methods to alleviate exercise heat stress (*McCubbin et al., 2020*; *Sawka et al., 2011*). A study showed that the body mass of participants decreased significantly after the first day of acute exercise-heat stress (*Willmott et al., 2019*). Our results showed that the NBM in all exercise groups decreased significantly from the pre-exercise values and showed significant difference with the values in the QC group, similar to the findings reported by *Willmott et al. (2019)*.

Body temperature is affected considerably by our environment and the work we do, and body surface temperature (*Periard, Racinais & Sawka, 2015*), sublingual temperature

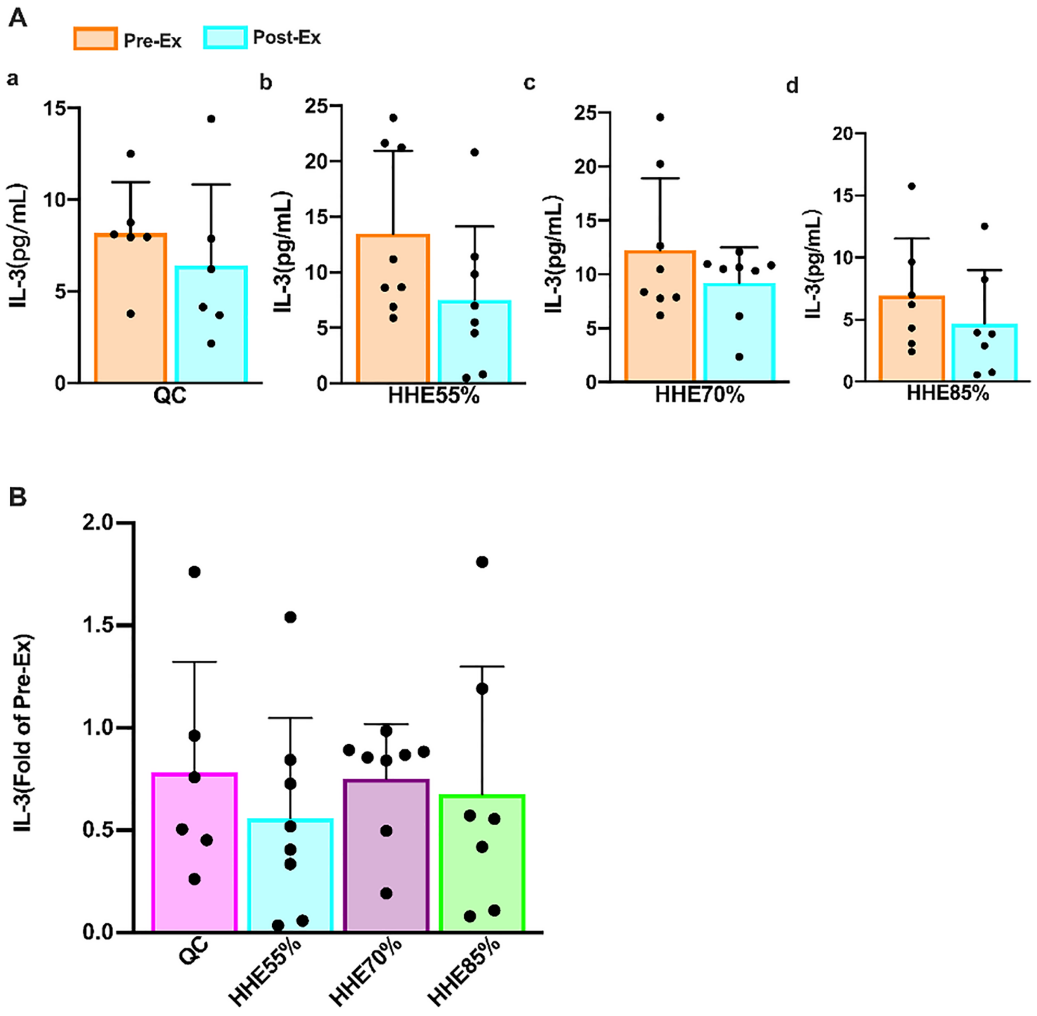

Figure 1 Effects of acute exercise on human serum IL-3 levels. (A) The IL-3 level in the QC, HHE55%, HHE70%, and HHE85% groups pre- and post-exercise. (B) The fold of IL-3 value in the QC, HHE55%, HHE70%, and HHE85% groups post-exercise compared with that pre-exercise. Abbreviations: Pre-Ex, pre-exercise; Post-Ex, post-exercise; IL-3, interleukin 3; QC, quiet control bout; HHE55%, 55% VO_2max intensity exercise bout; HHE70%, 70% VO_2max intensity exercise bout; HHE85%, 85% VO_2max intensity exercise bout. Full-size DOI: 10.7717/peerj.18603/fig-1

(*Singh & Kumar, 2019*), forehead temperature (*Dzien et al., 2021*), ear canal temperature (*Mueller et al., 2012*), and rectal body temperature (*Meade et al., 2020*; *Levy, Allender & Keller, 2020*) are commonly used as detection indices (*van der Vinne et al., 2020*). Prolonged exercise increases skin, muscle, and core body temperatures (*Ely et al., 2007*). A study of medical technician students during simulated work activities in a hot environment showed that the core temperature, skin temperature, and mean body temperature of participants were higher under heated conditions than under neutral conditions (*Gerhart et al., 2020*). Our results showed that the ST was unaffected by the exercise intensity in an HHE. The post-exercise ST in all groups was significantly greater than the pre-exercise ST, and the HHE55% group showed the least increase, indicating that low-intensity exercise may be more conducive to controlling the rise of body temperature in an HHE.

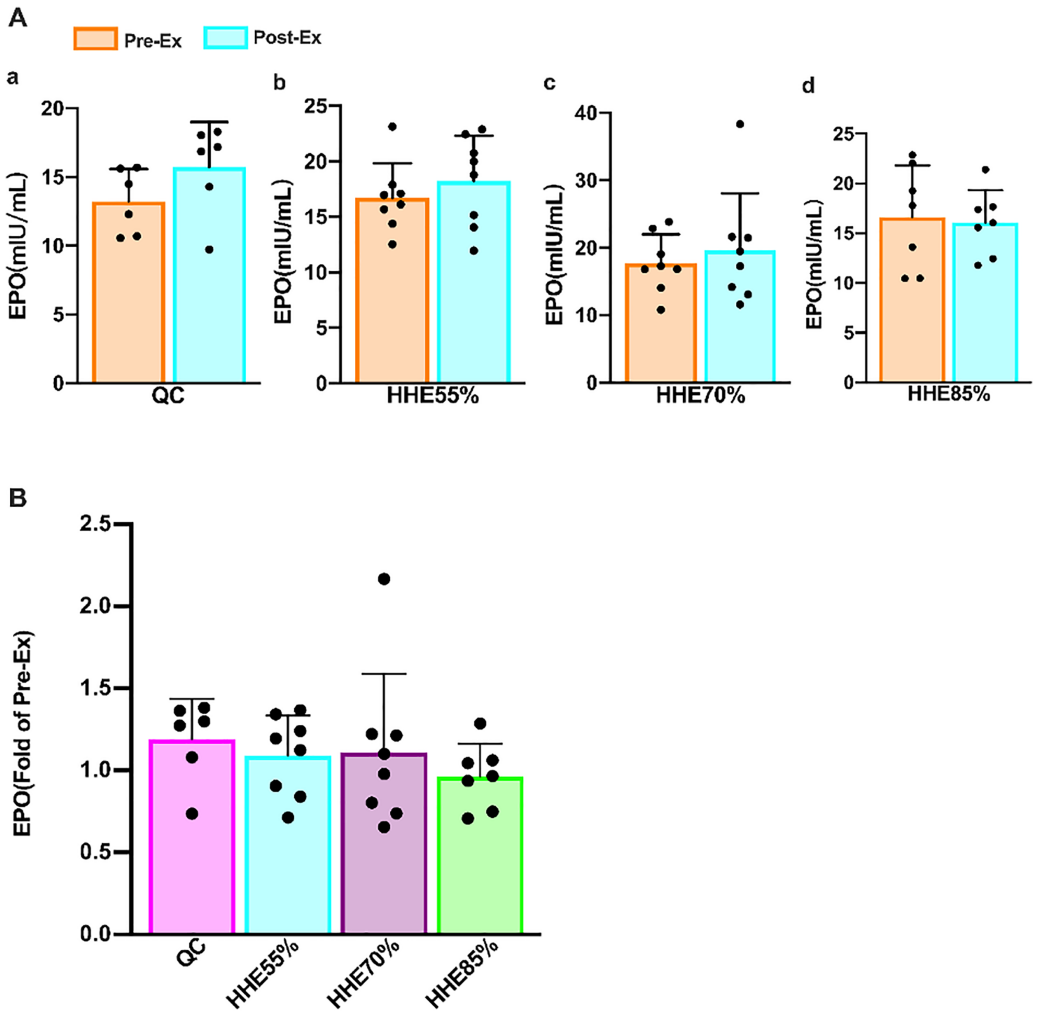

Figure 2 Effects of acute exercise on human serum EPO levels. (A) The EPO levels in the QC, HHE55%, HHE70%, and HHE85% groups pre- and post-exercise. (B) The fold of EPO level in the QC, HHE55%, HHE70%, and HHE85% groups post-exercise compared with that pre-exercise. Abbreviations: Pre-Ex, pre-exercise; Post-Ex, post-exercise; EPO, erythropoietin; QC, quiet control bout; HHE55%, 55% VO_2max intensity exercise bout; HHE70%, 70% VO_2max intensity exercise bout; HHE85%, 85% VO_2max intensity exercise bout. Full-size DOI: 10.7717/peerj.18603/fig-2

The SaO_2 indicates the physiological oxygen-carrying capacity. An individual's body temperature increases by 1 °C, whereas their blood oxygen saturation decreases by 0.27% (*Qiu et al., 2020*). Our findings showed that SaO_2 was unaffected by exercise intensity, whereas PI was affected significantly by the exercise intensity in an HHE. The PI levels in all exercise groups were significantly different from that in the QC group, but there was no significant differences among the values in any pair of exercise groups. The post-exercise SaO_2 in all exercise groups had decreased compared with the corresponding pre-exercise values, but only the change in the HHE55% group was significant, indicating that blood oxygen utilization is optimum at this exercise intensity. The post-exercise PI in all exercise groups was significantly higher than the pre-exercise PI, and the values were significantly

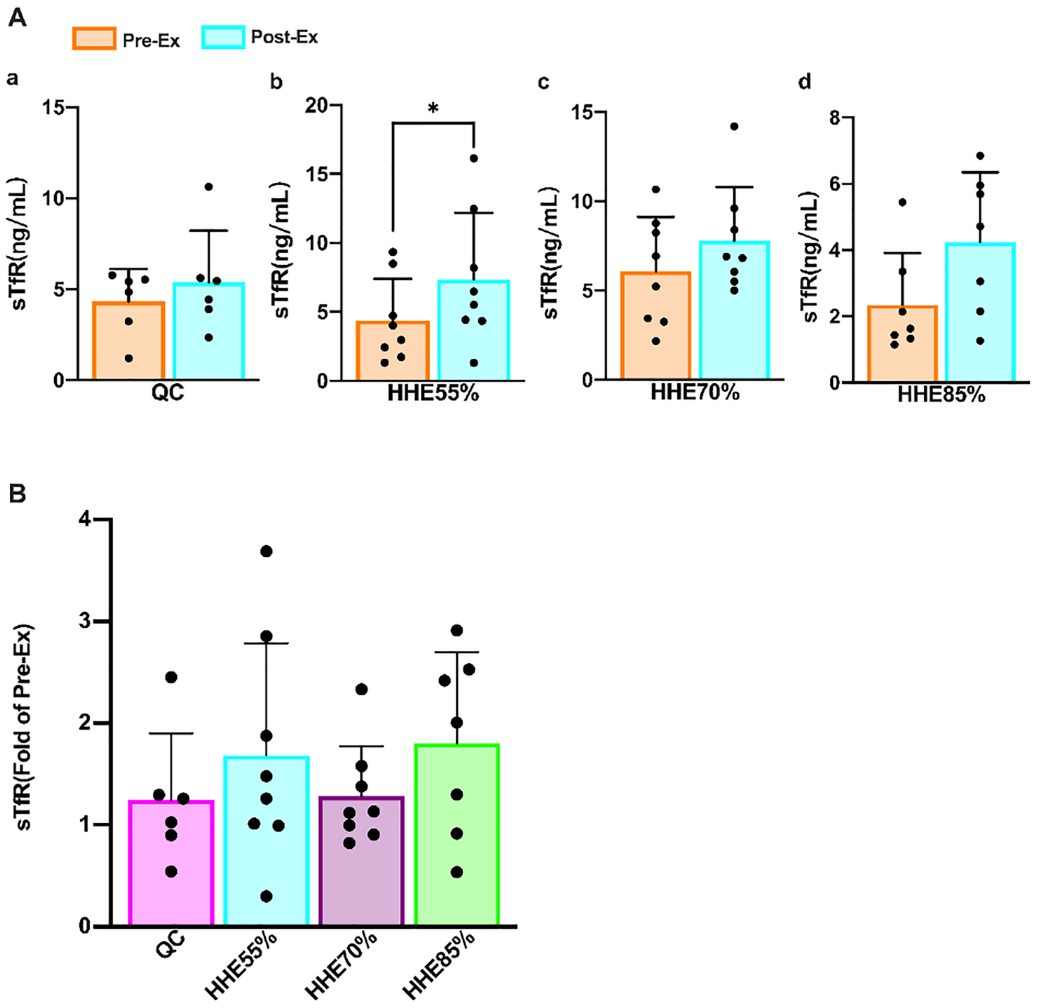

Figure 3 Effects of acute exercise on human serum sTfR levels. (A) The sTfR levels in the QC, HHE55%, HHE70%, and HHE85% groups pre- and post-exercise. (B) The fold of sTfR level in the QC, HHE55%, HHE70%, and HHE85% groups post-exercise and pre-exercise. Abbreviations: Pre-Ex, pre-exercise; Post-Ex, post-exercise; sTfR, soluble transferrin receptor; QC, quiet control bout; HHE55%, 55% VO_2max intensity exercise bout; HHE70%, 70% VO_2max intensity exercise bout; HHE85%, 85% VO_2max intensity exercise bout. Full-size DOI: 10.7717/peerj.18603/fig-3

different in all exercise groups compared to that in the QC group, suggesting that the choice of low- and moderate-intensity exercise may be more appropriate in an HHE.

Effects of SBOE on blood cells

The human body is more prone to dehydration, mineral loss, and weight loss in an HHE (*Adams et al., 2018*; *James et al., 2017*). Dehydration may also reduce cardiac filling and affect the body's ability to regulate blood pressure (*Stoehr et al., 2011*). Research showed that plasma volume decreases significantly during heat exposure and after exercise at a certain level of dehydration, whereas it is better maintained during and after exercise (*Jimenez et al., 1999*). Exercise can lead to acute and chronic increases in the HCT (*Pichon, Connes & Robach, 2016*). Meanwhile, the HGB and HCT values decrease in athletes after

single and repeated bouts of exercise (*Alberty et al., 2021*). Moreover, physical exercise research until exhaustion showed that erythrocytes do not undergo alterations (*Siquier-Coll et al., 2019*). *Boukelia, Gomes & Florida-James (2018)* used Bonferroni-adjusted *post hoc* tests to show that PLT is significantly affected by exercise and environmental conditions at two different times of day (09:00 hrs and 18:00 hrs). Our results showed that HGB, MCV, PLT, and PCT were affected significantly by exercise intensity, whereas others were not. The HGB, MCV, PLT, and PCT values in the HHE70% and HHE85% groups changed significantly compared with those in the QC group. The PCT values of the HHE70% and HHE85% groups were significantly different from those in the HHE55% group. The RBC, HGB, HCT, MCV, PLT, and PCT values in the HHE70% and HHE85% groups changed significantly compared with the pre-exercise values, whereas only the MCV changed significantly in the HHE55% group. We speculated that the decrease in MCV may be associated with exercise, which severely affected the RBC. Moderate exercise can strengthen the immunity of athletes, but the high intensity of long-term exercise temporarily weakens the immunity of athletes (*Simpson et al., 2006*). Exposure to heat may mobilize more white blood cells into the circulation to increase physiological demands (*Mitchell et al., 2002*). A study on highly trained runners who performed a 10 km time trial run in an HHE showed that the posttrial WBC, NE, LY, and MO increased significantly compared with the pretrial values (*Boukelia, Gomes & Florida-James, 2018*). Similarly, completing a 100-mile recreational cycling race in an HHE resulted in a significant increase in the total number of circulating white blood cells (*Luk et al., 2016*). We found that the WBC, LY, NE, MO, EO, and BA were not affected by exercise intensity during SBOE in an HHE. The WBC and NE in the HHE85% group and LY in the HHE70% group changed significantly compared with the pre-exercise values, whereas the other values did not change. This indicates that low-intensity exercise may be better for SBOE in an HHE, because low-intensity exercise may have a negligible effect on the blood.

Effects of SBOE on serum IL-3, EPO, and sTfR

IL-3 produced by monocytes and lymphocytes, also known as multiple colony-stimulating factors, indicates physiological hematopoietic function. Granulocyte-macrophage colony-stimulating factor (GM-CSF) plays a crucial role in bone marrow differentiation (*de Rezende et al., 2020*). To date, studies on IL-3 were primarily confined to hematopoietic, antitumor, and immunomodulatory effects (*Borriello et al., 2019*). Nevertheless, with more research, IL-3 has been found to play an essential role in diseases associated with inflammation (*Hu et al., 2019*). Although studies have shown that chronic exercise can boost immunity (*Sellami et al., 2018*), whereas acute exercise may reduce immunity (*Kakanis et al., 2010*; *Campbell & Turner, 2018*), findings from a recent study were contradictory (*Spielmann et al., 2016*). An early study showed that plasma IL-3 concentration is unaltered with training and/or with exercise (*Mucci et al., 2000*). We found that the IL-3 levels are unaffected by acute exercise and exercise intensity, consistent with the findings by *Mucci et al. (2000)*. IL-3 levels decreased after an SBOE in an HHE, but no significant difference was observed between any pair of exercise groups. After an SBOE, the WBC and NE of the HHE85% group increased significantly. These results indicate that

high-intensity exercise can increase the stimulation of the body, leading to acute infection or inflammation.

EPO acts as the primary regulator of red blood cell maturation. It is primarily synthesized in the kidney and to a lesser extent in the liver and brain (*Sgro et al., 2018*; *Jelkmann, 2011*). Its functions include promoting the proliferation and differentiation of erythrocyte progenitor cells, maintaining the number of erythrocytes and hemoglobin (*Jelkmann, 2011*), mediating apoptosis (*Kumral et al., 2006*), promoting axonal bud formation (*Garg, Sharma & Bansal, 2018*), facilitating immune-modulatory effects (*Nairz et al., 2012*), and improving heart function (*Zhang et al., 2016*). EPO was shown to help the Hb concentration remain relatively constant and accelerate the recovery of red blood cells after blood loss. Healthy individuals require limited amounts of EPO to maintain a stable state (*Jelkmann, 2011*). EPO is also used to treat various diseases, although it may exert adverse effects in patients with anemia and chronic heart failure, such as elevated blood pressure, thrombosis, and seizures (*Rao, Binbrek & Sobel, 2008*). A recent clinical study has shown that EPO is still relatively safe to treat chronic heart failure (*Zhang et al., 2016*). Recombinant human erythropoietin also has multiple functions, such as reducing the expression of inflammatory factors TNF-α and IL-1β after acute injury, improving motor function, reducing inflammation, and facilitating neuroprotection and functional recovery (*Zhou et al., 2017*). EPO generation depends on the partial pressure of tissue oxygen, and EPO expression is also activated when the arterial oxygen partial pressure decreases or O_2 affinity increases in the blood (*Jelkmann, 2011*). Under normal circumstances, a healthy serum EPO concentration is 6-32 IU/L, but significant differences are observed among individuals (*Jelkmann, 2011*; *Eckardt & Kurtz, 2005*). Heat stress resulting from endurance exercise in hypoxia (heat and hypoxic conditions) did not augment the EPO response (*Yatsutani et al., 2019*). Our data showed that EPO levels are unaffected by an acute exercise bout and exercise intensity. There was no significant difference between the values observed in different bouts and the pre-exercise values, consistent with the research findings of *Yatsutani et al. (2019)*. The EPO level in the HHE85% group decreased, whereas it increased in the other groups, indicating that high-intensity exercise in an HHE may decrease the EPO level, whereas low- and moderate-intensity may increase it.

With the improvement of our quality of life and understanding of science, people are becoming increasingly aware of the importance of exercise or physical activity. It is essential to pay attention to nutritional supplements and rest and take an appropriate approach to exercise. Young people may have a high demand for iron bioavailability owing to rapid growth and sports participation (*Shoemaker et al., 2019*). Some athletes may even have unsatisfactory iron reserves throughout the season (*Smith et al., 2020*), and iron overload can increase EPO resistance (*Zhang, 2020*). sTfR, red blood cell-free protoporphyrin, transferrin saturation (TS), serum iron (SI), and total iron-binding capacity play a significant clinical role in the diagnosis and differential diagnosis of abnormal iron metabolism (*Yang, Yang & Liang, 2013*). Physical exercise reduces the SI and TS and increases sTfR levels (*Di Santolo et al., 2008*), and sTfR levels exhibit a moderate correlation with the athletic performance of young female athletes, which may reflect the increase in their red blood cell production rate during their growth spurt

(*Di Santolo et al., 2008*; *Shoemaker et al., 2020*). Simultaneously, the relationship between hemoglobin levels and the performance of young male athletes was also relatively strong (*Shoemaker et al., 2019*). In addition, sTfR is a sensitive marker for erythropoiesis stimulation (*Brugnara, 2003*). Our findings showed the sTfR is unaffected by exercise intensity. After an SBOE in an HHE, the sTfR levels in all groups increased, and that in the HHE55% group increased significantly.

CONCLUSION

In conclusion, low-intensity exercise improves oxygen utilization, leads to a lower rise in the body temperature and a greater rise in the PI, exerts minimal effect on blood cells, and causes a significant increase in the sTfR levels. Low- and moderate-intensity SBOE can increase the serum EPO and sTfR levels and decrease the serum IL-3 level in an HHE. A high-intensity load could increase the risk of inflammation. Low-intensity exercise may be more appropriate for an SBOE in an HHE.

However, despite the strength of our findings, we acknowledge that there are multiple limitations in this study. First, in the laboratory setting of the study, participants were exposed to an HHE for only a limited period. Second, owing to complex and interrelated interactions, we cannot confirm the exact mechanisms underlying our findings. Third, we tested only a limited number of factors that promote hematopoiesis and only immediately after SBOE. In future studies, we can conduct continuous follow-up tests after exercise to understand how each indicator changes. In addition, we must also test the factors that inhibit hematopoiesis.

ACKNOWLEDGEMENTS

We are grateful to Ms. Yanan Jiang and Mr. Shaolin Chen for their assistance in recruiting experiment participants.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This study was supported by the 2024 Annual "Challenge Board" Research Project at Guangdong University of Education (2024JBGS004), the Sports Performance Analysis and Training Scientific Innovation Team (2024WCXTD030), the "National Natural Science Foundation of China" (32360218), the "Research Initiation Program for Doctoral Talents of Guangdong University of Education" (202304670451), the "Guangdong Youth Sports and Health Promotion Research Center", and the "Guangzhou Sports University Characteristic Innovation Project" (2014KTSCX123). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Guangdong University of Education: 2024JBGS004.
Sports Performance Analysis and Training Scientific Innovation Team: 2024WCXTD030.

National Natural Science Foundation of China: 32360218.
Guangdong University of Education: 202304670451.
Guangdong Youth Sports and Health Promotion Research Center.
Guangzhou Sports University Characteristic Innovation Project: 2014KTSCX123.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Yuhu Lv conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Lin Cheng analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Xiqian Zhang analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Fenglin Peng analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Yu Yuan performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Xiquan Weng conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Wen-Tao Lin conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

All human experiments were carried out in accordance with the Chinese Human Welfare Act. Ethical approval for these studies was obtained from the Ethics Committee of Guangzhou Sport University (No. 2018LCLL-10).

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.18603#supplemental-information.

REFERENCES

Adams JD, Sekiguchi Y, Suh H-G, Seal AD, Sprong CA, Kirkland TW, Kavouras SA. 2018. Dehydration impairs cycling performance, independently of thirst: a blinded study. *Medicine & Science in Sports & Exercise* **50(8)**:1697–1703 DOI 10.1249/mss.0000000000001597.

Alberty R, Pupis M, Vachalik V, Batovsky M. 2021. Diurnal variation in red blood cell variables in athletes after single and repeated bouts of exercise. *The Journal of Sports Medicine and Physical Fitness* **61(2)**:269–279 DOI 10.23736/S0022-4707.20.11174-5.

Ashworth ET, Cotter JD, Kilding AE. 2021. Impact of elevated core temperature on cognition in hot environments within a military context. *European Journal of Applied Physiology* **121(4)**:1061–1071 DOI 10.1007/s00421-020-04591-3.

Bartlett DB, Hanson ED, Lee JT, Wagoner CW, Harrell EP, Sullivan SA, Bates LC, Alzer MS, Amatuli DJ, Deal AM, Jensen BC, MacDonald G, Deal MA, Muss HB, Nyrop KA, Battaglini CL. 2021. The effects of 16 weeks of exercise training on neutrophil functions in breast cancer survivors. *Frontiers in Immunology* **12**:7 DOI 10.3389/fimmu.2021.733101.

Borriello F, Galdiero MR, Varricchi G, Loffredo S, Spadaro G, Marone G. 2019. Innate immune modulation by GM-CSF and IL-3 in health and disease. *International Journal of Molecular Sciences* **20(4)**:834 DOI 10.3390/ijms20040834.

Boukelia B, Gomes EC, Florida-James GD. 2018. Diurnal variation in physiological and immune responses to endurance sport in highly trained runners in a hot and humid environment. *Oxidative Medicine and Cellular Longevity* **2018(1)**:85 DOI 10.1155/2018/3402143.

Brugnara C. 2003. Iron deficiency and erythropoiesis: new diagnostic approaches. *Clinical Chemistry* **49(10)**:1573–1578 DOI 10.1373/49.10.1573.

Campbell JP, Turner JE. 2018. Debunking the myth of exercise-induced immune suppression: redefining the impact of exercise on immunological health across the lifespan. *Frontiers in Immunology* **9**:541 DOI 10.3389/fimmu.2018.00648.

Carneiro A, Viana-Gomes D, Macedo-da-Silva J, Oliveira Lima GH, Mitri S, Alves SR, Kolliari-Turner A, Zanoteli E, de Aquino Neto FR, Palmisano G, Pesquero JB, Moreira JC, Pereira MD. 2021. Risk factors and future directions for preventing and diagnosing exertional rhabdomyolysis. *Neuromuscular Disorders* **31(7)**:583–595 DOI 10.1016/j.nmd.2021.04.007.

Chan APC, Yang Y, Guo YP, Yam MCH, Song WF. 2016. Evaluating the physiological and perceptual responses of wearing a newly designed construction work uniform. *Textile Research Journal* **86(6)**:659–673 DOI 10.1177/0040517515591773.

Choudhary B, Udayraj. 2023. Effectiveness of air ventilation clothing in hot and humid environment for decreasing and intermittent activity scenarios. *Building and Environment* **239**:110436 DOI 10.1016/j.buildenv.2023.110436.

Cleary MA, Toy MG, Lopez RM. 2014. Thermoregulatory, cardiovascular, and perceptual responses to intermittent cooling during exercise in a hot, humid outdoor environment. *Journal of Strength and Conditioning Research* **28(3)**:792–806 DOI 10.1519/JSC.0b013e3182a20f57.

Crandall CG, Gonzalez-Alonso J. 2010. Cardiovascular function in the heat-stressed human. *Acta Physiologica* **199(4)**:407–423 DOI 10.1111/j.1748-1716.2010.02119.x.

De Jonge XAKJ, Thompson MW, Chuter VH, Silk LN, Thom JM. 2012. Exercise performance over the menstrual cycle in temperate and hot, humid conditions. *Medicine & Science in Sports & Exercise* **44(11)**:2190–2198 DOI 10.1249/MSS.0b013e3182656f13.

de Korte JQ, Eijsvogels TMH, Hopman MTE, Bongers CCWG. 2023. Thermoregulatory, cardiovascular and perceptual responses of spectators of a simulated football match in hot and humid environmental conditions. *Sports (Basel, Switzerland)* **11(4)**:78 DOI 10.3390/sports11040078.

De Miguel Z, Khoury N, Betley MJ, Lehallier B, Willoughby D, Olsson N, Yang AC, Hahn O, Lu NN, Vest RT, Bonanno LN, Yerra L, Zhang LC, Saw NL, Fairchild JK, Lee D, Zhang H, McAlpine PL, Contrepois K, Shamloo M, Elias JE, Rando TA, Wyss-Coray T. 2021. Exercise

plasma boosts memory and dampens brain inflammation via clusterin. *Nature* **600(7889)**:494–499 DOI 10.1038/s41586-021-04183-x.

de Rezende MM, Ng-Blichfeldt J-P, Justo GZ, Paredes-Gamero EJ, Gosens R. 2020. Divergent effects of Wnt5b on IL-3-and GM-CSF-induced myeloid differentiation. *Cellular Signalling* **67(10)**:109507 DOI 10.1016/j.cellsig.2019.109507.

Di Santolo M, Stel G, Banfi G, Gonano F, Cauci S. 2008. Anemia and iron status in young fertile non-professional female athletes. *European Journal of Applied Physiology* **102(6)**:703–709 DOI 10.1007/s00421-007-0647-9.

Dinh HC, Beyer I, Mets T, Onyema OO, Njemini R, Renmans W, De Waele M, Jochmans K, Vander Meeren S, Bautmans I. 2017. Effects of physical exercise on markers of cellular immunosenescence: a systematic review. *Calcified Tissue International* **100(2)**:193–215 DOI 10.1007/s00223-016-0212-9.

Dzien C, Halder W, Winner H, Lechleitner M. 2021. Covid-19 screening: are forehead temperature measurements during cold outdoor temperatures really helpful? *Wiener klinische Wochenschrift* **133(7–8)**:331–335 DOI 10.1007/s00508-020-01754-2.

Eckardt KU, Kurtz A. 2005. Regulation of erythropoietin production. *European Journal of Clinical Investigation* **35(S3)**:13–19 DOI 10.1111/j.1365-2362.2005.01525.x.

Ely MR, Cheuvront SN, Roberts WO, Montain SJ. 2007. Impact of weather on marathon-running performance. *Medicine & Science in Sports & Exercise* **39(3)**:487–493 DOI 10.1249/mss.0b013e31802d3aba.

Garg B, Sharma D, Bansal A. 2018. Systematic review seeking erythropoietin role for neuroprotection in neonates with hypoxic ischemic encephalopathy: presently where do we stand. *Journal of Maternal-Fetal & Neonatal Medicine* **31(23)**:3214–3224 DOI 10.1080/14767058.2017.1366982.

Garrett AT, Rehrer NJ, Patterson MJ. 2011. Induction and decay of short-term heat acclimation in moderately and highly trained athletes. *Sports Medicine* **41(9)**:757–771 DOI 10.2165/11587320-000000000-00000.

Gerhart HD, Fiorentini AB, Storti KL, Alman R, Bayles MP, Pesci L, Seo Y. 2020. Psychophysiological responses in emergency medical technician students during simulated work activities in a hot environment. *International Journal of Environmental Research and Public Health* **17(10)**:3443 DOI 10.3390/ijerph17103443.

Gerrett N, Kingma BRM, Sluijter R, Daanen HAM. 2019. Ambient conditions prior to Tokyo 2020 Olympic and Paralympic games: considerations for acclimation or acclimatization strategies. *Frontiers in Physiology* **10**:556 DOI 10.3389/fphys.2019.00414.

Golbabaei F, Heydari A, Moradi G, Dehghan H, Moradi A, Habibi P. 2022. The effect of cooling vests on physiological and perceptual responses: a systematic review. *International Journal of Occupational Safety and Ergonomics* **28(1)**:223–255 DOI 10.1080/10803548.2020.1741251.

Guy JH, Deakin GB, Edwards AM, Miller CM, Pyne DB. 2015. Adaptation to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes. *Sports Medicine* **45(3)**:303–311 DOI 10.1007/s40279-014-0277-4.

Hu J, Tang Z, Xu J, Ge W, Hu Q, He F, Zheng G, Jiang L, Yang Z, Tang W. 2019. The inhibitor of interleukin-3 receptor protects against sepsis in a rat model of cecal ligation and puncture. *Molecular Immunology* **109**:71–80 DOI 10.1016/j.molimm.2019.03.002.

James LJ, Moss J, Henry J, Papadopoulou C, Mears SA. 2017. Hypohydration impairs endurance performance: a blinded study. *Physiological Reports* **5(12)**:e13315 DOI 10.14814/phy2.13315.

Jelkmann W. 2011. Regulation of erythropoietin production. *The Journal of Physiology* **589(6)**:1251–1258 DOI 10.1113/jphysiol.2010.195057.

Jimenez C, Melin B, Koulmann N, Allevard AM, Launay JC, Savourey G. 1999. Plasma volume changes during and after acute variations of body hydration level in humans. *European Journal of Applied Physiology and Occupational Physiology* **80(1)**:1–8 DOI 10.1007/s004210050550.

Kakanis MW, Peake J, Brenu EW, Simmonds M, Gray B, Hooper SL, Marshall-Gradisnik SM. 2010. The open window of susceptibility to infection after acute exercise in healthy young male elite athletes. *Exercise Immunology Review* **16**:119–137 DOI 10.1016/j.jsams.2010.10.642.

Kumral A, Genc S, Ozer E, Yilmaz O, Gokmen N, Koroglu TF, Duman N, Genc K, Ozkan H. 2006. Erythropoietin downregulates bax and DP5 proapoptotic gene expression in neonatal hypoxic-ischemic brain injury. *Biology of the Neonate* **89(3)**:205–210 DOI 10.1159/000089951.

Lee W, Moon M, Kim HG, Lee TH, Oh MS. 2015. Heat stress-induced memory impairment is associated with neuroinflammation in mice. *Journal of Neuroinflammation* **12(1)**:701 DOI 10.1186/s12974-015-0324-6.

Levy I, Allender MC, Keller KA. 2020. Comparison of axillary and inguinal body temperature to rectal temperature in healthy guinea pigs (*Cavia porcellus*). *Journal of Exotic Pet Medicine* **34**:1–5 DOI 10.1053/j.jepm.2020.03.016.

Li D, Wang X, Liu B, Liu Y, Zeng Z, Lu L, Zheng Z, Li B, Zheng Z. 2014. Exercises in hot and humid environment caused liver injury in a rat model. *PLOS ONE* **9(12)**:e111741 DOI 10.1371/journal.pone.0111741.

Luk H-Y, McKenzie AL, Duplanty AA, Budnar RG, Levitt D, Fernandez A, Lee EC, Armstrong LE, Vingren JL. 2016. Leukocyte subset changes in response to a 164-km road cycle ride in a hot environment. *International Journal of Exercise Science* **9(1)**:34–46 DOI 10.70252/FXYL1232.

Mathot E, Liberman K, Hung Cao D, Njemini R, Bautmans I. 2021. Systematic review on the effects of physical exercise on cellular immunosenescence-related markers—an update. *Experimental Gerontology* **149(1)**:149 DOI 10.1016/j.exger.2021.111318.

McCubbin AJ, Allanson BA, Odgers JNC, Cort MM, Costa RJS, Cox GR, Crawshay ST, Desbrow B, Freney EG, Gaskell SK, Hughes D, Irwin C, Jay O, Lalor BJ, Ross MLR, Shaw G, Periard JD, Burke LM. 2020. Sports dietitians Australia position statement: nutrition for exercise in hot environments. *International Journal of Sport Nutrition and Exercise Metabolism* **30(1)**:83–98 DOI 10.1123/ijsnem.2019-0300.

McFarlin BK, Henning AL, Venable AS. 2017. Clothing woven with titanium dioxide-infused yarn: potential to increase exercise capacity in a hot, humid environment? *Journal of the Textile Institute* **108(1)**:1259–1263 DOI 10.1080/00405000.2016.1239329.

McFarlin BK, Henning AL, Venable AS, Williams RR, Sampson JNB. 2016. A shirt containing multistage phase change material and active cooling components was associated with increased exercise capacity in a hot, humid environment. *Ergonomics* **59(8)**:1019–1025 DOI 10.1080/00140139.2015.1108460.

Meade R, Notley S, D'Souza A, Rutherford M, Binet E, Boulay P, Kenny G. 2020. Blunted effects of elevated serum osmolality on whole-body heat loss and rectal temperature in middle-aged-to-older men exercising in dry heat. *The FASEB Journal* **34(S1)**:1 DOI 10.1096/fasebj.2020.34.s1.01900.

Melikov AK, Kaczmarczyk J. 2012. Air movement and perceived air quality. *Building and Environment* **47(2)**:400–409 DOI 10.1016/j.buildenv.2011.06.017.

Mitchell JB, Dugas JP, McFarlin BK, Nelson MJ. 2002. Effect of exercise, heat stress, and hydration on immune cell number and function. *Medicine & Science in Sports & Exercise* **34(12)**:1941–1950 DOI 10.1097/00005768-200212000-00013.

Mucci P, Durand F, Lebel B, Bousquet J, Prefaut C. 2000. Interleukins 1-beta, -8, and histamine increases in highly trained, exercising athletes. *Medicine & Science in Sports & Exercise* **32(6)**:1094–1100 DOI 10.1097/00005768-200006000-00009.

Mueller EN, Bergmann LK, Anciuti AN, Tillmann MT, Nobre MdO. 2012. Study of the difference in rectal and ear canal temperature according to the conformation of the acoustic conch in dogs. *Semina-Ciencias Agrarias* **33(5)**:1907–1910 DOI 10.5433/1679-0359.2012v33n5p1907.

Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G. 2012. The pleiotropic effects of erythropoietin in infection and inflammation. *Microbes and Infection* **14(3)**:238–246 DOI 10.1016/j.micinf.2011.10.005.

Navalta JW, Mohamed R, El-Baz A, McFarlin BK, Lyons TS. 2010. Exercise-induced immune cell apoptosis: image-based model for morphological assessment. *European Journal of Applied Physiology* **110(2)**:325–331 DOI 10.1007/s00421-010-1504-9.

Neal RA, Corbett J, Massey HC, Tipton MJ. 2016. Effect of short-term heat acclimation with permissive dehydration on thermoregulation and temperate exercise performance. *Scandinavian Journal of Medicine & Science in Sports* **26(8)**:875–884 DOI 10.1111/sms.12526.

Nybo L. 2008. Hyperthermia and fatigue. *Journal of Applied Physiology* **104(3)**:871–878 DOI 10.1152/japplphysiol.00910.2007.

Peake JM, Neubauer O, Walsh NP, Simpson RJ. 2017. Recovery of the immune system after exercise. *Journal of Applied Physiology* **122(5)**:1077–1087 DOI 10.1152/japplphysiol.00622.2016.

Pedersen BK, Ullum H. 1994. NK cell response to physical activity: possible mechanisms of action. *Medicine & Science in Sports & Exercise* **26(2)**:140–146 DOI 10.1249/00005768-199402000-00003.

Periard JD, Racinais S, Sawka MN. 2015. Adaptations and mechanisms of human heat acclimation: applications for competitive athletes and sports. *Scandinavian Journal of Medicine & Science in Sports* **25(S1)**:20–38 DOI 10.1111/sms.12408.

Pichon AP, Connes P, Robach P. 2016. Effects of acute and chronic hematocrit modulations on blood viscosity in endurance athletes. *Clinical Hemorheology and Microcirculation* **64(2)**:115–123.

Poirier M, Friesen BJ, Hardcastle SG, Kenny GP. 2013. The effect of progressive heat acclimation on change in body heat content in young males. *The FASEB Journal* **27(S1)**:1201.20 DOI 10.1096/fasebj.27.1_supplement.1201.20.

Qiu H, Xi X, Man CL, Ko FWS, Yim SHL, Kwok TCY, Ho K-F. 2020. Real-time monitoring of the effects of personal temperature exposure on the blood oxygen saturation level in elderly people with and without chronic obstructive pulmonary disease: a panel study in Hong Kong. *Environmental Science & Technology* **54**:6869–6877 DOI 10.1021/acs.est.0c01799.

Randall CA, Ross EZ, Maxwell NS. 2015. Effect of practical precooling on neuromuscular function and 5-km time-trial performance in hot, humid conditions among well-trained male runners. *Journal of Strength and Conditioning Research* **29(7)**:1925–1936 DOI 10.1519/jsc.0000000000000840.

Rao KNS, Binbrek AS, Sobel BE. 2008. Heart disease and erythropoietin. *Future Cardiology* **4(1)**:57–64 DOI 10.2217/14796678.4.1.57.

Rubin R. 2019. In hot, humid weather, fans benefit health and environment. *Journal of the American Medical Association* **322**:1340–1341 DOI 10.1001/jama.2019.14684.

Sardeli AV, Mori MA, Lord JM. 2022. Effect of exercise on acute senescent lymphocyte counts: a systematic review and meta-analysis. *Gerontology* **68(9)**:961–975 DOI 10.1159/000520528.

Sawka MN, Leon LR, Montain SJ, Sonna LA. 2011. Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Comprehensive Physiology* **1**:1883–1928 DOI 10.1002/cphy.c100082.

Sellami M, Gasmi M, Denham J, Hayes LD, Stratton D, Padulo J, Bragazzi N. 2018. Effects of acute and chronic exercise on immunological parameters in the elderly aged: can physical activity counteract the effects of aging? *Frontiers in Immunology* **9**:515 DOI 10.3389/fimmu.2018.02187.

Sgro P, Sansone M, Sansone A, Romanelli F, Di Luigi L. 2018. Effects of erythropoietin abuse on exercise performance. *The Physician and Sportsmedicine* **46(1)**:105–115 DOI 10.1080/00913847.2018.1402663.

Shoemaker ME, Gillen ZM, McKay BD, Bohannon NA, Gibson SM, Koehler K, Cramer JT. 2019. Sex-specific relationships among iron status biomarkers, athletic performance, maturity, and dietary intakes in pre-adolescent and adolescent athletes. *Journal of the International Society of Sports Nutrition* **16(1)**:1596 DOI 10.1186/s12970-019-0306-7.

Shoemaker ME, Gillen ZM, McKay BD, Koehler K, Cramer JT. 2020. High prevalence of poor iron status among 8-to 16-year-old youth athletes: interactions among biomarkers of iron, dietary intakes, and biological maturity. *Journal of the American College of Nutrition* **39(2)**:155–162 DOI 10.1080/07315724.2019.1621229.

Simpson RJ, Cosgrove C, Chee MM, McFarlin BK, Bartlett DB, Spielmann G, O'Connor DP, Pircher H, Shiels G. 2010. Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. *Exercise Immunology Review* **16**:40–55.

Simpson RJ, Florida-James GD, Whyte GP, Guy K. 2006. The effects of intensive, moderate and downhill treadmill running on human blood lymphocytes expressing the adhesion/activation molecules CD54 (ICAM-1), CD18 (beta2 integrin) and CD53. *European Journal of Applied Physiology* **97(1)**:109–121 DOI 10.1007/s00421-006-0146-4.

Singh DK, Kumar G. 2019. Comparison of the subgingival temperature of smokers and nonsmokers in healthy and diseased sites of gingiva in association with sublingual body temperature. *Journal of Family Medicine and Primary Care* **8(10)**:3166–3172 DOI 10.4103/jfmpc.jfmpc_516_19.

Siquier-Coll J, Bartolome I, Perez-Quintero M, Grijota FJ, Robles MC, Munoz D, Maynar-Marino M. 2019. Influence of a physical exercise until exhaustion in normothermic and hyperthermic conditions on serum, erythrocyte and urinary concentrations of magnesium and phosphorus. *Journal of Thermal Biology* **80**:1–6 DOI 10.1016/j.jtherbio.2018.12.020.

Skwarczynski MA, Melikov AK, Kaczmarczyk J, Lyubenova V. 2010. Impact of individually controlled facially applied air movement on perceived air quality at high humidity. *Building and Environment* **45(10)**:2170–2176 DOI 10.1016/j.buildenv.2010.03.017.

Smith S, Sims ST, Thorpe H, Baker D, Haszard J, Badenhorst C, Black KE. 2020. Hepcidin and iron: novel findings for elite female rugby Sevens players. *The Journal of Sports Medicine and Physical Fitness* **60(2)**:289–293 DOI 10.23736/S0022-4707.19.10096-5.

Spielmann G, Bollard CM, Kunz H, Hanley PJ, Simpson RJ. 2016. A single exercise bout enhances the manufacture of viral-specific T-cells from healthy donors: implications for allogeneic adoptive transfer immunotherapy. *Scientific Reports* **6(1)**:285 DOI 10.1038/srep25852.

Stelzer I, Kroepfl JM, Fuchs R, Pekovits K, Mangge H, Raggam RB, Gruber HJ, Prueller F, Hofmann P, Truschnig-Wilders M, Obermayer-Pietsch B, Haushofer AC, Kessler HH,

Maechler P. 2015. Ultra-endurance exercise induces stress and inflammation and affects circulating hematopoietic progenitor cell function. *Scandinavian Journal of Medicine & Science in Sports* **25(5)**:E442–E450 DOI 10.1111/sms.12347.

Stoehr EJ, Gonzalez-Alonso J, Pearson J, Low DA, Ali L, Barker H, Shave R. 2011. Dehydration reduces left ventricular filling at rest and during exercise independent of twist mechanics. *Journal of Applied Physiology* **111(3)**:891–897 DOI 10.1152/japplphysiol.00528.2011.

Than Tran T, Riera F, Rinaldi K, Briki W, Hue O. 2015. Ingestion of a cold temperature/menthol beverage increases outdoor exercise performance in a hot, humid environment. *PLOS ONE* **10(4)**:e0123815 DOI 10.1371/journal.pone.0123815.

Timmerman KL, Flynn MG, Coen PM, Markofski MM, Pence BD. 2008. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? *Journal of Leukocyte Biology* **84(5)**:1271–1278 DOI 10.1189/jlb.0408244.

Tsutsumi H, Tanabe SI, Harigaya J, Iguchi Y, Nakamura G. 2007. Effect of humidity on human comfort and productivity after step changes from warm and humid environment. *Building and Environment* **42(12)**:4034–4042 DOI 10.1016/j.buildenv.2006.06.037.

van der Vinne V, Pothecary CA, Wilcox SL, McKillop LE, Benson LA, Kolpakova J, Tam SKE, Krone LB, Fisk AS, Wilson TS, Yamagata T, Cantley J, Vyazovskiy VV, Peirson SN. 2020. Continuous and non-invasive thermography of mouse skin accurately describes core body temperature patterns, but not absolute core temperature. *Scientific Reports* **10(1)**:335 DOI 10.1038/s41598-020-77786-5.

Wijekulasuriya GA, Coffey VG, Badham L, O'Connor F, Sharma AP, Cox GR. 2022. Effect of acetaminophen on endurance cycling performance in trained triathletes in hot and humid conditions. *International Journal of Sports Physiology and Performance* **17(6)**:917–925 DOI 10.1123/ijspp.2021-0475.

Willmott AGB, Hayes M, James CA, Gibson OR, Maxwell NS. 2019. Heat acclimation attenuates the increased sensations of fatigue reported during acute exercise-heat stress. *Temperature* **7(2)**:178–190 DOI 10.1080/23328940.2019.1664370.

Willmott AGB, Hayes M, Waldock KAM, Relf RL, Watkins ER, James CA, Gibson OR, Smeeton NJ, Richardson AJ, Watt PW, Maxwell NS. 2017. Short-term heat acclimation prior to a multi-day desert ultra-marathon improves physiological and psychological responses without compromising immune status. *Journal of Sports Sciences* **35(22)**:2249–2256 DOI 10.1080/02640414.2016.1265142.

Wright HE, Larose J, McLellan TM, Miller S, Boulay P, Kenny GP. 2013. Do older firefighters show long-term adaptations to work in the heat? *Journal of Occupational and Environmental Hygiene* **10(12)**:705–715 DOI 10.1080/15459624.2013.821574.

Yang Y, Yang B, Liang Z-P. 2013. Clinical study of iron metabolism indicators in ineffective hematopoiesis of myelodysplastic syndrome. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* **21**:948–952.

Yatsutani H, Mori H, Ito H, Hayashi N, Okazaki K, Goto K. 2019. Erythropoietin response to endurance exercise under heat and hypoxic conditions. *Medicine & Science in Sports & Exercise* **51(6S)**:465–466 DOI 10.1249/01.mss.0000561899.77809.dc.

Yu S-Y, Matzarakis A, Lin T-P. 2020. A study of the thermal environment and air quality in hot-humid regions during running events in Southern Taiwan. *Atmosphere* **11(10)**:1101 DOI 10.3390/atmos11101101.

Zhang Y. 2020. Effect of iron chelation therapy on EPO-STAT5 signalling pathway and EPO resistance in iron-overloaded low-risk myelodysplastic syndrome patients. *Hematology* **25(1)**:1–10 DOI 10.1080/16078454.2019.1700330.

Zhang H, Zhang P, Zhang Y, Yani J, Dongi P, Wang Y, Niue X. 2016. Effects of erythropoiesis-stimulating agents on heart failure patients with anemia: a meta-analysis. *Postepy W Kardiologii Interwencyjnej* **12**:247–253 DOI 10.5114/aic.2016.61647.

Zhou Z-w, Li F, Zheng Z-t, Li Y-d, Chen T-h, Gao W-w, Chen J-l, Zhang J-n. 2017. Erythropoietin regulates immune/inflammatory reaction and improves neurological function outcomes in traumatic brain injury. *Brain and Behavior* **7(11)**:1031 DOI 10.1002/brb3.827.