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Research Article

New Sports Nutritious Food for Health Improvement and Antioxidant Function

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Abstract: The research aimed to explore the influence of new sports nutritious food (Hydrogen Water) on exercise-induced oxidative stress injury and athletic ability of power organic skeletal muscle that is susceptible to oxidation attacks and in vivo basis and possible mechanism of hydrogen water in the process of selective oxidation. This study randomly divided 80 healthy male SD rats into 8 groups of quiet control group (EC, group A), exercise control group (EE, group B), hydrogen water injection before exercise group (EH, group C) and joint hydrogen water injections before and after exercise group (EM, group D), with 10 rats in each group. The rest groups conducted adaptive low intensive treadmill running for 1 time every day before a week of the experiment except EC (group A). EH (group C) conducted intraperitoneal injection of hydrogen water with 10 mL/kg before 1 min of exercise before formal experiment. EM (group D) conducted intraperitoneal injection of hydrogen water immediately in 1 min before and after exercise. EE (group B) conducted intraperitoneal injection with same volume normal saline. EE, EH and EM group conducted a one-time heavy intensive exhausted treadmill running by using Marra project. Group B, C, D conducted quantitative treadmill running, detected 3-NT, 8-OHdG value of skeletal muscle after 3 h of exercise in group A, B, C, D using Elisa method and measured MDA, SOD, GSH, T-AOC and level of O2-, H2O2 and •OH using chemical colorimetry. The results showed that hydrogen water can significantly prolong the duration of exercise to exhaustion of rats, which had significant anti-fatigue effect; and can significantly inhibit the aggravation of motility of O2-, H2O2 and •OH, remove in vivo cytotoxic substances •OH effectively, improve SOD and GSH, enhance T-AOC, improve minicirculation and reduce the damage of cells; joint hydrogen water injections before and after exercise had synergistic effect for the prevention and control of exercise-induced oxidative stress injury. These results confirm that hydrogen water has anti-fatigue effect and protective effect of exercise-induced oxidative stress injury, which is conducted through multiple targets on the basis of selective antioxidant effect of hydrogen water.

Keywords: Antioxidant, exercise-induced oxidative damage, hydrogen water, skeletal muscle

INTRODUCTION

Sports nutritious food refers to a kind of food that can provide special nutritional requirements for human movement; adjust the metabolic state and help to improve physical fitness and health. Common functional factors of sports nutritious food include protecting articular cartilage, improving muscle mass, regulating incretion, reducing and controlling weight, boosting immunity and enhancing antioxidant ability, etc (Lin *et al*., 2011). The body fatigue and tissue damage are the key factors of affecting health, sports ability and sports life of athletes and sports enthusiasts, thereby postponing the occurrence of exercise fatigue, improving sports ability and extending sports life are the research focuses of sports medicine.

Numerous studies have shown that oxygen free radicals increase in the body in acute heavy intensive exercise, which will lead to the accumulation of lipid peroxidation in the body, decline of antioxidant enzymes activity and rise of nitrite concentration in the body, thus causing the lipid peroxidation damage of collective organization and cells and protein damage, thereby bringing about the body fatigue, tissue injury and athletic ability decline, etc (Li, 2012). In recent years, domestic and foreign studies have shown that hydrogen is a kind of high-quality antioxidants, which has the features of non-toxic, non-residue, easy preparation, low cost and selective oxidability. Namely free radicals with strong oxidation are chosen to react directly, which can prevent and relieve liver disease, atherosclerosis and other disease using the function of oxidation reduction of hydrogen molecular caused by free radicals (Sun *et al*., 2014; Tan and Zhang, 2012; Lu and Li, 2014; Qin *et al*., 2013). Japan and the European developed countries have had drinking water of “hydrogen water” and use it to reach the purposes of regulating, preventing and relieving their own body. Through observing and analyzing the corresponding index changes of hydrogen water injection of oxidative stress of skeletal muscle in rats after exhaustion exercise, Li *et al*. (2011) confirmed that hydrogen water can lower the metabolic level of free radical of skeletal muscle, improve antioxidant enzymes activity of skeletal muscle and enhance the total antioxidant capacity of skeletal muscle, which plays protective role in exercise-induced oxidative stress injury of skeletal muscle. Through exploring the influence of hydrogen water lavage on oxidative stress injury of skeletal muscle in rats after the heavy intensive and exhaustive exercise and its possible mechanism, (Tian and Gu, 2011) verified that hydrogen water can effectively reduce the ultra structure damage on skeletal muscle after heavy intensive and exhaustive exercise, protect its structural integrity and improve the total antioxidant capacity, inhibit the autophagy of cells and reduce oxidative stress injury effectively. On the above basis, research further explored that the influence of hydrogen water on exercise-induced oxidative stress injury of skeletal muscle, antioxidant defense system, sports ability, etc, in order to confirm the protective effect of hydrogen water on exercise-induced oxidative stress injury and provide certain reference basis for prevention of exercise-induced oxidative injury and beverage development in the future.

MATERIALS AND METHODS

Experimental materials and equipments: SD rats, hydrogen water, MDA test kit, rats 3-NT enzyme linked immunosorbent assay test kit, rats 8-OHdG enzyme linked immunosorbent assay test kit, SOD test kit, GSH test kit, T-AOC test kit, anti O2- activity test kit, H2O2test kit, inhibition ability of •OH test kit.

BCPT-98type electric animals running platform, sport timer, high-pressure sterilizer, DK-g A type thermostatic water tank, THZ. C constant temperature oscillator, low temperature refrigerated centrifuge, frozen stamping disruptor, adjustable high-speed tissue homogenate, dry heat sterilization oven, SK-1 mixer, UNICO 7200spectrophotometer, enzyme linked immunosorbent assay detector, Elisa plate, automatic washing machine, Ineucell 55 type incubator, one-way micropipettor, 12 micropipettor, sartorius BS110S electronic scale, Eppend of tube.

Experimental methods:

Experimental groups: Divide80 clean and healthy male SD rats that were in the same germ line and batch randomly into 8 groups after pairing according to the weight and follow the processes as shown in Table 1.

Experimental treatment: A week before the experiment, in addition to the EC group and the group A, the rest of the groups conducted adaptive low intensive treadmill running every day for one time for 10 min with 0° grade and 10 m/min speed. In formal experiment, EH and group C conducted intraperitoneal injection of hydrogen water with 10 mL/kg before 1 min of exercise; EM and group D conducted intraperitoneal injection of hydrogen water with 10 mL/kg before and after 1 min of exercise, respectively; EE and group B conducted intraperitoneal injection of normal saline with the same volume. EE, EH and EM group conducted a one-time heavy intensive exhausted treadmill running by using Marra project. Group B, C, D conducted quantitative treadmill running (Its duration was determined based on test results of the exhausted treadmill running). Gastrocnemius muscle of hindlimb of group A, B, C, D was taken after 3 h of exercise, detected 3-NT, 8-OHdG value of skeletal muscle using Elisa method and measured MDA, SOD, GSH, T-AOC and level of O2-, H2O2 and •OH using chemical colorimetry.

Index testing:

Weight of rats: Weigh and record the weight of all rats immediately after rats were bought considering as the observed value of weight before the experiment. Weigh at the same time in the every morning before and after the exercise as the basis of dosage of the intraperitoneal injection of hydrogen water. Weigh the last time immediately after exhaustive exercise as the observed value of weight after the experiment. Compare weight of rats before and after the experiment.

Exercise duration of rats: According to exhaustion standard of treadmill running, if the animal can't stick to the original running speed at the end of exercise and strand the 1/3 track for more than 3 times, the stimulation and drive were confirmed as invalid, signs after stopping running characterized by polypnea, tired, ventral decubitus, sluggish reaction to stimulation, which determined the rats exhausted. Record the exercise duration of rats to exhausted.

Conduct tests of protein content: 3-NT, MDA, 8-OHdG, SOD activity and GSH content, T-AOC, anti O2-activity, H2O2 content, inhibition ability of •OH according to operating instructions in kit.

Table 1: Groups of rats

Group	Number	Exertion factors (Treatment)
Quiet control group (EC, group A)	10	Without exertion factors
Exercise control group (EE, group B)	10	Intraperitoneal injection of normal saline with 10 mL/kg before 1 min of exercise
Hydrogen water injection before exercise group (EH, group C)	10	Intraperitoneal injection of hydrogen water with 10 mL/kg before 1 min of exercise
Joint hydrogen water injections before and after exercise group (EM, group D)	10	Intraperitoneal injection of hydrogen water with 10 mL/kg before and after 1 min of exercise respectively

Table 2: The influence of weight of rats in each group

Group	Number	Weight before the experiment (g)	Weight after the experiment (g)
EC	10	389.4±33.4	396.9±28.4
EE	10	390.7±28.3	391.6±34.4
EH	10	390.4±30.2	387.7±29.9
EM	10	390.8±29.8	388.6±42.3

Table 3: Comparison of exercise duration of rats in each group

Group	Number	Exercise duration (min)	Rate of Change (%)
EE	10	47.3±10.8	
EH	10	60.7±9.4☆☆	28.3
EM	10	58.9±7.8☆	24.5

☆: Expresses that there is significant difference compared with exercise control group ($p<0.05$); ☆☆: Expresses that there is very significant difference compared with exercise control group ($p<0.01$); similarly hereinafter

RESULTS AND DISCUSSION

The influence of hydrogen water on exercise capacity in rats after acute exhaustive exercise: Weight of rats in each group had no obvious difference ($p>0.05$) before experiment by observing and analyzing it before and after experiment; weight in EH and EM group had slight decline without obvious significance ($p>0.05$) compared with EC and EE group after the experiment, which showed that the influence of hydrogen water on weight of rats was not significant in the short term, please refer to Table 2.

The results explained that the time of exercise to exhaustion in EH and EM group extended significantly compared with EE group, 28.3 and 24.5%, respectively through observing the influence of hydrogen water on exercise duration of acute exhaustive exercise in rats. By t test, there was significant difference between EH, EM group and EE group ($p<0.01$), which indicated that the injection of hydrogen water before exercise and joint injection of hydrogen water before and after the exercise can significantly extend the duration of exercise and improve sports ability, which had obvious anti-fatigue effect. EH, EM group had no significant difference ($p>0.05$) and the comparison was shown in Table 3.

Weight is an index that reflects the development degree and obesity degree of the body's bones and muscles, the change of which can reflect the body's nutritional status and muscle's development situation, also measure the body's growth and health. Duration of exhaustive exercise is the commonly used indicator that reflects the sport ability and the improvement of sport ability is the strongest macro reflection of the enhancement of body's anti-fatigue ability. Study showed that the decline of weight in rats in EH and EM group had no significant difference, which indicated that hydrogen water had no significant effect on weight of rats after exhaustive exercise. It might be caused by short experimental period by analyzing its reason. In addition, the research also showed that EE group had relatively short exercise duration; exercise duration in EH and EM group were significantly prolonged compared with in EE group. It might be that rats produced a large number of free radicals after acute exhaustive exercise by analyzing the causes, which not only can cause oxidative damage of cell membrane, but also can react with biomacromolecules, such as lipid, protein, nucleic acid, etc, affecting the normal biological functions of cells and inducing fatigue (Xiong, 2010). Supplement of hydrogen water helped to delay the occurrence of fatigue and enhance the sports ability. It might be the reason that the antioxidant effect of hydrogen water inhibited generation of free radicals in the exercise tissue, thus improving the endogenous antioxidant enzyme activity of skeletal muscle in rats, reducing the lipid peroxidation of skeletal muscle and sports injuries, eventually delaying fatigue and improving the effect of exercise capacity (Li *et al*., 2011).

The influence of hydrogen water on oxidative stress injury of skeletal muscle in rats after acute heavy intensive sports: 3-NT, MDA, 8-OHdG in group B rose significantly ($p<0.01$) compared with group A; and 3-NT in group C and A had no obvious difference ($p>0.05$). The significance of MDA was lower than group A ($p<0.05$), but the significance of 8-OHdG concentration was higher than group A ($p<0.05$), the rest in group D was lower than group A except 3-NT ($p<0.05$), MDA and 8-OHdG had no significant difference compared with group C ($p>0.05$); 3-NT and MDA in group C declined significantly ($p<0.01$) compared with group B, 8-OHdG decreased notably and had significant difference ($p<0.05$); 3-NT, MDA, 8-OHdG in group D declined significantly, especially MDA and 8-OHdG had great significant difference ($p<0.05$, $p<0.01$, $p<0.01$). Compared with group C, 3-NT in group D decreased notably and had significant difference; MDA increased slightly while 8-OHdG decreased, but there was no obvious difference ($p<0.01$, $p>0.05$, $p>0.05$). 3-NT, MDA, 8-OHdG were the trademark products of protein oxidation, lipid peroxidation damage and DNA oxidative stress injury, respectively. The above results showed that the acute heavy intensive exercise caused the damage of protein in skeletal muscle, DNA and lipid peroxidation stress; hydrogen water had significant protective effect, which was remarkable especially in joint injection of hydrogen water before and after exercise group as shown in Table 4.

Oxidative stresses refer to the generation and increase of body tissue or oxygen free radicals in cells and/or reduce of clear ability, leading to the process of oxidative damage caused by the accumulation of active oxygen in the body or cell. Oxygen free radicals in body have instability and high chemical reactivity, which can attack biological molecules such as proteins,

Table 4: Comparison of oxidative damage of skeletal muscle in rats in each group

Group	n	3-NT (nmol/L)	MDA (nmol/mgprot)	8-OHdG (pg/mL)
A	10	47.76±21.35	0.92±0.20	12.06±13.24
B	10	79.82±20.53**	1.27±0.21**	36.55±9.32**
C	10	58.73±24.01☆	0.76±0.13*☆☆	25.02±10.21*☆
D	10	31.87±10.03*☆☆# #	0.83±0.25☆☆	17.97±6.23☆☆

Compared with quiet control group: *p<0.05, **p<0.01; compared with exercise control group; ☆: p<0.05, ☆☆: p<0.01; compared with injection of hydrogen water before exercise group: #p<0.05, ##p<0.01, similarly hereinafter

Table 5: Comparison of pointer of antioxidant defense system of skeletal muscle in rats in each group

Group	n	SOD (U/mgprot)	GSH (mgGSH/gprot)	T-AOC (U/mgprot)
A	10	56.42±3.15	0.75±0.01	0.58±0.12
B	10	53.13±2.96*	0.65±0.07**	0.42±0.11**
C	10	57.80±3.07☆☆	1.07±0.05**☆☆	0.77±0.10**☆☆

Compared with quiet control group: *p<0.05, ** p<0.01; compared with exercise control group: ☆: p<0.05, ☆☆: p<0.01

lipids and DNA and form a series of oxidative damage products (Li *et al*., 2011). In the field of sports science research, MDA is a commonly used indicator for preventing oxidative damage in sports and the study on 3-NT, 8-OHdG is very little. A large number of studies have shown that exhaustive exercise will affect free metabolism of skeletal muscle (Li, 2012; Yang *et al*., 2009; Sun *et al*., 2013; Zu and Zhu, 2013; Li *et al*., 2010), which embody in that heavy intensive exhaustive exercise for a long time causes the imbalance of free radical in oxidation system; endogenous free radicals of multiple tissues in body increase, especially skeletal muscles and exhaustive exercise is positively related to exercise intensity and duration closely. This experimental study shows that exhaustive exercise plays increasing role in the generation of free radicals of skeletal muscle in the body, which leads to protein oxidation of skeletal muscle, lipid peroxidation and DNA damage in rat; hydrogen water can reduce the above damage and protect oxidative stress injure of skeletal muscle. Its protection has correlation to the generation of free radicals caused by hydrogen water inhibiting acute strenuous exercise (Li, 2012).

The influence of hydrogen water on antioxidant defense system of skeletal muscle in rats after acute heavy intensive exercise: Compared with group A, SOD activity in group B declined sharply and group C had no obvious difference and the significance of group D was higher than group A (p<0.05, p>0.05, p<0.01); moreover, GSH content in group B decreased significantly and the significance of group C and D was higher than group A (p<0.01); in addition, T-AOC in group B declined while in group C and D increased obviously and has great significant difference (p<0.01); compared with group B, SOD in group C and D was higher than group B (p<0.01), GSH content rose significantly (p<0.01) and the rate of change of GSH content was +44.6%, +81.5%, respectively; furthermore, the rate of change of T-AOC in group C and D reached +83.3%, +145.2%, all had great significant difference (p<0.01) and group D was higher than group C (p<0.01); compared with group C, the significance of SOD in group D was higher than group C (p<0.05), the significance of GSH content was higher than group C (p<0.01) and with the rate of change of +25.5%. The above results explained that SOD activity, GSH content and T-AOC of skeletal muscle after acute heavy intensive exercise declined significantly and hydrogen water can improve SOD activity, GSH content and T-AOC of skeletal muscle, which had protective effect on exercise-induced oxidative stress injury of skeletal muscle in rats and joint injection of hydrogen water before and after exercise had significant effect and meaning on improving the ability of antioxidant defense system. Please refer to Table 5.

Body's antioxidant capacity depends on the dynamic balance of degree of lipid peroxidation and antioxidant protection, most of the studies on the body's antioxidant ability are based on the determination of certain antioxidant content of tissue or blood currently, for example, SOD and GSH levels and the antioxidant ability is reflected by them. SOD is an indispensable enzyme in the human body that has the special biological activity, which plays an important role in the balance of oxidation and anti-oxidant of the body and its change can indirectly reflect the generation of free radicals and metabolism of substance in the body. GSH as a kind of low molecular free-radical scavenger is exogenous and endogenous antioxidant, which can be absorbed by small intestine and synthesized in the body. Because the changes of single component of SOD and GSH do not indicate that the change of antioxidant ability and lipid peroxidation damage has occurred and cannot make a comprehensive evaluation on the body's antioxidant system together with complete antioxidant capacity. This study also adopted the T-AOC as evaluation index of reflecting the body's total antioxidant and scavenging the ability of free radicals, except SOD and GSH. The research showed that the content of SOD and GSH of skeletal muscle in group B after one-time exhaustive exercise declined and the reason might be related to the increased release of glucagon, catecholamine and vasopressin in exercise. Drop of T-AOC might be related to the occurrence of ROS high level in exercise, change of blood flow and decline of the energy supply and energy utilization

Table 6: Comparison of change of ROS of skeletal muscle in rats in each group

		O2-		H2O2		•OH	
Group	n	Anti O2 activity (U/g prot)	Inhibition ratio (%)	Content (mmol/g prot)	Rate of change (%)	Inhibiting ability of •OH (U/mg prot)	Inhibition ratio (%)
A	10	121.89±10.01	36.91	6.53±0.65		58.92±7.54	79.74
B	10	47.19±5.38**	9.13	7.26±2.10		38.79±7.54**	17.39
C	10	116.70±12.15☆☆	32.51	5.35±0.80☆☆	-26.31	56.65±3.88☆☆	73.52
D	10	113.86±7.59☆☆	32.16	4.86±0.71☆☆	-33.06	60.40±1.87☆☆	83.33

Compared with quiet control group: *p<0.05, ** p<0.01; compared with exercise control group: ☆: p<0.05, ☆☆: p<0.01

ability. The rise of SOD, GSH and T-AOC in group H and M might be related to the characteristics of hydrogen. The hydrogen has the characteristics of low density, small molecules and easy to spread and it also can effectively improve the microcirculation and reduce the microcirculation impedance; thus it increases oxygen supply, reduces the production of free radicals and enhances the SOD activity of the body, increases the GSH content and also improves the total antioxidant capacity of skeletal muscle, decreases oxidative stress and improves exercise capacity. On the other hand, energy metabolism is the source of antioxidant, thus hydrogen water may be involved in energy metabolism, affecting the generation of NADPH oxidase, which plays a signalization role (Li, 2012; Sun *et al*., 2013, 2014).

Selective antioxidation of hydrogen water: Compared with group A, anti O2- activity in group B declined significantly (p<0.01), inhibition ratio reduced to 9.13 from 36.91%, respectively anti O2- activity in group C and D had no obvious difference (p>0.05); H2O2 content in group B showed a trend of increase, H2O2 content in group C and D showed a trend of decrease and they all had no obvious difference (p>0.05); furthermore, inhibiting ability of •OH in group B decreased sharply (p<0.01), inhibition ratio reduced to 17.39 from 79.74%, respectively inhibiting ability of •OH in group C showed a trend of decrease, inhibiting ability of •OH in group D showed a trend of increase and they all had no obvious difference (p>0.05); compared with group B, anti O2- activity in group C and D increased obviously (p<0.01), inhibition ratios were 32.51 and 32.16%, respectively and H2O2 content reduced sharply by 26.31 and 33.06%, respectively, there was great significant difference (p<0.01); in addition, inhibiting ability in group C and D increased obviously (p<0.01), inhibition ratio reached 73.52 and 83.33% respectively; the changes of anti O2- activity, H2O2 content and inhibiting ability of •OH in group D had no significant difference compared with group C (p>0.05). Please refer to Table 6.

Free radical is atoms, radical or molecules containing unpaired electrons, which has two sides. On the one hand, it is the necessary material to maintain normal life and the basis of energy metabolism, part of the free radical is important signal molecule in the cell. On the other hand, it is also the dangerous killer of biological macromolecules and cells. O2- is the main source of ROS in biological body, the free radical firstly generated by oxygen metabolism in the biological body and also predecessor of all OFR. H2O2 is one of the metabolism product of organisms, the most important product of reaction of human respiratory chain. •OH is a free radical with extreme damage effects known as invasiveness and is the most active ROS with strongest toxicity. The results of the study showed that hydrogen water can inhibit and prevent lipid free radicals and •OH and involve in regulating and improving ability of antioxidant system of SOD, GSH, TAOC of skeletal muscle, so as to ease the protein damage of skeletal muscle, lipid peroxidation damage and DNA damage.

CONCLUSION

The study researches the effect of hydrogen water on protein oxidative damage of skeletal muscle, lipid peroxidation damage, DNA damage, the antioxidant defense system and its selective antioxidant effect from the perspective of the skeletal muscle by adopting the animal experiments of exhaustive exercise and acute heavy intensive exercise. Study confirms that hydrogen water has anti-fatigue effect, which plays a protective role in exercise-induced oxidative stress injury. Its protective effect is conducted through the multiple targets on the basis of the selective antioxidant effect of hydrogen water. The research explores function and mechanism of selective antioxidant effect of hydrogen water on oxidative damage of athletes from its perspective and establishes its protective effect on exercise-induced oxidative stress injury, which provides reference basis for prevention of exercise-induced oxidative damage and research and development of beverage in the future.

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