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Original Article

Effect of Cordyceps Sinensis on repeated-sprint performance following repeated-sprint training in hypoxia in female soccer players

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Abstract:
This study aimed to explore the combined effects of *Cordyceps sinensis* (Cs) supplementation during 4 weeks of repeated-sprint training in hypoxia (RSH) on physical performance and hematological responses of female soccer players. A total of 39 female soccer players were randomly divided into four groups: hypoxic repeated sprints [RSH (FiO_2 14.5%) with Cs (RSH-Cs, n = 11) or placebo (RSH-P, n = 11)], and normoxic repeated sprints [RSN (FiO_2 20.9%) with Cs (RSN-Cs, n = 8) or placebo (RSN-P, n = 9)]. All participants were instructed to consume 3 g of Cs or placebo daily while completing 12 RS sessions of repeated sprints training (RSH or RSN) over the 4 weeks. Performance and blood tests were measured at baseline and following the 4 weeks. Incremental exercise and repeated sprint ability tests were performed at sea level. The results showed the main effect of the supplement, regardless of the condition, on time to exhaustion ($p = 0.04$), peak power ($p = 0.04$), and fatigue index ($p = 0.03$) was higher in the Cs groups than in the placebo groups; however, no significant change in VO_2max was observed. There were significant improvements on peak power, mean power, fatigue index, and blood lactate, compared with baseline in RSH-Cs (all $p < 0.05$); but even so, RSN-Cs significantly improved both mean power and blood lactate (all $p < 0.05$). Interestingly, only mean power was significant in both the main effect of the supplement and condition, with a greater improvement in RSH-Cs than its respective control. However, these improvements were not associated with hematological variables (all $p > 0.05$). The current findings indicate the potential of Cs to help in maintaining tolerance under hypoxia, which increases aerobic and anaerobic performance.
Key Words: **Adaptogen, Hypoxic training, Repeated sprint ability, Hematologic response**

Introduction

Soccer is an intermittent sport that requires repeated maximal or near-maximal efforts in sprints with short recovery intervals between sprints during the game (Aughey, 2011; Bishop et al., 2013). Recently, scientific literature has paid increasing attention to women's soccer because of the markedly increased popularity of female soccer over the decade (Andersson et al., 2010; Randell et al., 2021). Although physiological demands during match competition were similar between male and female players, women's entire match was represented by a decrement in high-speed running within halves (Datson et al., 2014; Milanović et al., 2017). Especially, players may experience considerable fatigue after the multiple-sprint work during a match play (Bishop et al., 2013). Thus, the ability to maintain sprint speed during the game, i.e., repeated-sprint ability (RSA), is crucial among athletes (Hamlin et al., 2017).

To date, several hypoxic training strategies are widely used to augment physiological adaptations or improved exercise performance (Brocherie et al., 2015; Goods et al., 2014; Morrison et al., 2015). RS training in hypoxia (RSH) is one of the hypoxic training methods, which involves the repetitions of "all-out" efforts of short (<30 s) duration interspersed with incomplete recoveries, which affect physical performance upon return to sea level (Faiss et al., 2013). Practically, RSH benefits have been consistently shown for many team sports. Considering the game's intermittent nature, the ability to sprint repeatedly may be valuable to its outcome. Indeed, RSH is an effective training means in improving VO_2max, anaerobic power, buffering capacity, and RSA when compared with similar training in normoxia (RSN) (Brocherie et al., 2017).

For instance, some previous studies showed that RSH further enhanced effectively the peak power output during the RS test compared with RSN (Hamlin et al., 2017; Kasai et al., 2015). Conversely, two recent studies did not find an additional effect on RSA of RSH versus RSN (Goods et al., 2014; Montero et al., 2017). Interestingly, growing evidence indicated the improvement in aerobic and anaerobic performance in RSH, and we believe that RSH would lead to greater performance improvement than similar training at sea level, induced by a multifactorial cascade of hematological or nonhematological response (Faiss et al., 2013; Girard et al., 2020). Generally, hypoxic training has a positive effect on hematological adaptations, resulting in greater oxygen delivery by inducing red blood cell proliferation (Park et al., 2022). On the contrary, RSH training was believed to upregulate nonhematological peripheral adaptations for improving multiple sprint performance (Girard et al.,

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2017). For this end, RSA improvement was attributable to increasing blood perfusion (Faiss et al., 2013). Additionally, increasing angiogenesis, glucose transport, pH regulation, and glycolysis at the muscular level may partially contribute to better performance (Gore et al., 2007).

Although numerous factors contribute to peak athletic performance, robust evidence shows that several supplements may be considered to enhance sports performance. For instance, Huang et al. (2023) revealed that 7-day beetroot juice supplementation may improve time to exhaustion (TTE) in young active winter triathletes. Recently, evidence for a few specific ergogenic aids for performance enhancement at sea level has been growing (Peeling et al., 2018); however, very few studies have focused on hypoxic conditions. Indeed, ergogenic aid strategies may have potential practical use in hypoxic conditions (Caris et al., 2019; Stellingwerff et al., 2019). Additionally, supplementation along with RSH training is being unexplored. Nowadays, there is increasing interest in the role of natural adaptogen that can regulate the physiological functions; thus, several adaptogens are considered to improve physical performance in athletes. *Cordyceps sinensis* (Cs), one of the *Cordyceps spp*., is a type of traditional Chinese herbal medicine. It has beneficial properties and various health functions. Typically, Cs is believed to exert its ergogenic effect presumably through its bioactive component adenosine (Siu et al., 2004). Interestingly, however, Cs plays important roles in regulating physiological processes associated with vasodilating effect (Xiang et al., 2016), angiogenesis-producing muscle capillary (Kumar et al., 2011), and antioxidant properties (Singh et al., 2013).

In addition, Cs showed a stimulating effect on lactate dehydrogenase activity, producing sufficient ATP for exercise under anaerobic conditions (Li et al., 2009). In one previous study, 12-week Cs supplementation demonstrated aerobic improvement in a 5k test run, even though VO_2 max was unaffected in amateur marathoners (Savioli et al., 2022). In addition, Cs plus *Rhodiola rosea* (RR) supplementation during concurrent training for 14-week duration resulted in enhanced aerobic fitness (Kreipke et al., 2021). Despite this, there is still limit information regarding the effect of Cs on anaerobic performance. Additionally, to the best of our knowledge, the combined effects of Cs supplementation on physiological responses while performing RSH have not yet been fully explored. Thus, this study aimed to evaluate the combined effect of Cs during RS training on the aerobic performance, repeated sprint ability, and hematological response under hypoxic versus normoxic conditions in female soccer players.

Material & methods

Participants

The study participants were 44 female university league soccer players with a training background of at least 1 year. These players provided informed consent. None had a history of exposure to altitude >1,500 m in the previous 6 months. Participants did not consume any supplements for at least 3 months before and during the study. Ethical approval was obtained from the Research Ethics Review Committee for Research Involving Human Research Participants Group 1 Chulalongkorn University (no. 219.1/14) under the Declaration of Helsinki. Participants were randomly allocated to one of the following four groups (n = 11/group): RS training in hypoxia with Cs (RSH-Cs) or placebo (RSH-P) and RS training in normoxia with Cs (RSN-Cs) or placebo (RSN-P). After enrolment, one participant withdrew due to injury, and four did not complete the 12 training sessions. Consequently, 39 participants completed the protocol as described in Table 1.

Table 1. Participant characteristics

	RSH-Cs (n = 11)	**RSN-Cs (n = 8)**	**RSH-P (n = 11)**	**RSN-P (n = 9)**	**p value**
Age (year)	20.00±1.55	20.87±0.99	21.45±2.38	21.00±0.70	0.22
Body weight (kg)	59.56±9.28	58.40±12.41	62.41±11.80	61.57±8.59	0.84
Height (cm)	165.18±5.42	161.56±7.09	163.91±5.13	162.11±5.42	0.49
BMI (kg/m2)	21.75±2.57	22.27±3.86	23.15±3.81	24.11±4.16	0.50
Body fat (%)	25.57±4.24	26.17±5.83	27.03±4.08	28.71±5.42	0.53
VO_2max (ml/min/kg)	39.36±3.44	40.87±4.79	37.82±2.93	37.00±4.27	0.17

Data are presented as mean ± SD. BMI, body mass index, VO_2max, maximal oxygen uptake; RSH-Cs, RS in hypoxia with Cs; RSN-Cs, RS in normoxia with Cs; RSH-P, RS in hypoxia with placebo; RSN-P, RS in normoxia with placebo

Study design

A single-blinded, randomized, control design was applied. The study was carried out in a simulated hypoxic room (ATS-5KHP 750 SYSTEM, Altitude Training Systems, NSW, Australia) at approximately 24°C–25°C and relative humidity of approximately 40%–42% under hypoxic (FiO_2 = 14.5%) or normoxic (FiO_2 = 20.9%) condition. Performance tests were assessed at sea level (Barometric pressure of approximately 756–759 mmHg). Performance and blood tests were performed at baseline (pre) and 4 weeks after the intervention (post) as shown in Figure 1.

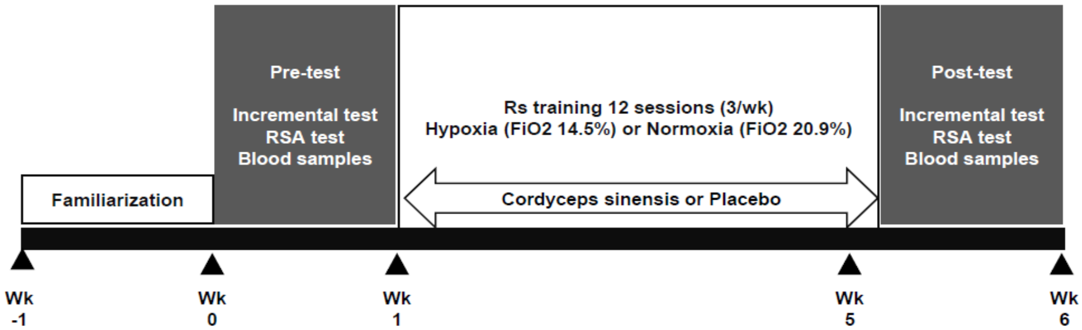

Figure 1. Schematic illustration of the study

Procedure

Supplementation

The dosing regimen was prepared by dissolving 3 g of Cs (China Jiangsu International Economic and Technical Cooperation Group, Ltd.) in 100 mL of beverage (one serving size), or 3 g of maltodextrin (Facobis Co., Ltd.) with a similar additive flavor and color as the placebo. This dose is recommended for use as a natural health product by the FDA in Thailand (www.food.fda.moph.go.th.). Both supplements were formulated by Innovative Health Food, Thailand Institute of Scientific and Technological Research, Thailand, and manufactured by the Faculty of Food Engineering, King Mongkut's Institute of Technology Ladkrabang, Thailand. All samples were sterilized and subjected to contaminant tests. Participants were instructed to consume one serving size once a day after a meal in the evening for four consecutive weeks. Compliance was monitored weekly by a phone call, and participants were asked to return the packages at the end of the experiment.

Incremental exercise test

Under normoxic conditions (FiO_2 = 20.9%) in a simulated hypoxic room, maximal oxygen uptake (VO_2max) was evaluated using the modified Bruce ramp protocol on a motorized treadmill, and environmental conditions were set. The test began with a 4-min warm-up at an initial 5.0 km/h speed at 0% grade. Then, the speed was adjusted to 10 km/h running speed with a 1% grade and increased by 1.0 km/h every minute until volitional exhaustion (Beltz et al., 2016). Respiratory gas exchange was measured breath-by-breath using a wireless portable gas analyzer MetaMax 3B (Cortex, Germany). Heart rate was continuously monitored using Polar H7 (Kampele, Finland), and peripheral oxygen saturation (SpO_2) was measured by a pulse oximeter (Nonin Pulse Oximeters, Nonin Medical Inc., Plymouth, USA) before and during the test. Verbal encouragement was given to each participant throughout the test. Participants were instructed to avoid strenuous activities for 24 h and refrain from caffeine intake before testing.

RSA test

The RSA was evaluated by an outdoor field test. Briefly, the RSA test consisted of 6 × 35-m sprints, with 10 s of passive recovery between sprints. The time from the start point through the 35-m finished sprint was recorded using a stopwatch by two independent research assistants. Power output was calculated using the following formula: Power output (W) = (body mass x distance $(m)^2$)/$time^3$ (Zagatto et al., 2009). The fatigue index (FI) was calculated as FI = [(total sprint time / ideal sprint time*) × 100] − 100. *Ideal sprint time = Number of sprints × fastest sprint time (Glaister et al., 2008).

Training intervention

During the RS training sessions, a simulated hypoxic room was set in hypoxic FiO_2 14.5% (RSH) or normoxic FiO_2 20.9% (RSN). The experiments were carried out simultaneously under similar environmental conditions. Each session consisted of a 5-min warm-up at 6 km/h, followed by three sets of 6 × 6s at a velocity corresponding to 140% vVO_2max on a motorized treadmill with 1% incline, 30 s interval of passive recovery, and 5-min rest period between sets. The E:R ratio was 1:5 for each sprint. After the last sprint, participants were asked to remain in the chamber for 5–10 min rest. The heart rate and fingertip oxygen saturation were continuously monitored throughout the training session. All participants performed three sessions per week for 4 weeks (total of 12 sessions) under the supervision of sports scientists.

Blood samples

A venous blood sample (6 mL) was collected from the antecubital vein and drawn into an ethylenediaminetetraacetic acid EDTA tube for complete blood count analysis. The blood samples were handled and analyzed by medical technologists at the Faculty of Allied Health Sciences Laboratory. To determine the blood lactate concentration, a capillary blood sample was taken from the participants' fingertips at rest and immediately after the RSA test using a portable lactate analyzer (Lactate Scout+, Germany).

Statistical analysis

All data are expressed as means ± SD. Statistical analysis was identified using IBM SPSS Statistics for Windows version 28.0 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to test data normality. A two-way multivariate analysis of variance was applied to clarify the interaction and main effect of supplementation and condition for all dependent variables. When significance was identified, a Bonferroni post hoc test was used for multiple comparisons. The significance was set at $p < 0.05$ was all analyses.

Results

Effects on aerobic performance

As shown in Table 2, no significant interaction and main effect for supplement and condition were found on any cardiorespiratory variables (all $p > 0.05$). However, only a significant main effect for supplement, but not for condition, on TTE ($p = 0.04$) was found, with a trend in the Cs groups greater than that in the placebo groups. Furthermore, the magnitude of the percentage changes (relative to pretraining values) in the cardiorespiratory variables could be seen on the maximal velocity at VO_2max (vVO_2max) and TTE in the Cs groups (all $p < 0.05$), but not in the placebo. SpO_2 was also significantly improved in all groups after 4 weeks of intervention (all $p < 0.05$), with no significant difference was observed between the groups.

Table 2. Aerobic performance variables during an incremental exercise test before (pre) and after (post) the 4-week intervention in four groups.

	RSH-C (n = 11)		**RSN-C (n = 8)**		**RSH-P (n = 11)**		**RSN-P (n = 9)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
VO_2max (ml/min/kg)	39.36±3.44	41.00±4.63	40.87±4.79	40.00±5.81	37.82±2.93	38.27±2.28	37.00±4.27	38.33±5.41
vVO_2max (km/h)	12.08±0.68	12.39±0.52*	11.79±0.32	11.99±0.56*	12.04±0.51	12.01±0.26	11.71±0.65	11.80±0.33
TTE (min)	10.54±1.20	11.33±1.51*	9.83±0.64	10.33±1.06*	10.64±1.13	10.60±0.55	10.04±1.66	10.26±1.27
HRmax (bpm)	183.09±7.71	185.36±8.77	181.62±5.07	182.37±4.31	183.18±9.12	181.91±7.95	182.78±3.53	181.11±5.32
RER	1.07±0.06	1.11±0.05	1.09±0.06	1.08±0.07	1.11±0.05	1.09±0.11	1.14±0.07	1.11±0.08
SpO2 (%)	93.90±2.38	95.45±1.97*	95.37±0.92	96.75±1.16*	95.27±2.61	97.54±1.13*	94.78±1.39	95.11±1.27*

Data are presented as means ± SD. RER, respiratory exchange ratio; SpO_2, peripheral oxygen saturation; TTE; time to exhaustion; VO_2max, maximal oxygen uptake; vVO_2max, maximal velocity at VO_2max. * Significant from pre-test, $p < 0.05$.

Effect on RSA performance

Table 3 showed the peak power, mean power, FI, and blood lactate concentration during the RSA test. No significant interaction was found for all measured variables. The supplement significantly affected peak power ($p = 0.04$), mean power ($p = 0.01$), FI ($p = 0.03$), and blood lactate ($p = 0.02$), which were higher in the Cs groups than in the placebo groups, regardless of the condition. Interestingly, only mean power showed the main effect of the condition ($p = 0.01$). Compared with baseline, significant improvements were found in peak power, mean power, FI, and blood lactate in RSH-Cs (all $p < 0.05$); however, RSN-Cs significantly improved both mean power and blood lactate concentration (all $p < 0.05$). Interestingly, only RSH-Cs showed a greater increase in mean power than their respective control (i.e., RSH-P and RSN-P) (all $p < 0.05$) as shown in Figure 2.

Table 3. RSA performance before (pre) and after (post) the 4-week intervention in four groups

	RSH-Cs (n = 11)		**RSN-Cs (n = 8)**		**RSH-P (n = 11)**		**RSN-P (n = 9)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
PP (W/kg)	4.56±0.63	5.55±1.03*	4.24±0.86	4.65±0.84	4.72±0.68	4.70±0.72	4.54±0.63	4.71±1.12
MP (W/kg)	3.65±0.36	4.77±0.74*, β, †	3.30±0.68	3.83±0.63*	3.85±0.47	4.52±0.32*, †	3.62±0.42	3.70±0.79
FI (%)	8.16±3.47	5.37±1.89*	8.79±3.74	6.98±4.10	7.58±2.93	7.10±2.46	8.55±2.70	9.33±3.25
BL (mmol/L)	13.20±2.05	11.85±1.32*	12.77±0.92	11.27±1.11*	12.83±2.04	12.46±2.15	11.98±1.93	12.10±1.75

Data are presented as mean ± SD. BL, blood lactate; FI, fatigue index; MP, mean power; PP, peak power; *Significantly different from the pre-test values, $p < 0.05$; β Significantly different from RSN-Cs, $p < 0.05$; †Significantly different from RSN-P, $p < 0.05$

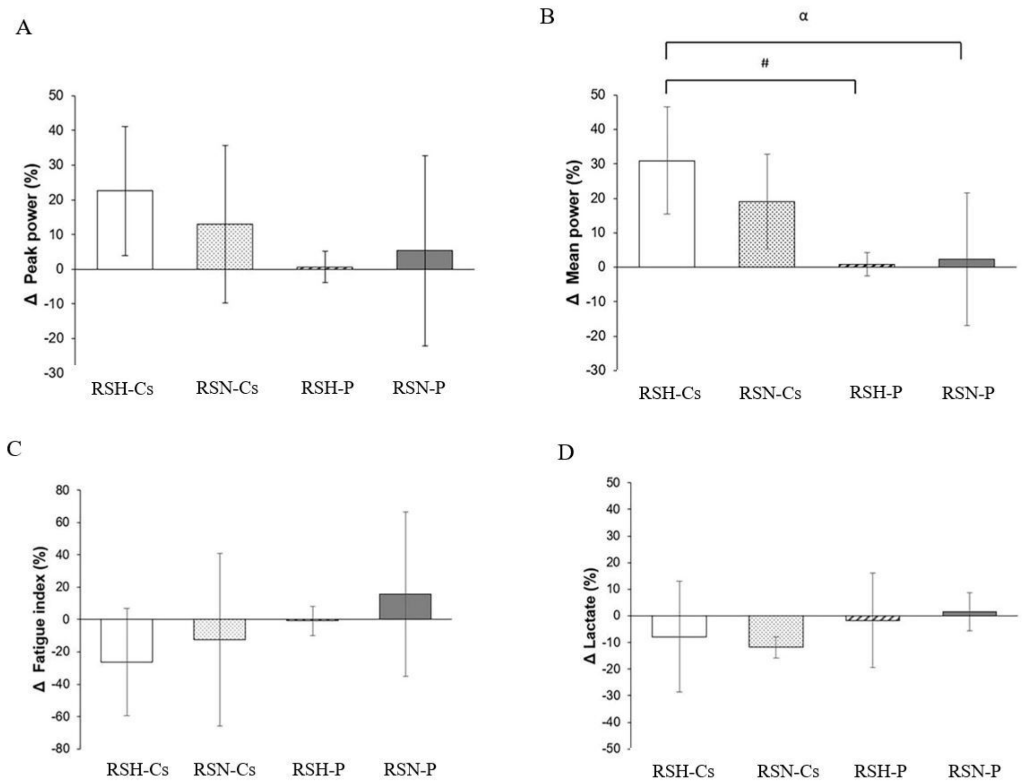

Figure 2. Changes in RSA performance: peak power (A), mean power (B), fatigue index (C), and lactate concentration (D) after the 4-week intervention in four groups. Data are presented as relative changes from the baseline (Δ). # Significantly different from RSH-P, $p < 0.05$, α Significantly different from RSN-P; $p < 0.05$.

Effect on hematological parameters

As shown in Figure 3, no interaction and main effect were found for the supplement and condition in hemoglobin, hematocrit, red blood cell, and platelet count after the 4-week intervention (all $p > 0.05$). These hematological results were unchanged compared with the baseline values.

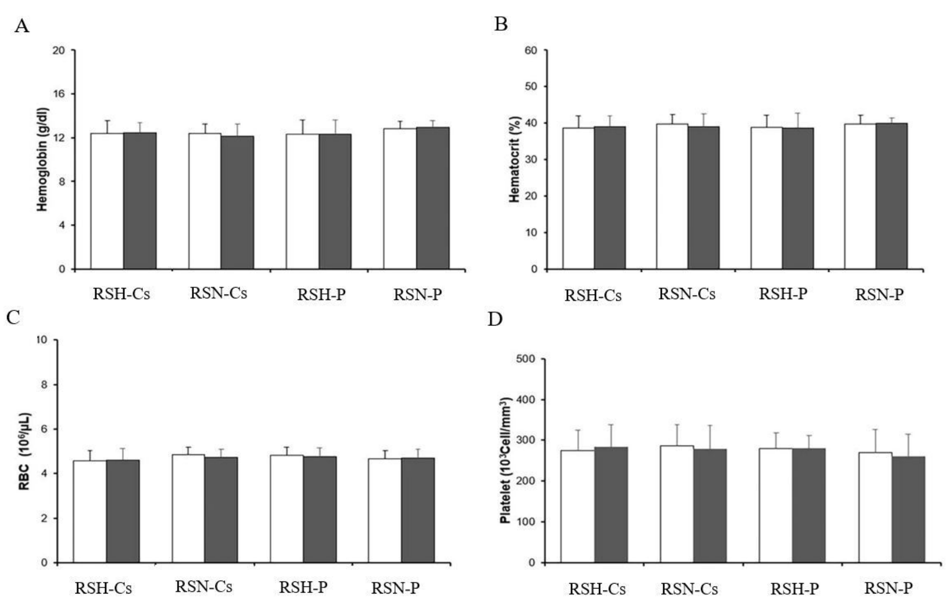

Figure 3. Hematological parameters before (□) and after (■) the 4-week intervention. Hemoglobin (A), Hematocrit (B), Red blood cell (C), Platelet count (D)**.** Data are presented as mean ± SD.

Discussion

This study explored the influence of Cs supplementation during 4-week RSH training on physical performance and hematologic responses in female soccer players. The main findings were as follows: 1) Cs supplementation elicited greater improvement in TTE but not VO_2max compared with placebo. 2) Cs supplementation induced greater RSA performance, particularly mean power, in RSH than in RSN and its placebo. 3) These changes were not associated with alterations in hematological variables.

To our knowledge, this may be the first study to focus on Cs supplementation in combination with RSH training on aerobic and RSA performance in female soccer players. Although several studies have evaluated the ergogenic effect of Cs supplementation on aerobic and physical performance in amateur athletes, these findings were still inconclusive (Savioli et al., 2022; Tsuk et al., 2018). To confirm the effectiveness of Cs supplementation in improving aerobic performance in these athletes, this study revealed that 4-week Cs supplementation elicited an improvement in the TTE and a trend for increasing vVO_2 max during an incremental exercise without VO_2max changes compared with placebo. This finding agreed with the results of some previous studies, for example, Savioli et al. (2022) revealed an aerobic improvement in the 5K test run after 12-week Cs supplementation along with aerobic training sessions. In addition, our previous study showed that aerobic response after 2-week Cs consumption elicited greater TTE and VO_2max in long-distance runners (Thongsawang et al., 2021). In contrast to above findings, Liao et al. (2019) demonstrated that 8-week Cs and *Rhodiola rosea* (RR) consumption combined with endurance exercise training did not affect VO_2 max in the young sedentary group compared with placebo. However, another study demonstrated that the total running time improved after Cs and RR supplementation during concurrent training, whereas VO_2max did not change in the 14-week study (Kreipke et al., 2021). In the study by Tsuk et al. (2018) of male cyclists, the 5-week Cs (3 g/day) supplementation without exercise did not induce anaerobic capacity on both peak and mean power performance. Although RSH has shown efficacy in enhancing sea-level performance compared with normoxia (RSN) (Faiss et al., 2013; Girard et al., 2017); however, in the present study, no significant aerobic improvement in VO_2 max was found during RSH compared with that during RSN. In line with this finding, Kasai et al. (2017) reported that VO_2 max did not significantly improve after 4-week RS training in hypoxia (FiO_2 14.5%), which was similar to the present experiment. The lack of such improvement was not as expected but it could be attributed to the specific adaptation from the RS training protocol, hypoxic dose, and/or training status of the participants. The specific mechanisms that affect VO_2 max are currently unknown. A novel finding of the present study was the significant improvement in all RSA variables, mainly in mean power, over 4 weeks of RSH with Cs supplementation compared with RSN and its placebo. This finding was consistent with the findings of Hamlin et al. (2017) showing that a 3-week RS training in hypoxia (FiO_2 14.5%) significantly improved RSA but not YYIR1 performance in well-trained rugby players. Similarly, Girard et al. (2017) revealed that RSA was more effective at FiO_2 <14.5 % than normoxia. Nevertheless, Montero et al. (2017) illustrated that the mean power, peak power, and number of RS in endurance-trained men did not respond to hypoxic (FiO_2 = 13.8 %) and normoxic (FiO_2 = 20.9 %) training. Altogether, the beneficial effects on sprint performance after such altitude training are still debated and warrant further investigation.

Apart from the above findings, the present study indicated that RSH can elicit hematological and nonhematological responses through some potential mechanisms for aerobic performance improvement (Brocherie et al., 2017). Although several studies have supported the possibility that Hb mass increased when athletes are exposed to moderate altitude (Faiss et al., 2013; Wehrlin et al., 2016), the present study did not detect changes in hematological variables. Our results agreed with those of a previous study by Humberstone-Gough et al. (2013), who reported that intermittent hypoxia was insufficient to accelerate erythropoiesis and did not elicit changes in Hb mass. Because no hematological changes were noted during the intervention period, the improvements observed in RSA were mainly due to significant increases associated with the nonhematological response in the hypoxic group. These findings expand the observations reported by Holliss et al. (2014) in highly trained runners, providing a means of submaximal exercise improvement with no hematological alteration after an 8-week intermittent hypoxic training. According to Pramkratok et al. (2022), RSH could improve aerobic and RSA performance without erythropoiesis, possibly through enhancing angiogenesis via the hypoxia inductor factor-1 (HIF-1α) and the vascular endothelial growth factor (VEGF). In addition, Chen et al. (2014) did not find a significant difference in hematological profiles after 2-week Cs supplementation along with endurance high-altitude training, whereas an effect on aerobic performance was found. Accordingly, one of the possible mechanisms is the activation of HIF-1 pathway, leading to the upregulation of VEGF gene expression related to oxygen transport (Nava et al., 2022), glycolysis (Nagao et al., 2019), and mitochondrial biogenesis (Faiss et al., 2013). These molecular adaptations to RSH may explain some of the performance benefits of RSH. In addition, Faiss et al. (2015) reported similar effects, in which high blood perfusion affected the number of sprints after RSH training. In addition, Cs supplementation has putative benefits during RSH because it may modify the increase in tolerance under hypoxia by activating VEGF that promotes angiogenesis (Long et al., 2021), which agreed with the results of Kumar et al. (2011), demonstrating better skeletal angiogenesis via VEGF expression with Cs supplementation during exercise compared with exercise alone. Furthermore, Cs supplementation may inhibit hypoxia-induced oxidative stress by maintaining an antioxidant status (Singh et al., 2013). Nevertheless, other mechanisms, such as glucose transport and lactate turnover at the muscular level, could not be ruled out

(Kumar et al., 2011). Based on these results, the present findings indicate that Cs supplementation may effectively produce an ergogenic effect during RSH exercise, which could be due to its molecular mechanism.

Conclusions

This study demonstrates that there was a trend toward an improvement in aerobic and anaerobic capacity with the 4 weeks of Cs supplementation combined with RSH training compared with RSN and its placebo in female soccer players. Specifically, time to exhaustion and repeated sprint ability were improved after the intervention. Such an improvement did not appear to be associated with changes in hematologic response. The present findings may be of interest to coaches, sport nutritionist and sports professional in applying Cs supplementation with RSH to optimize physiological and metabolic adaptations and maximize athletic performance. However, further studies may investigate the optimal degree of hypoxic dose and the time course of evaluation to effectively stimulate hematological adaptations.

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Conflict of interest

The authors declare no conflict of interest

References

Andersson, H. Å., Randers, M. B., Heiner-Møller, A., Krustrup, P., & Mohr, M. (2010). Elite female soccer players perform more high-intensity running when playing in international games compared with domestic league games. *The Journal of Strength and Conditioning Research24*(4), 912-919. doi:http://dx.doi.org/10.1519/JSC.0b013e3181d09f21

Aughey, R. (2011). Applications of GPS technologies to field sports. *International Journal of Sports Physiology and Performance, 6*(3), 295-310.

Beltz, N. M., Gibson, A. L., Janot, J. M., Kravitz, L., Mermier, C. M., & Dalleck, L. C. (2016). Graded exercise testing protocols for the determination of VO 2 max: historical perspectives, progress, and future considerations. *Journal of Sports Medicine*. doi:http://dx.doi.org/10.1155/2016/3968393

Bishop, D., & Girard, O. (2013). Determinants of team-sport performance: implications for altitude training by team-sport athletes. *British Journal of Sports Medicine, 47*(Suppl 1), i17-i21. doi:http://dx.doi.org/10.1136/bjsports-2013-092950

Brocherie, F., Girard, O., Faiss, R., & Millet, G. (2015). High-intensity intermittent training in hypoxia: a double-blinded, placebo-controlled field study in youth football players. *The Journal of Strength and Conditioning Research, 29*(1), 226-237. doi:http://dx.doi.org/10.1519/JSC.0000000000000590

Brocherie, F., Girard, O., Faiss, R., & Millet, G. (2017). Effects of repeated-sprint training in hypoxia on sea-level performance: a meta-analysis. *Sports Medicine, 47*(8), 1651-1660. doi:http://dx.doi.org/10.1007/s40279-017-0685-3

Caris, A. V., & Santos, R. V. T. (2019). Performance and altitude: ways that nutrition can help. *Nutrition, 60*, 35-40. doi:http://dx.doi.org/10.1016/j.nut.2018.09.030

Chen, C.-Y., Hou, C.-W., Bernard, J. R., Chen, C.-C., Hung, T.-C., Cheng, L.-L., Liao, Y.-H., & Kuo, C.-H. (2014). Rhodiola crenulata-and Cordyceps sinensis-based supplement boosts aerobic exercise performance after short-term high altitude training. *High Altitude Medicine and Biology, 15*(3), 371-379. doi:http://dx.doi.org/10.1089/ham.2013.1114

Datson, N., Hulton, A., Andersson, H., Lewis, T., Weston, M., Drust, B., & Gregson, W. (2014). Applied physiology of female soccer: an update. *Sports Medicine, 44*(9), 1225-1240. doi:http://dx.doi.org/10.1007/s40279-014-0199-1

Faiss, R., Léger, B., Vesin, J.-M., Fournier, P.-E., Eggel, Y., Dériaz, O., & Millet, G. P. (2013). Significant molecular and systemic adaptations after repeated sprint training in hypoxia. *PlosOne, 8*(2), e56522. doi:http://dx.doi.org/10.1371/journal.pone.0056522

Faiss, R., Willis, S., Born, D.-P., Sperlich, B., Vesin, J.-M., Holmberg, H.-C., & Millet, G. P. (2015). Repeated double-poling sprint training in hypoxia by competitive cross-country skiers. *Medicine and Science in Sports and Exercise, 47*(4), 809-817. doi:http://dx.doi.org/10.1249/MSS.0000000000000464

Girard, O., Brocherie, F., Goods, P. S., Millet, G. P., & Living, A. (2020). An Updated Panorama of “Living Low-Training High” Altitude/Hypoxic Methods. *Frontiers in Sports and Active Living, 2*, 26. doi:http://dx.doi.org/10.3389/fspor.2020.00026

Girard, O., Brocherie, F., & Millet, G. P. (2017). Effects of altitude/hypoxia on single-and multiple-sprint

performance: a comprehensive review. *Sports Medicine, 47*(10), 1931-1949. doi:http://dx.doi.org/10.1007/s40279-017-0733-z
Glaister, M., Howatson, G., Pattison, J. R., & McInnes, G. (2008). The reliability and validity of fatigue measures during multiple-sprint work: an issue revisited. *The Journal of Strength and Conditioning Research, 22*(5), 1597-1601. doi:http://dx.doi.org/10.1519/JSC.0b013e318181ab80
Goods, P. S., Dawson, B. T., Landers, G. J., Gore, C. J., & Peeling, P. (2014). Effect of different simulated altitudes on repeat-sprint performance in team-sport athletes. *International Journal of Sports Physiology Performance, 9*(5), 857-862. doi:http://dx.doi.org/10.1123/ijspp.2013-0423
Gore, C. J., Clark, S. A., & Saunders, P. U. (2007). Nonhematological mechanisms of improved sea-level performance after hypoxic exposure. *Medicine and Science in Sports and Exercise, 39*(9), 1600-1609. doi:http://dx.doi.org/10.1249/mss.0b013e3180de49d3
Hamlin, M. J., Olsen, P. D., Marshall, H. C., Lizamore, C. A., & Elliot, C. A. (2017). Hypoxic repeat sprint training improves rugby player's repeated sprint but not endurance performance. *Frontiers in Physiology, 8*, 24. doi:http://dx.doi.org/10.3389/fphys.2017.00024
Holliss, B. A., Burden, R. J., Jones, A. M., & Pedlar, C. R. (2014). Eight weeks of intermittent hypoxic training improves submaximal physiological variables in highly trained runners. *The Journal of Strength and Conditioning Research, 28*(8), 2195-2203. doi:http://dx.doi.org/10.1519/JSC.0000000000000406
Huang, X., Zhang, Z., Wang, X., Wang, G., Wang, Y., Tang, K., & Gao, B. (2023). Influence of chronic nitrate-rich beetroot juice supplementation on the endurance performance of active winter triathletes: A randomized controlled trial. *Journal of the American Nutrition Association, 42*(2), 195-206. doi:http://dx.doi.org/10.1080/07315724.2021.2021562
Humberstone-Gough, C. E., Saunders, P. U., Bonetti, D. L., Stephens, S., Bullock, N., Anson, J. M., & Gore, C. J. (2013). Comparison of live high: train low altitude and intermittent hypoxic exposure. *Journal of Sports Science and Medicine, 12*(3), 394.
Kasai, N., Kojima, C., Sumi, D., Takahashi, H., Goto, K., & Suzuki, Y. (2017). Impact of 5 days of sprint training in hypoxia on performance and muscle energy substances. *International Journal of Sports Medicine, 38*(13), 983-991. doi:http://dx.doi.org/10.1055/s-0043-117413
Kasai, N., Mizuno, S., Ishimoto, S., Sakamoto, E., Maruta, M., & Goto, K. (2015). Effect of training in hypoxia on repeated sprint performance in female athletes. *Springerplus, 4*(1), 1-7. doi:http://dx.doi.org/10.1186/s40064-015-1041-4
Kreipke, P., Vince C, Moffatt, P., Robert J, Tanner, M., Charles J, & Ormsbee, P., Michael J (2021). Effects of concurrent training and a multi-ingredient performance supplement containing Rhodiola rosea and Cordyceps sinensis on body composition, performance, and health in active men. *Journal of Dietary Supplements, 18*(6), 597-613.
Kumar, R., Negi, P., Singh, B., Ilavazhagan, G., Bhargava, K., & Sethy, N. K. (2011). Cordyceps sinensis promotes exercise endurance capacity of rats by activating skeletal muscle metabolic regulators. *Journal of Ethnopharmacology, 136*(1), 260-266. doi:http://dx.doi.org/10.1016/j.jep.2011.04.040
Li, T., & Li, W. (2009). Impact of polysaccharides from Cordyceps on anti-fatigue in mice. *Scientific Research and Essays, 4*(7), 705-709.
Liao, Y. H., Chao, Y. C., Sim, B. Y., Lin, H. M., Chen, M. T., & Chen, C. Y. (2019). Rhodiola/Cordyceps-Based Herbal Supplement Promotes Endurance Training-Improved Body Composition But Not Oxidative Stress and Metabolic Biomarkers: A Preliminary Randomized Controlled Study. *Nutrients, 11*(10). doi:10.3390/nu11102357
Long, H., Qiu, X., Cao, L., Liu, G., Rao, Z., & Han, R. J. J. o. E. (2021). Toxicological safety evaluation of the cultivated Chinese cordyceps. *Journal of Ethnopharmacology, 268*, 113600. doi:http://dx.doi.org/10.1016/j.jep.2020.113600
Milanović, Z., Sporiš, G., James, N., Trajković, N., Ignjatović, A., Sarmento, H., Trecroci, A., & Mendes, B. M. B. (2017). Physiological demands, morphological characteristics, physical abilities and injuries of female soccer players. *Journal of Human Kinetics, 60*, 77. doi:http://dx.doi.org/10.1515/hukin-2017-0091
Montero, D., & Lundby, C. (2017). No improved performance with repeated-sprint training in hypoxia versus normoxia: a double-blind and crossover study. *International Journal of Sports Physiology and Performance, 12*(2), 161-167. doi:http://dx.doi.org/10.1123/ijspp.2015-0691
Morrison, J., McLellan, C., Minahan, C., & medicine. (2015). A clustered repeated-sprint running protocol for team-sport athletes performed in normobaric hypoxia. *Journal of Sports Science and Medicine, 14*(4), 857.
Nagao, A., Kobayashi, M., Koyasu, S., Chow, C. C., & Harada, H. (2019). HIF-1-dependent reprogramming of glucose metabolic pathway of cancer cells and its therapeutic significance. *International Journal of Molecular Sciences, 20*(2), 238. doi:http://dx.doi.org/10.3390/ijms20020238
Nava, R. C., McKenna, Z., Fennel, Z., Berkemeier, Q., Ducharme, J., de Castro Magalhães, F., Amorim, F. T., & Mermier, C. (2022). Repeated sprint exercise in hypoxia stimulates HIF-1-dependent gene expression in skeletal muscle. *European journal of applied physiology, 122*(4), 1097-1107.

doi:http://dx.doi.org/10.1007/s00421-022-04909-3

Park, H.-Y., Jung, W.-S., Kim, S.-W., Kim, J., & Lim, K. (2022). Effects of interval training under hypoxia on hematological parameters, hemodynamic function, and endurance exercise performance in amateur female runners in Korea. *Frontiers in Physiology, 13*, 1011. doi:http://dx.doi.org/10.3389/fphys.2022.919008

Peeling, P., Binnie, M. J., Goods, P. S., Sim, M., & Burke, L. M. (2018). Evidence-based supplements for the enhancement of athletic performance. *International Journal of Sport Nutrition and Exercise Metabolism, 28*(2), 178-187. doi:http://dx.doi.org/10.1123/ijsnem.2017-0343

Pramkratok, W., Songsupap, T., & Yimlamai, T. (2022). Repeated sprint training under hypoxia improves aerobic performance and repeated sprint ability by enhancing muscle deoxygenation and markers of angiogenesis in rugby sevens. *European Journal of Applied Physiology* 1-12. doi:http://dx.doi.org/10.1007/s00421-021-04861-8

Randell, R. K., Clifford, T., Drust, B., Moss, S. L., Unnithan, V. B., De Ste Croix, M. B., Datson, N., Martin, D., Mayho, H., & Carter, J. M. (2021). Physiological characteristics of female soccer players and health and performance considerations: a narrative review. *Sports Medicine, 51*, 1377-1399. doi:http://dx.doi.org/10.1007/s40279-021-01458-1

Savioli, F. P., Zogaib, P., Franco, E., de Salles, F. C. A., Giorelli, G. V., & Andreoli, C. V. (2022). Effects of cordyceps sinensis supplementation during 12 weeks in amateur marathoners: A randomized, double-blind placebo-controlled trial. *Journal of Herbal Medicine, 34*, 100570. doi:http://dx.doi.org/10.1016/j.hermed.2022.100570

Singh, M., Tulsawani, R., Koganti, P., Chauhan, A., Manickam, M., & Misra, K. (2013). Cordyceps sinensis increases hypoxia tolerance by inducing heme oxygenase-1 and metallothionein via Nrf2 activation in human lung epithelial cells. *BioMed Research International*.

Siu, K. M., Mak, D. H., Chiu, P. Y., Poon, M. K., Du, Y., & Ko, K. M. (2004). Pharmacological basis of 'Yin-nourishing'and 'Yang-invigorating'actions of Cordyceps, a Chinese tonifying herb. *Life Sciences, 76*(4), 385-395.

Stellingwerff, T., Peeling, P., Garvican-Lewis, L. A., Hall, R., Koivisto, A. E., Heikura, I. A., & Burke, L. M. (2019). Nutrition and altitude: strategies to enhance adaptation, improve performance and maintain health: a narrative review. *Sports Medicine, 49*, 169-184. doi:http://dx.doi.org/10.1007/s40279-019-01159-w

Thongsawang, S., Krataithong, T., ChorCharoenying, S., Norchai, P., & Nokkaew, N. (2021). Applying Cordyceps sinensis to Boost Endurance Performance in Long-Distance Runners. *Journal of Exercise Physiology Online, 24*(3), 1-13.

Tsuk, S., Lev, Y. H., Rotstein, A., Zeev, A., Carasso, R., & Steiner, G. (2018). Effects of a commercial supplement of Ophiocordyceps sinensis and Ganoderma lucidum on physiological responses to maximal exercise in healthy young participants. *International Journal of Medicinal Mushrooms, 20*(4). doi:http://dx.doi.org/10.1615/IntJMedMushrooms.2018025989

Wehrlin, J. P., Marti, B., & Hallén, J. (2016). *Hemoglobin mass and aerobic performance at moderate altitude in elite athletes*.

Xiang, F., Lin, L., Hu, M., & Qi, X. (2016). Therapeutic efficacy of a polysaccharide isolated from Cordyceps sinensis on hypertensive rats. *International Journal of Biological Macromolecules, 82*, 308-314. doi:http://dx.doi.org/10.1016/j.ijbiomac.2015.09.060

Zagatto, A. M., Beck, W. R., & Gobatto, C. A. (2009). Validity of the running anaerobic sprint test for assessing anaerobic power and predicting short-distance performances. *The Journal of Strength and Conditioning Research, 23*(6), 1820-1827. doi:http://dx.doi.org/10.1519/JSC.0b013e3181b3df32