EFFECTS OF A 4-WEEK MICRO-HYPERBARIC OXYGEN INTERVENTION ON OXIDATION-ANTIOXIDATION SYSTEM FUNCTION

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EFEITOS DA INTERVENÇÃO DE OXIGÊNIO MICRO-HIPERBÁRICO DE 4 SEMANAS NA FUNÇÃO DO SISTEMA OXIDANTE-ANTIOXIDANTE

EFECTOS DE LA INTERVENCIÓN CON OXÍGENO MICRO-HIPERBÁRICO DURANTE 4 SEMANAS EN LA FUNCIÓN DEL SISTEMA OXIDACIÓN-ANTIOXIDACIÓN

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ABSTRACT

Introduction: Hyperbaric oxygen intervention has an important effect on the function of the body's oxidation-antioxidant system. Objective: To verify the effects of a 4-week micro-hyperbaric oxygen intervention on oxidation-antioxidation system function in skeleton athletes. Methods: The experimental group underwent a 1.3 ATA HBO intervention for 4 weeks and the control group underwent natural recovery. The levels of MDA, PC, SOD, CAT, GSH-PX, T-AOC, BU, CK, T, and C of the two groups were measured at Week 0, Week 2, and Week 4. Results: The MDA, PC, and CK of the Exp group were significantly lower than Con group ($P < 0.05$) in Week 4. The SOD, CAT, and T-AOC of the Exp group were significantly higher in Week 4 than in Week 0 ($P < 0.05$) and significantly higher than the Con group values ($P < 0.05$). Conclusions: A four-week 1.3 ATA HBO intervention decreased the level of oxidative stress, increased the activity of antioxidant enzymes, and reduced the degree of exercise fatigue in skeleton athletes. ***Level of Evidence II; Therapeutic studies - Investigating treatment results.***

Keywords: Hyperbaric oxygenation; Oxidative stress; Muscle fatigue.

RESUMO

Introdução: A intervenção de oxigênio hiperbárico tem um efeito importante na função do sistema oxidativo--antioxidante do corpo. Objetivo: Verificar os efeitos de uma intervenção de oxigênio micro-hiperbárico de 4 semanas na função do sistema oxidante-antioxidante em atletas de skeleton. Métodos: O grupo de teste foi submetido a uma intervenção de oxigênio micro-hiperbárico a 1,3 ATA de 4 semanas, 4 vezes por semana e o grupo controle passou por recuperação natural. Os níveis de malondialdeído (MDA), proteína carbonila (PC), superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GSH-PX), capacidade antioxidante total (T-AOC), uréia sanguínea (BU), creatina quinase (CK), testosterona (T) e cortisol (C) foram medidos na semana 0, semana 2 e semana 4 para ambos os grupos de atletas. Resultados: MDA, PC e CK do grupo de teste foram significativamente menores do que os valores do grupo controle (p <0,05) na semana 4. SOD, CAT e T-AOC foram significativamente maiores no grupo de teste na semana 4 do que na semana 0 (p < 0,05) e significativamente maiores do que os valores para o grupo controle (p < 0,05). Conclusão: A intervenção de oxigênio micro-hiperbárico de 4 semanas a 1,3 ATA reduziu significativamente os níveis de estresse oxidativo, aumentou a atividade enzimática antioxidante e reduziu os níveis de fadiga relacionada ao exercício nos atletas de skeleton. ***Nível de evidência II; Estudos terapêuticos - Investigação dos resultados do tratamento.***

Descritores: *Oxigenação hiperbárica; Estresse oxidativo; Fadiga muscular.*

RESUMEN

Introducción: La intervención con oxígeno hiperbárico tiene un efecto importante en la función del sistema oxidación-antioxidación del organismo. Objetivo: Verificar los efectos de una intervención con oxígeno micro-hiperbárico durante 4 semanas en la función del sistema oxidación-antioxidación en atletas de skeleton. Métodos: El grupo de prueba se sometió a una intervención con oxígeno micro-hiperbárico a 1,3 ATA durante 4 semanas, 4 veces a la semana, y el grupo de control se sometió a una recuperación natural. Se midieron los niveles de malondialdehído (MDA), carbonilo proteico (PC), superóxido dismutasa (SOD), catalasa (CAT), glutatión peroxidasa (GSH-PX), capacidad antioxidante total (T-AOC), urea en sangre (BU), creatina quinasa (CK), testosterona (T), y cortisol (C) en la semana 0, en la semana 2 y en la semana 4 para ambos grupos de atletas. Resultados: Los valores de MDA, CP y CK del grupo de prueba fueron significativamente inferiores a los del grupo de control (p <0,05) en la semana 4. Los valores de SOD, CAT y T-AOC fueron significativamente mayores en el grupo de prueba en la semana 4 que en la semana 0 (p < 0,05) y significativamente mayores que los valores del grupo de control (p < 0,05) Conclusión: La intervención con oxígeno hiperbárico a 1,3 ATA durante 4 semanas redujo significativamente

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los niveles de estrés oxidativo, aumentó la actividad de las enzimas antioxidantes y redujo los niveles de fatiga relacionada con el ejercicio en los atletas de skeleton. ***Nivel de evidencia II; Estudios terapêuticos - Investigación de los resultados del tratamiento.***

Descriptores: *Oxigenación hiperbárica; Estrés oxidativo; Fatiga muscular.*

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INTRODUCTION

Hyperbaric oxygen (HBO) therapy refers to placing the human body in a pure oxygen environment where the pressure is higher than the atmospheric pressure to increase the physical oxygen dissolution and oxygen partial pressure in the blood, oxygen partial pressure difference between blood and cells, and effective diffusion distance of oxygen.[1] In exercise training, HBO is mainly used for athletes' sports injury rehabilitation.[2,3] In addition, several studies have also applied HBO to eliminate exercise-induced fatigue and recover physical function in athletes undergoing exercise training.[4,5] After high-intensity exercise, the lactic acid and heart rate recovery speeds in the hyperbaric oxygen intervention group was significantly faster than that in the control group, suggesting that hyperbaric oxygen can promote the elimination of peripheral acute fatigue.[4] Another study suggest that HBO contributes to sustained force production due to suppressing the muscle fatigue progression, and HBO can contribute to the prevention of excess fatigue of agonist muscles for specific exercises involving repeated jumping.[5] However, some studies have found that the intervention of high dose HBO (the pressure>2ATA) increases the formation of free radicals, reduces the function of oxidation-antioxidation system, and eventually leads to lipid peroxidation, organ damage, resulting in the occurrence of chronic fatigue.[6,7]

Exercise-induced fatigue is a complex physiological phenomenon that is produced via the interaction of many factors. Free radical damage theory, which is accepted by many scholars, states that excessive exercise leads to increased production of free radicals, causes imbalance between oxidation stress and anti-oxidation ability, breaks down coordination and functions in the endocrine system, nervous system, and immune system, causes the decline of body function, and induces the generation of exercise-induced fatigue.[8,9] The enhancement of oxidative stress damages the physical function of athletes, which is not conducive to recovery after exercise or the elimination of fatigue. High-dose hyperbaric oxygen intervention can enhance the level of oxidative stress in the body, but low-dose intervention may not significantly impact the level of oxidative stress or even mobilize the antioxidant enzyme system to protect against oxidative damage.

Studies have shown that micro-hyperbaric oxygen of 1.3ATA can significantly reduce serum reactive oxygen metabolites and average visual analog scale score to eliminate exercise-induced fatigue;[10] and micro-hyperbaric oxygen intervention of 1.25ATA, 1.7ATA can regulate antioxidant system function and protect against oxidative damage in diabetic patients.[11,12] In conclusion, the effects of hyperbaric oxygen on oxidative-antioxidant system function may depend on the level of pressure; micro-hyperbaric oxygen intervention may have positive effects on oxidative-antioxidant system function but further studies are necessary to confirm this.

A winter ski racing event called "skeleton racing" will be one of China's main events in the 2022 Winter Olympic Games. At present, the Chinese skeleton team is in the critical stage of preparing for the 2022 Winter Olympics. In the training process, the stimulation of high-intensity exercise load may cause the increase of free radicals , destroy the oxidative-antioxidant system function, cause oxidative stress damage, and lead to the generation of exercise fatigue in athletes. Therefore, timely intervention after training to reduce the level of oxidative stress is helpful for the recovery of athletes' physical function and the elimination of fatigue. The purpose of the present study is to explore whether a four- week micro-hyperbaric oxygen intervention regime can enhance oxidative-antioxidant system function in skeleton athletes during summer training. We hypothesize that the four-week micro-hyperbaric oxygen intervention will enhance the oxidative-antioxidant system function, promote the recovery of body function, and reduce the degree of exercise-induced fatigue.

METHODS

Participants

Eighteen athletes currently preparing for the 2022 Beijing Winter Olympics were recruited from the Chinese National Skeleton Team. All athletes were physically and mentally healthy. They were randomly divided into two groups: an experimental group and a control group. This study is in accord with the Declaration of Helsinki (2000) of the World Medical Association and has got the permit from the Ethics Committee of Shanghai University of Sport (Approval number: 102772020RT079). All athletes involved in the study have signed the informed consent. Body composition was tested using an X-SCAN II body composition analyzer (South Korea). The participants' demographics and basic physical condition data are shown in Table 1.

Training arrangement

The summer training stage (held between August 23 and September 21) of the Chinese skeleton team in 2020 was treated as the experimental stage. All the athletes were trained by the same coach group and their training plan, diet, and rest time were identical. The training content consisted of land skid-pushing simulation training and physical training.

1.3ATA micro-hyperbaric oxygen intervention

On Monday, Wednesday, Friday, and Sunday evenings (19:00-20:00) of each week, a portable hyperbaric oxygen chamber and related software (OxyMu International Hi-Tech Co., Ltd., Beijing) were utilized to conduct micro-hyperbaric oxygen intervention for the athletes in the experimental group at a pressure of 1.3 ATA. The athletes lay flat in a cabin in which pressure rose to 1.3 ATA within 5 min and lasted for 60 min. Athletes experiencing claustrophobia were given professional

Table 1. Participant Demographics.

Index	Experimental group	Control group
Number (male/female)	8 (4/4)	8 (4/4)
Age (year)	23.13±1.25	22.50±1.69
Height (m)	1.76±0.06	1.81±0.06
Weight (kg)	73.38±10.68	75.89±10.68
BMI (weight/height^2)	23.66±2.44	23.22±2.55
Body fat percentage (%)	14.57±2.96	13.71±2.12
Body fat mass (kg)	10.54±1.86	10.31±1.74
Lean body mass (kg)	62.83±10.47	65.57±10.00
Professional training (years)	3.93±1.54	3.79±1.68

Note: Data are means±SD. There was no significant difference in the indicators between the two groups ($P > 0.05$) after the T-test.

psychological counseling to avoid any psychological changes associated with the intervention. Baseline blood pressure and heart rate (HR) were measured before entering the cabin.

Blood serum measurements

Five milliliters of blood were collected at three-time points: before the experiment, at Week 2, and at Week 4 after the athletes had fasted for 12 h. The blood was centrifuged at room temperature (3500 r/s, 5 min) and the supernatant was stored at -80°C within 30 min. Levels of MDA, PC, SOD, CAT, GSH-PX, and T-AOC were determined by microplate ultraviolet colorimetry, immunosorbent assay, visible light, colorimetry, and microplate assay, respectively. Levels of BU and CK were determined on a three-channel whole blood dry biochemical analyzer and matching reagent strip (Reflotron Plus, USA). Levels of T and C were determined by chemiluminescence. All tests were performed by a professional experimenter.

Data analysis

Relevant data are presented here as means and standard deviations (means±SD). SPSS 25.0 (IBM, United States) was used to analyze the data. A Kolmogorov-Smirnov test was used to determine the normality of the data. A two-way (treatment and time) analysis of variance (ANOVA) with repeated measures was used to analyze the changes of each index in the two groups in different weeks, with a Bonferroni test for post comparison. The significance level was set to P<0.05.

RESULTS

As shown in Table 2, there were significant group-by-time interactions on MDA, PC, SOD, CAT, and T -AOC. MDA and PC in Week 4 that were significantly lower than in Week 0 (P < 0.05); SOD, CAT, and T-AOC in Week 4 were significantly higher than in Week 0 (P < 0.05). In Week 4, MDA and PC in the experimental group were significantly lower than in the control group (P < 0.05); SOD, CAT, and T-AOC in the experimental group were significantly higher than those of the control group (P < 0.05). There were no significant group-by-time interactions on GSH-PX (P>0.05).

As shown in Table 3, there were significant group-by-time interactions on CK. In the control group, CK in Week 4 was significantly higher than in Week 0 (P < 0.05). In Week 4, CK in the control group was significantly higher than in the experimental group (P < 0.05). There were no significant group-by-time interactions on BU, T, C, or T/C (P>0.05), but the increment of BU in the control group. The T/C in the experimental group was slightly increased, while T/C in the control group was decreased.

DISCUSSION

Consistent with our hypothesis, a 4-week 1.3 ATA micro-hyperbaric oxygen intervention regime significantly reduced the level of oxidative stress and improved the antioxidant capacity, which can interfere positively the recovery of body functional states, and reduce the degree of exercise fatigue in skeleton athletes during summer training. As is well-known, the high-intensity training can increase the level of oxidative stress, overload the Ca^{2+} in the cytoplasm, reduce muscle excitability and muscle contraction function, induce the occurrence of exercise fatigue. In addition, oxidative stress damage can also cause vasodilation dysfunction and blood flow decreased.[13] The decrease of microvascular blood flow not only affects the timely elimination of metabolic waste after exercise, but also affects the transportation and exchange of matter and energy during exercise, which can induce the generation of exercise-induced fatigue and affects the exercise performance. Therefore, it is of great significance to take intervention measures to improve the function of the oxidation-antioxidant system after training.

In our study, MDA and PC in Exp group were significantly reduced in four weeks of micro-hyperbaric oxygen intervention, indicating that the intervention reduced the level of oxidative stress; this is consistent with the results of Kim's 2011 study, where micro-hyperbaric oxygen treatment

Table 2. Oxidative stress, antioxidant capacity indices of two groups by week.

Index	Group	Week 0	Week 2	Week 4	Interaction -Time × Group
MDA (nmol/ml)	Con	8.33±1.56	7.85±1.35	8.84±1.07	F = 9.686, p = 0.002
	Exp	8.50±0.92	7.49±1.12	5.82±0.99*#	
PC (nmol/mgprot)	Con	1.25±0.07	1.22±0.07	1.27±0.09	F = 11.486, p = 0.001
	Exp	1.24±0.13	1.19±0.07	1.06±0.08*#	
SOD (U/mL)	Con	16.76±2.72	16.46±2.05	15.96±1.82	F = 7.593, p = 0.006
	Exp	16.22±2.40	18.63±2.45	19.96±1.42*#	
CAT (U/mL)	Con	6.98±0.64	6.22±2.75	5.98±0.84	F = 4.029, p = 0.041
	Exp	6.53±1.16	6.84±1.16	8.34±0.46*#	
GSH-PX (mmol/L)	Con	18.85±3.43	21.89±2.89	21.17±3.30	F = 0.910, p = 0.425
	Exp	19.70±2.80	21.25±2.36	23.59±2.61	
T-AOC (mmol/L)	Con	0.70±0.04	0.74±0.14	0.66±0.07	F = 5.812, p = 0.015
	Exp	0.71±0.11	0.75±0.11	0.90±0.07*#	

Note: * P < 0.05 vs. corresponding indicators in Week 0 of experiment group. # P<0.05 vs. corresponding indicators in Week 4 of control group.

Table 3. Exercise fatigue monitoring indices of two groups by week.

Index	Group	Week 0	Week 2	Week 4	Interaction - Time × Group
BU (nmol/ml)	Con	6.41±1.06	5.30±1.34	7.89±0.86	F = 2.942, p = 0.086
	Exp	6.49±0.90	5.34±1.36	6.62±0.83	
CK (U/mL)	Con	182.00±22.17	200.63±66.33	280.50±41.05*#	F = 9.360, p = 0.003
	Exp	177.75±22.88	196.88±50.71	192.19±27.39	
T (ng/dl)	Con	366.63±361.38	350.13±332.93	317.00±295.33	F = 1.664, p = 0.225
	Exp	358.38±348.30	363.75±348.08	363.63±336.04	
C (ug/dl)	Con	20.08±3.09	20.16±2.66	19.31±1.37	F = 0.298, p = 0.747
	Exp	19.60±3.50	18.83±3.50	19.00±2.87	
T/C	Con	18.99±19.37	17.37±17.04	16.29±15.17	F = 0.794, p = 0.472
	Exp	18.85±19.60	20.66±21.46	20.12±19.30	

Note: * P < 0.05 vs. corresponding indicators in Week 0 of control group; # P<0.05 vs. corresponding indicators in Week 4 of experiment group.

reduced oxidative stress and VAS fatigue score while promoting fatigue elimination.[10] Hypoxia is an important factor in the generation of oxygen free radicals. Hyperbaric oxygen can improve the dissolution of blood oxygen and oxygen partial pressure, expand the oxygen partial pressure difference between blood and tissue, improve the body's hypoxia state and oxygen supply, speed up the scavenging of free radicals, and reduce the level of oxidative stress. However, some studies have shown that micro-hyperbaric oxygen intervention has no significant effect on the levels of reactive oxygen metabolites[14] or8-OHdG,[15] which is a marker of DNA oxidative damage. In addition, another study found that psychological fatigue induced by high-intensity exercise can be improved early without changes in oxidative stress by micro-hyperbaric oxygen intervention.[16]The differences in our findings may be related to the dose of the intervention. Most of the above studies were conducted over one or fewer micro-hyperbaric oxygen interventions, which may not have been sufficient to produce significant effects on the body. In this study, we conducted 16 separate micro-hyperbaric oxygen intervention iterations with athletes over a four-week regime, thus showing positive effects.

In addition, some studies have shown that HBO intervention can increase the level of oxidative stress. In the absence of antioxidant substances,high-dose HBO intervention can enhance the level of oxidative stress and increase the level of DNA strand breaks. Further, there is a positive dose-response relationship with hyperbaric oxygen pressure and intervention time.[17] Long-term HBO therapy (15 sessions) resulted in significant increases in the levels of ROM and MDA in the absence of antioxidant supplementation in a previous study.[18] Long-term HBO intervention does appear to enhance the level of oxidative stress, but previous scholars' use of HBO intervention exceeded 2ATA, which is a significant pressure difference compared to our micro-hyperbaric oxygen (1.3ATA). We believe that hyperbaric oxygen pressure over 2ATA may increase the production of active free radicals and enhance the level of oxidative stress. When the pressure is kept within an appropriate range, it will not increase the production of free radicals and may accelerate the scavenging of free radicals and reduce the level of oxidative stress. However, our conclusion still lacks sufficient research evidence and needs to be confirmed by further studies.

In our study, SOD, CAT, and T-AOC significantly increased in Exp group after micro-hyperbaric oxygen intervention for four weeks, indicating that this intervention up-regulated the antioxidant system function and reduced the damage of free radicals to cells,this is consistent with the findings of some scholars.[11,12] Micro-hyperbaric oxygen intervention of 1.25ATA,[11] 1.7ATA[12]can regulate antioxidant system function and protect against oxidative damage in diabetic patients. However, other scholars hold different conclusions. In a previous study, HBO treatment (15 sessions) significantly reduced SOD and CAT activities but produced no significant change in GSH-Px in the absence of antioxidant supplements.[18] The SOD activity of subjects all decreased after 24 h of exposure to 2 ATA and 2.4 ATA, respectively, but the level of GSH-Px only decreased in the 2.4ATA environment.[19] We believe that the pressure of HBO is still the main reason for the difference between our results and those of the above-mentioned studies, the activity of antioxidant enzymes appears to decrease once the pressure exceeds a certain level. However, this conclusion also needs to be confirmed by further studies.

The improvement of oxidation - antioxidant system function can accelerate the scavenging of free radicals, promote the recovery of the body and the elimination of exercise fatigue.[8,9] In order to evaluate the changes of athletes' physical function, we tested the levels of BU, CK, T and C. BU is the product of protein decomposition, which is an important index for evaluating exercise-induced fatigue. In our study, the level of BU was significantly higher in Con group after four weeks of training, indicating that the training regimen caused physical fatigue in the athletes. However, the BU level of Exp group did not change significantly under the same training load, indicating that the four-week micro-hyperbaric oxygen intervention promoted the recovery of glycogen energy supply and reduced the post--fatigue functional proportion of proteins. HBO improves kidney hypoxia, promotes the recovery of kidney function, accelerates the elimination of urea, thus promoting athletes' body recovery and relieving exercise fatigue.

CK is used to evaluate muscle adaptation to exercise intensity and injury after exercise.[20,21] When the muscle membrane is damaged or its permeability changes, CK flows into the blood from the membrane – this process is intensified if the cell membrane is damaged due to oxidative stress. In our experiment, the level of CK was significantly increased in Con group after four weeks of training, indicating that the high-intensity summer training caused certain damage to the muscle cells of the athletes. However, the level of CK was not significantly increased in Exp group, indicating that the four-week micro-hyperbaric oxygen intervention repaired the damage to muscle cell membranes and eliminate fatigue in the skeletal muscle. Our result is consistent with the results of Aunampai 's 2011 study, where three sessions of 1.7ATA HBO therapy did reduce CK level after repeated maximum vertical jumps.[22]

However, most scholars believe that HBO intervention cannot effectively reduce the level of CK after intense exercise.[23-25] Four sessions of 1.2ATA, 2ATA[23] and five sessions of 2.5ATA[24] HBO intervention failed to improve muscle pain and CK levels induced by centrifugal contractions in previous studies. Navid et al. reached a similar conclusion.[25] For different research results, the intervention dose of HBO and exercise pattern may be important reasons but further studies are necessary to confirm this.

Testosterone and cortisol are important indicators of athletes' physical functioning.[26,27] When athletes are fatigued, testosterone levels drop and cortisol levels rise leading to increased catabolism and decreased anabolism. The intervention effects of hyperbaric oxygen on serum testosterone and cortisol are still controversial, mainly because the changes of testosterone and cortisol are not only related to the stimulation of exercise load (intensity and quantity) but also affected by psycho-emotional states and metabolism.[28] For judo athletes, HBO intervention showed no significant effect on cortisol or total testosterone after high-intensity training.[29] However, the level of testosterone in divers increased after HBO rehabilitation treatment.[30] In our study, testosterone/cortisol in Con group decreased significantly before and after training, indicating that athletes were in a state of fatigue and their catabolism was strengthened during summer training. However, testosterone/cortisol in Exp group increased, indicating that HBO intervention promoted anabolism, inhibited catabolism, and improved body functions throughout the training process.

CONCLUSIONS

The results of this study show that a four-week 1.3 ATA HBO intervention improved the oxidative-antioxidant system function, which can interfere positively the recovery of body functional states, and reduce the degree of exercise fatigue in skeleton athletes during summer training. Therefore, we believe that 1.3ATA hyperbaric oxygen intervention can be used to promote physical function recovery and fatigue elimination in athletes.

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