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Selective protective effect of hydrogen water on free radical injury of athletes after high-intensity exercise.

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Abstract

Objective: This study aims to investigate the selective protective effect of hydrogen water on the free radical injury of athletes after high-intensity exercise and to provide a reliable method for reducing oxidative stress injury of athletes.

Methods: A total of 60 athletes from the swimming team in our city were selected as the research subjects. They were divided into the control group and hydrogen water group according to different intervention methods. The athletes in the control group were treated with placebo, and the athletes in the hydrogen water group were supplemented with hydrogen water. The serum superoxide anions, Serum Superoxide Dismutase (SOD) activities, and total antioxidant capacities of athletes were compared between the two groups.

Results: The serum superoxide anions, serum SOD activities, and total antioxidant capacities of athletes during and after training were significantly superior to those of the control group (P<0.05), and the difference was statistically significant.

Conclusion: Hydrogen water supplement could effectively reduce the oxidized substances in athletes before, during, and after exercise and could prevent the free radical injury caused by high-intensity exercise.

Keywords: Hydrogen water, Athletes, High intensity exercise, Free radical injury.

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Introduction

Hydrogen water is one of the antioxidants. Its low price, nontoxic side effects, being non-stimulant, and other benefits provide a decisive advantage in clinical application [1]. Clinical study indicated that injection or drinking of hydrogen water in the human body or animals or breathing hydrogen has a therapeutic effect for periodontitis, foot swelling, traumatic pancreatitis, intestinal ischemia reperfusion injury, brain injury, and other diseases caused by oxidative stress [2]. One-time injection of hydrogen water had a protective effect on the biological membrane damage of free radical after acute exhaustive exercise in rat. Meanwhile, they first proved the collective selective oxidation of hydrogen water [3]. However, previous research on hydrogen water used animal experiments, and research in the field of sport medicine is still in the initial exploratory stage. The systematic analysis of athletes undergoing professional high-intensity exercise is yet to be conducted [4]. In this study, 60 athletes from our swimming team in our city were selected as the research subjects. They were supplemented with hydrogen water at different time phases. The antioxidant effects were compared, and the detailed discussion of the research follows.

Materials and Methods

General data

A total of 60 athletes from the swimming team in our city were selected as the research subjects. They were divided into the control group and hydrogen water group according to the different intervention methods. The athletes in the control group were treated with placebo, and the athletes in the hydrogen water group were supplemented with hydrogen water. Every group had 30 male athletes. In the control group, the athletes were aged 14-22 years old with average of (18.1 ± 1.3) years old and had the following characteristics: height 172-196 cm, average (180.2 ± 6.3) cm; body weight 62-78 kg, average (68.2 ± 4.5) kg; and exercise duration 1-7 years, average (4.1 ± 0.5) years. In the hydrogen water group, the athletes were aged 15-22 years old, average (17.9 ± 1.5) years old, and had the following characteristics: height 174-192 cm, average (179.8 ± 6.5) cm; body weight 65-76 kg, average (68.0 ± 4.3) kg; exercise duration 2-7 years, average (3.7 ± 0.7) years. No statistical difference in athlete age, height, weight, and exercise duration (P>0.05) was noted between the two groups.

Intervention methods

The hydrogen water in this study was purchased from Japan. It was authenticated as neither stimulant nor banned substance by the analeptic inspection center. All athletes were in good health during the intervention period and did not take any antioxidants, including vitamins C and E. The heart rates of athletes in the two groups were monitored. Meanwhile, the blood lactic acid of athletes was measured after exercise to ensure that exercise intensity was adequate. The study lasted for 8 d. A total of 5 ml fasting venous blood was drawn in the morning of the first day. The athletes were treated with the placebo (mineral water) and hydrogen water before, during, and after high-intensity exercise, tid, 200 ml each time. Venous blood was drawn after 2 h exercise. The intensities and amounts of training of all athletes were consistent in the study. The venous blood was labelled, naturally coagulated, and centrifuged by 3000 r/min in the refrigerated centrifuge. The separated serum was preserved in the refrigerator. The athletes were instructed to be mindful of their diet, and antioxidant nourishment was prohibited.

Determining indexes

The selective antioxidant indexes (superoxide anion (O^{2-})), antioxidant defense system indexes (Superoxide Dismutase (SOD)), and serum Total Antioxidant Capacity (T-AOC) of athletes were monitored in the two groups.

The activity of resisting superoxide anion was measured through colorimetric method. The operation was in accordance with the kit instruction of Nanjing Bioengineering Institute, and the OD value of each tube was measured. The formula was as follows: anti O^{2-} activity (U/L)=(OD value of the control tube-OD value of the measured tube)/(OD value of the control tube-OD value of the standard tube) × 1000 ml × concentration of standard sample × diluted times of the sample before test.

The SOD level *in vivo* was tested through biotin double-antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The operation was in accordance with the kit instruction of human SOD from Shanghai Lianshuo Biological Technology Co., Ltd. SOD concentration was positively correlated with the color.

The T-AOC *in vivo* was determined through biotin double-antibody sandwich ELISA. The operation was in accordance with the kit instruction of human SOD from Shanghai Lianshuo Biological Technology Co., Ltd. T-AOC concentration was positively correlated with the color.

Statistical methods

In this study, all the data were imputed into the Excel table and analysed using the SPSS19.0 statistical software. The measurement data were expressed with ($\chi \pm s$) and compared using t test. $P<0.05$ showed that the difference was statistically significant.

Results

Comparison of serum superoxide anion activities of athletes between the two groups

The serum antisuperoxide anion activities of athletes were not different between the two groups before exercise. Meanwhile, the serum antisuperoxide anion activities of athletes in the two groups decreased after exercise. However, the serum antisuperoxide anion activity of athletes in the hydrogen water group was decreased compared with that of the control group during and after exercise, as shown in Table 1.

Table 1. Comparison of serum antisuperoxide anion activities of athletes in the two groups ($\chi \pm s$; U/ml).

Group	N	Before exercise	During exercise	After exercise	P value
Blank group	30	146.60 ± 9.31	139.67 ± 9.07	117.17 ± 15.27	<0.05
Hydrogen water group	30	143.18 ± 7.88	95.86 ± 12.85	98.86 ± 8.30	<0.05
t value	/	1.53	15.25	5.77	/
P value	/	0.13	0.00	0.00	/

Note: The serum superoxide anion activities of athletes between the two groups were compared during and after exercise; P<0.05 indicates the difference was statistically significant.

Comparison of serum superoxide dismutase activities of athletes between the two groups

The SOD activities of athletes were not different between the two groups before exercise. Meanwhile, the SOD of athletes in the blank group was decreased after exercise. However, the SOD activity of athletes in the hydrogen water group during and after exercise was significantly higher than that of the control group and higher than that before and during exercise, as shown in Table 2.

Table 2. Comparison of serum superoxide dismutase activities of athletes between the two groups ($\chi \pm s$; U/L).

Group	N	Before the training	During the training	After the training	P value
Blank group	30	57.07 ± 7.08	47.86 ± 7.31	45.65 ± 7.63	<0.05
Hydrogen water group	30	55.79 ± 9.20	56.88 ± 4.83	66.92 ± 6.70	<0.05
t value	/	0.60	5.63	11.47	/

P value	/	0.55	0.00	0.00	/

Note: The Serum Superoxide Dismutase (SOD) activities of athletes were compared between the two groups during and after exercise. P<0.05 indicates that the difference was statistically significant.

Comparison of serum total antioxidant capacities of athletes between the two groups at different time phases

The serum total antioxidant capacities of athletes was not different between the two groups before exercise. Meanwhile, the serum T-AOC of athletes in the blank group fluctuated after exercise. However, the serum T-AOC of athletes in the hydrogen water group during and after exercise was significantly higher than that of the control group and higher than that before and during exercise, as shown in Table 3.

Table 3. *Comparison of serum total antioxidant capacities of athletes between the two groups at different time points (χ ± s; U/ml).*

Group	N	Before exercise	During exercise	After exercise	P value
Blank group	30	2.48 ± 0.11	2.28 ± 0.16	2.35 ± 0.11	<0.05
Hydrogen water group	30	2.46 ± 0.13	2.52 ± 0.19	3.36 ± 0.12	<0.05
t value	/	0.64	5.29	33.98	/
P value	/	0.52	0.00	0.00	/

Note: The total antioxidant capacities of athletes were compared between the two groups during and after exercise; P<0.05 indicates that the difference was statistically significant.

Discussion

Free radicals are a kind of substance produced by the normal metabolism in the human body. They do not contain paired electrons, so its nature is lively. Free radicals will offensively target all cells and induce injury. The free radical has two types, and 95% of free radicals belong to oxygen free radicals [5]. It has normal biological functions, such as sterilization, playing an important role in embryonic development, regulating angiotensin, and involvement in the biological initiation of various biological factors as a second messenger. However, the free radical is also cytotoxic. A large number of research [6] have reported that the free radical is closely related to cancer, inflammation, Alzheimer's disease, depression, protein oxidative pyrolysis, and lipid peroxidation. Therefore, the free radical is regarded as a "double-edged sword," and too much or too little will cause adverse effects or even damage. Superoxide anion free radical is a source of various free radicals. Free radicals will absorb the electrons in the endoplasmic reticulum, mitochondria, and nucleus through both non-enzymatic and enzymatic reaction; produce all kinds of oxygen free radicals; and cause damage [7]. Under normal circumstances, the content of plasma Hb is little. However, after high-intensity exercise, a large number of free radicals generate in the body, and the erythrocyte membrane permeability is increased, resulting in the release of Hb into the blood. After drinking the hydrogen water, the antisuperoxide anion activity of athletes was significantly lower than that of the control group (P<0.01), suggesting that hydrogen water could inhibit the antisuperoxide anion activity to a certain extent and reduce the oxidative stress injury.

SOD is an important substance of antioxidant system in body. It can effectively eliminate the superoxide anion during metabolism; prevent lipid peroxidation, aging, fatigue, and injury; and improve athletic ability. Monitoring SOD activity can effectively investigate the quantity of free radicals *in vivo* [8]. The study found that the SOD activity of athletes in the blank group was decreased after exercise. However, the SOD activities of athletes in the hydrogen water group during and after exercise were significantly higher than those of the control group and also higher than those before and during exercise (P<0.01). T-AOC is a comprehensive index. It can measure the intergraded function of the antioxidant system in body. Its value is closely related to the body's defense system and can directly reflect the health of the body [9]. At present, reports on SOD activity after exercise are inconsistent. Compared with before exercise and other periods, the serum SOD activity was significantly increased. Meanwhile, the serum SOD activities were not significantly different among other time phases. The serum SOD activities were significantly decreased after anaerobic and aerobic exercises. The study found that in the blank group, serum T-AOC of athletes fluctuated after exercise [10]. However, the serum T-AOC of athletes in the hydrogen group was significantly higher than that of the control group and higher than that before and during exercise (P<0.05).

Conclusion

Hydrogen water supplement can effectively reduce the oxidizing substance before, during, and after exercise, preventing free radical damage caused by high-intensity exercise. Whether or not it can be generally used in athletes still requires further research with a large sample size.

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