

Original Article

Effect of haematococcus pluvialis on exercise-related renal ischemia-reperfusion injury and ECM expression

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Abstract: This paper was to study the effect of haematococcus pluvialis on exercise-related renal ischemia reperfusion injury, rat renal tissue inflammatory factors and ECM expression. In the experiment, the rats were provided with 450 mg/kg haematococcus pluvialis intragastric administration for 56 d and were given with incremental load swimming training additionally. The glomerular ECM deposition and test serum urea nitrogen, creatinine, renal tissue inflammatory factor protein, gene expression, TGF- β 1 gene expression and other biochemical indicators were observed after 24 h of the last exercise. The results showed that the rats suffered from exercise-related renal ischemia reperfusion, reinforcement of ECM deposition and renal function injury after 8 weeks' overtraining. Of glomerulus ECM deposition, it showed no significant difference ($P > 0.05$) among the quiet control group (Group C), general training group (Group M) and overtraining group (Group OM). Compared with Group C and Group M, overtraining group (Group OM) significantly increased ($P < 0.01$); haematococcus pluvialis+ overtraining group (Group HM) was significantly lower than that in Group OM ($P < 0.05$). (2) With respect to serum urea nitrogen and creatinine level, OM group and HM group were significantly higher than those of Group C ($P < 0.01$) and HM group was significantly lower than that in the OM group ($P < 0.05$); as for renal tissue TNF α , IL-1 β , IL-6 and IL-18 protein expression, the OM group and HM group were significantly higher than those of Group C ($P < 0.01$) and HM group was significantly lower than that in the OM group ($P < 0.05$); as for renal tissue TNF- α mRNA and IL-1 β mRNA and IL-6 mRNA and IL-18 mRNA expression, OM group and HM group were significantly higher than those of group C ($P < 0.01$) and HM group was significantly lower than that in the OM group ($P < 0.05$); as far as renal tissue TGF- β 1 mRNA expression was concerned, HM group ($P < 0.05$) and OM group ($P < 0.01$) were significantly higher than those of C group and HM group was significantly lower than that in the OM group ($P < 0.05$). It is demonstrated that Haematococcus pluvialis can effectively inhibit inflammatory factor expression in the renal tissue so as to reduce kidney tissue TGF- β 1 expression in case of exercise-related renal ischemia reperfusion induced by the overtraining, maintain ECM homeostasis and delay or avoid the damage to the kidney.

Keywords: Haematococcus pluvialis, exercise-related renal ischemia reperfusion injury, inflammatory factor, TGF- β 1, extracellular matrix

Introduction

As a high perfusion organ, kidney is sensitive to ischemia and ischemia reperfusion. The initial study shows that the rats may suffer from exercise-related renal ischemia-reperfusion injury caused by long-time and high-intensity training. (exercise-related renal ischemia-reperfusion injury, ERRIRI) [1-4]. Inflammatory factor expression caused by the renal ischemia reperfusion injury can promote the proliferation of renal inherent cells and stimulate the expression adhesion molecules and generate surplus ex-

tracellular matrix (extracellular matrix, ECM), thereby it breaks the homeostasis of the renal extracellular matrix metabolism and directly impact the changes of tissue cell structure and function [5]. Natural astaxanthin widely survives in nature and it is the highest level product synthesized by carotenoid, with the special structure. At home and abroad, a large number of studies have confirmed that astaxanthin has strong antioxidant activity, but also it can improve the immunity, prevent occurrence and development of liver and kidney injury, tumor, cardiovascular disease and other diseases and

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delay aging [6]. Because of its multi-target and multi-pathway, without toxic and side-effect and stimulants, it has been applied in sports medicine and sports nutrition field from time to time. Haematococcus is an algae food with rich nutritional value and medicinal value in the scientific field after the spirulina and chlorella. Haematococcus pluvialis is recognized as the best biology for producing natural astaxanthin in the nature. Astaxanthin content is 1.5%~10.0% and it is seen as the natural astaxanthin concentration. In this experiment, it intends to observe effect of Haematococcus pluvialis on renal tissue TGF- β 1 gene expression, inflammatory cell factor protein and gene expression, its role in the renal metabolism and ECM deposition adjustment and then study its protection effect and mechanism in the exercise-related renal ischemia reperfusion injury caused by the overtraining.

Materials and instruments

Experiment animals

Grade-SPF 75 male Wistar rats, which are 49 days old and their weight is 222.1 ± 10.9 g, are provided by the Experimental Animal Science Department of Peking University Health Science Center. Animal production certificate number: SCXK (Jing) 2006-0008. In the whole experiment, the temperature of the laboratory is kept at $(22 \pm 2)^{\circ}\text{C}$; the relative humidity is 55%~75% and the light time changes with the natural change. Rats are fed with basic feed (provided by the Experimental Animal Science Department of Peking University Health Science Center) and the distilled water as their routine feeding, additionally, they are free diet. The experimental time lasts for 63 days and the official training lasts for 56 days. Animal experiment is finished in the sports nutrition laboratory of Beijing Sport University.

Trial medication

Haematococcus pluvialis (Haematococcus pluvialis) is given by Shaanxi's natural products co., LTD. Haematococcus pluvialis is broken to pieces by the homogenizer and prepared to the desired concentration with sterile distilled water and stored at 4°C for use.

Instruments

CM-2000B biomedical image analysis system (Beijing University of Aeronautics and Astron-

autics), BS224S type electronic analysis balance (German Sartorius); optical microscope (Japan Olympus); ALCYON300 automatic biochemical analyzer (USA Abbott Laboratories); LKB-V ultrathin flaker (Swedish LKB company).

Animal grouping

After the rats are provided with adaptability feeding for 4 days, they do 20 minutes/day exercise for 3 days. Then eliminate the individual rats that do not adapt to swim and reject rats that do not conform to the experimental requirements. The remaining rats are divided into 4 groups by the digital random grouping method: a quiet control group (C group, 12 rats), general training group (group M, 12 rats), overtraining group (OM group, 24 rats) and Haematococcus pluvialis +overtraining group (HM group, 24 rats) [1-4]. The professional gavage device is used to feed rats once a day. Haematococcus pluvialis intervention group dose is 450 mg/kg (pre-experiment to obtain the optimal dose) but other group rats are fed with equal-volume sterile distilled water.

Training and testing programs: Quiet control group is fed ordinarily, without exercise and intervention. The general training group is provided with the moderate intensity swimming training for 8 weeks (without load) 6 days a week and once a day. At the first time, they swim in the water for 20 minutes and then gradually increase the time, for example, they swim for 60 minutes a day at the first weekend. At the second weekend, the swimming time is increased to 90 minutes a day; at the third weekend, it is increased to 120 minutes a day. In the sequent 5 weeks, maintain the exercise intensity. In the first 3 weeks, the training time of overtraining group and Haematococcus pluvialis intervention group is same with that of the general training group. From the fourth week, high intensity training is arranged for overtraining group and Haematococcus pluvialis intervention group. From the first week to the third week, their weight is reduced by 0.5%; from the fourth week, its weight is reduced by 1% and from the fifth week, its weight is reduced by 2%. Training is provided once a day. From the sixth week, its weight is reduced by 2% and training is provided once at every morning and afternoon respectively. From the seventh to eighth week, training is provided once at every morning, afternoon and night respectively. After that, its weight is reduced by 5%

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[1-4]. The rats swim with loads each time until they are exhausted. The standard of exhaustion is that rats don't float on the water surface after it is sunk for 10 s. Rats of quiet control group and general training group grow normally, without accidental death. Some rats of overtraining group and *Haematococcus pluvialis* intervention group, are incapable of recovering in time due to loads in their tail, fatigue and exhaustion and then accidental death. At the eighth week-end, only 11 rats survive among 24 rats in the overtraining group and only 14 rats among 24 rats in the *Haematococcus pluvialis* intervention group.

Index determination: After 24 hours of the last swimming training, rats are adequately anaesthetized by ether and then blood is taken from the carotid artery and sodium citrate solution is added for anticoagulation. Blood is given with the water bath for 30 min at 37°C and then is centrifuged for 10 min at the speed of 3000 r/min at 4°C. Blood is separated for preparing the serum and stored in -20°C refrigerator for inspection. Rapidly remove two kidneys and fascia and place them in the pre-cooled saline to wash bloodstain. Observe the renal size, color and texture. Cut the renal tissue and then pack with a sterile aluminum foil and quickly place in liquid nitrogen for temporary storage. Later, store it in -70°C refrigerator for freezing and measurement [4]. Use Jaffe picric acid method to determine serum creatinine, diacetyl monoxime method to determine serum urea nitrogen and immunohistochemical method to determine renal tissue TNF- α , IL-1 β , IL-6 and IL-18 protein expression. Meanwhile, use RT-PCR method to determine the renal tissue TNF- α , IL-1 β , IL-6, IL-18 and TGF- β 1 gene expression. The above kits are provided by Shanghai Hengyuan Biological Technology Co., Ltd. The above indicators shall be determined in strict accordance with the kit instructions. For calculation formula and other details, see the kit instructions.

PAS staining section image analysis: Take a part of kidney tissues and it is fixed by 10% formalin and embedded by paraffin. Cut 4 μ m thick section and carry out PAS staining and histopathological analysis. Collect and cut the glomerulus of urinary pole and blood vessel pole in PAS stained sections by BUAA image acquisition module software at 400 \times objective lens (Each section has about 8-10 glomerulus).

Then adopt Beihang University medical pathological image analysis software to describe the glomerulus capillary contour, distinguish matrix and cellular components and measure the average area of a single glomerulus and its matrix area.

Semi-quantitative score evaluation of immunohistochemistry results: Semi-quantitative score evaluation of immunohistochemistry results are based on the staining degree and scope. It is 0 score if it is not colored basically; 1 score in case of light coloring; 2 scores in case of obvious coloring; 3 scores in case of deep coloring; for the coloring scope, see Riera [7] and other standards. It is 0 score if it is negative. It is 1 score if coloring scope $\leq 25\%$; 2 scores if coloring scope $> 25\% \leq 50\%$; 3 scores if coloring scope $> 50\% \leq 75\%$; 4 scores if coloring scope $> 75\%$. Multiply the staining degree of each section by its scope to obtain its final score. If it is ≤ 1 , it is negative (-); it is weakly positive (+) if $> 1 \text{ score} \leq 3 \text{ scores}$; it is positive (++) if $> 3 \text{ scores} \leq 5 \text{ scores}$; it is strongly positive (+++) if $> 5 \text{ scores}$. Semi-quantitative scores of immunohistochemistry results are calculated by two technicians in a way of the double blind and then their average value is taken.

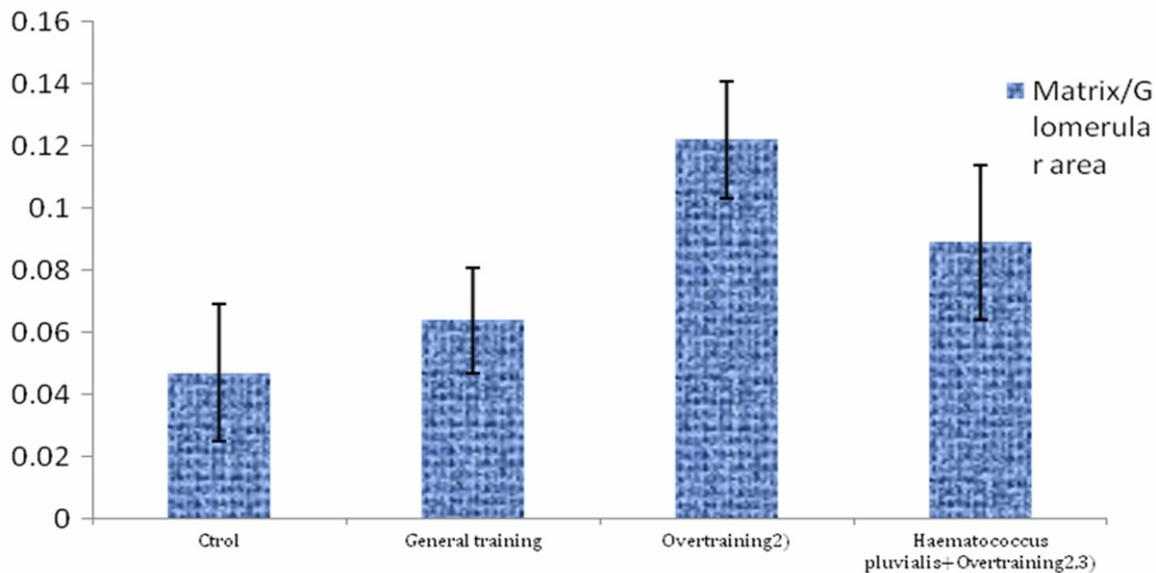
PCR product analysis: According to the requirements of Trizol kit instructions, the total RNA of the rats' renal tissues was extracted. Then cDNA is synthesized according to the requirements of the reverse transcription amplification kit. According to the PCR amplification conditions of different indicators, amplify corresponding PCR. The amplified product is given with electrophoresis (stable voltage 5 V/cm) by 2% agarose gel for 1 h. After it is stained by 0.5 μ g/ml ethidium bromide for 20 min, observe it under the ultraviolet lamp. Use UVP gel imaging system to scan and analyze the electrophoresis results. Use the ratio of amplification belt gray and GAPDH amplification gray to carry out semi quantitative analysis for mRNA expression subject to measurement.

Data statistics

Use SPSS12.0 software to analyze and process all of the data. The data is expressed by average value \pm standard difference ($\bar{x} \pm s$). Adopt mean variance to analyze the inter-group difference. Rank count data is analyzed by rank test and the correlation analyzed by Pearson corre-

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Table 1. Glomerular ECM deposition of rats in all groups



Note: ¹ $P < 0.05$, ² $P < 0.01$, compare with the quiet control group, ³ $P < 0.05$, ⁴ $P < 0.01$, compare with overtraining group.

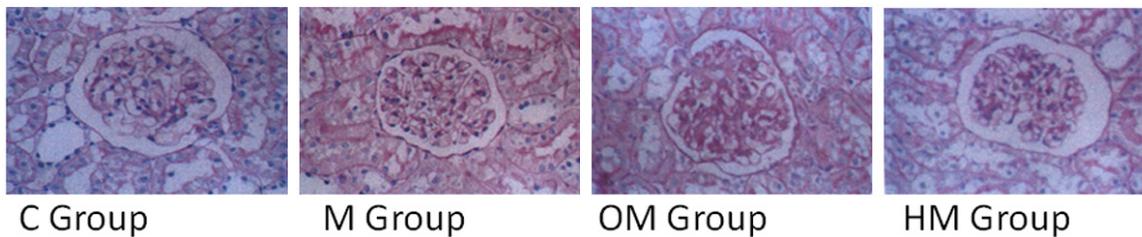


Figure 1. The glomerular ECM deposition (PAS, $\times 400$) of four groups.

Table 2. Comparison of serum urea nitrogen and creatinine levels of rats in all groups

Group	n	BUN (mmol/L)	SCr ($\mu\text{mol/L}$)
Ctrl	12	8.41 \pm 1.03	31.29 \pm 6.01
General training	12	9.25 \pm 0.35	36.60 \pm 8.04
Overtraining	11	14.29 \pm 1.96 ²⁾	81.21 \pm 15.75 ²⁾
Haematococcus pluvialis+Overtraining	14	11.93 \pm 1.45 ^{2,3)}	53.21 \pm 12.63 ^{2,3)}

Note: ¹ $P < 0.05$, ² $P < 0.01$, compare with control group, ³ $P < 0.05$, ⁴ $P < 0.01$, compare with overtraining group.

lation. The significance level $P < 0.05$ and the extraordinary significance level $P < 0.01$.

Results

Renal tissue PAS staining

Use renal tissue PAS stained section to analyze glomerular ECM deposition. Glomerular ECM deposition of rats in the quiet control group and

the general training group shows no significant difference ($P > 0.05$); compared with the quiet control group, glomerular ECM deposition of rats in the overtraining is increased significantly ($P < 0.01$); Glomerular ECM deposition of rats in Haematococcus pluvialis intervention group

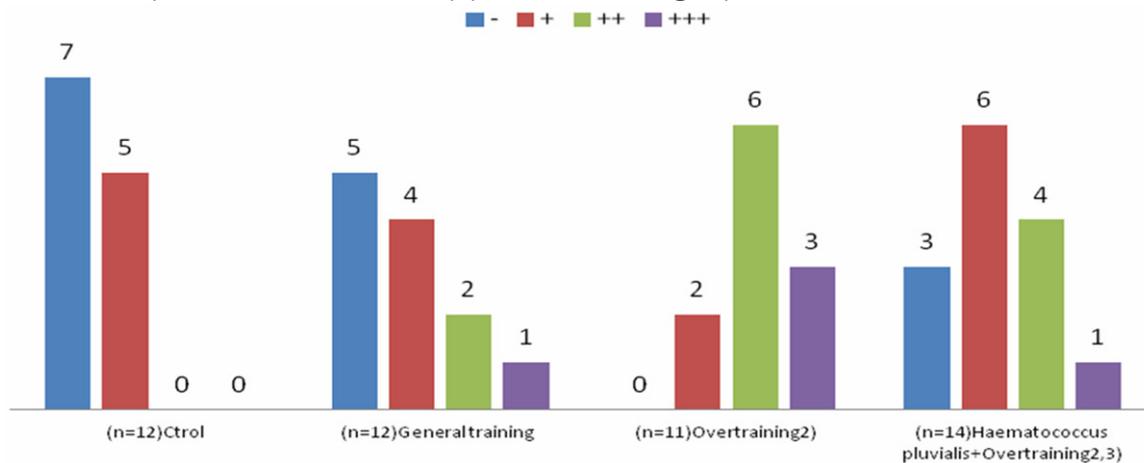
is significantly lower than that of the overtraining group ($P < 0.05$) (See **Table 1** and **Figure 1**).

Serum urea nitrogen and creatinine

Table 2 shows serum urea nitrogen and creatinine levels and there is no significant difference between the quiet control group and the general training group ($P > 0.05$). The general training group and Haematococcus pluvialis inter-

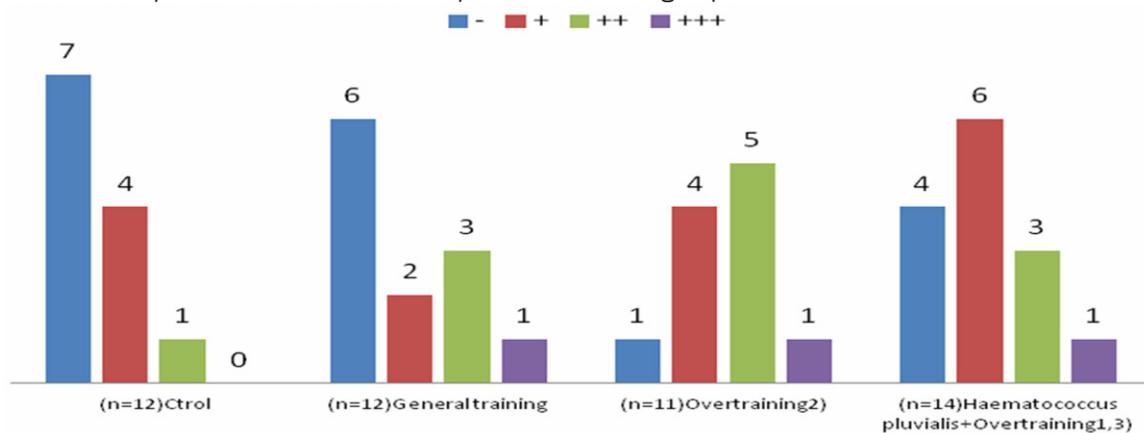
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Table 3. Comparison of renal tissue IL-1 β protein level in all groups



Note: ¹ $P < 0.05$, ² $P < 0.01$, compare with control group, ³ $P < 0.05$, ⁴ $P < 0.01$, compare with overtraining group.

Table 4. Comparison of renal tissue IL-6 protein level in all groups



Note: ¹ $P < 0.05$, ² $P < 0.01$, compare with control group, ³ $P < 0.05$, ⁴ $P < 0.01$, compare with overtraining group.

vention group are significantly higher than the quiet control group ($P < 0.01$); Haematococcus pluvialis intervention group is significantly lower than the overtraining group ($P < 0.05$).

IL-1 β , IL-6, TNF- α , IL-18 protein levels of renal tissue

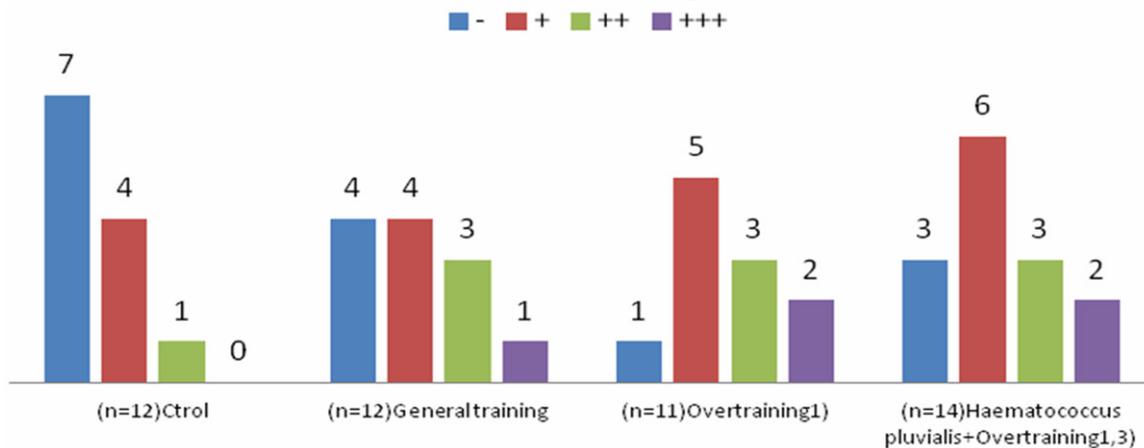
IL-1 β protein level: Table 3 shows that IL-1 β level of the renal tissue of the rats in the quiet control group and the general training group is only mildly expressed and there is no significant difference between the groups ($P > 0.05$). The other two groups show significant difference from the quiet control group ($P < 0.01$). Positive expression of the overtraining group is strong but its expression of haematococcus pluvialis intervention group is reduced in comparison with it ($P < 0.05$).

IL-6 protein level: Table 4 shows that IL-6 protein level of the renal tissue of the rats in the quiet control group and the general training group is only mildly expressed and there is no significant difference between the groups ($P > 0.05$). The other two groups show significant difference from the quiet control group (respectively $P < 0.01$ and $P < 0.05$). Positive expression of the overtraining group is strong but its expression of haematococcus pluvialis intervention group is reduced in comparison with it ($P < 0.05$).

TNF- α protein level: Table 5 shows that TNF- α protein level of the renal tissue of the rats in the quiet control group and the general training group is only mildly expressed and there is no significant difference between the groups ($P > 0.05$). The other two groups show significant

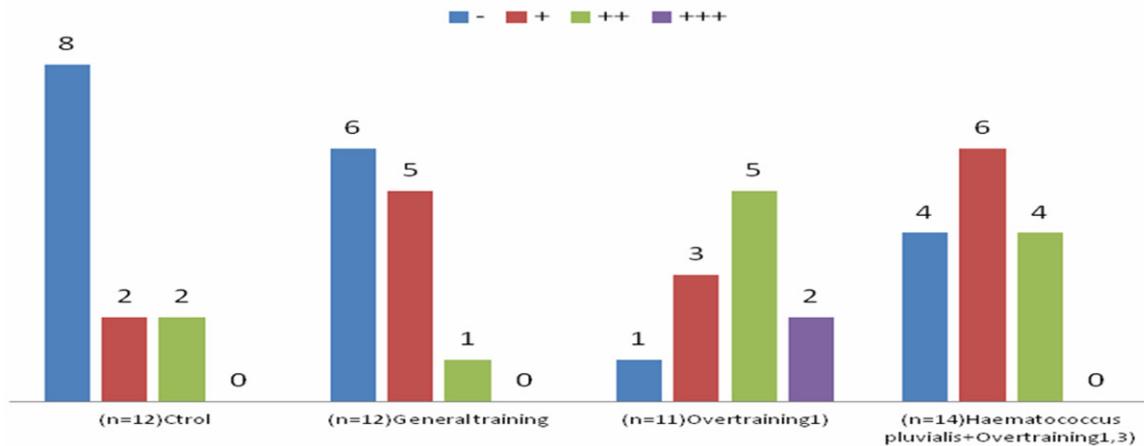
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Table 5. Comparison of renal tissue TNF- α protein level in all groups



Note: ¹ $P < 0.05$, ² $P < 0.01$, compare with control group, ³ $P < 0.05$, ⁴ $P < 0.01$, compare with overtraining group.

Table 6. Comparison of renal tissue IL-18 protein level in all groups



Note: ¹ $P < 0.05$, ² $P < 0.01$, compare with control group, ³ $P < 0.05$, ⁴ $P < 0.01$, compare with overtraining group.

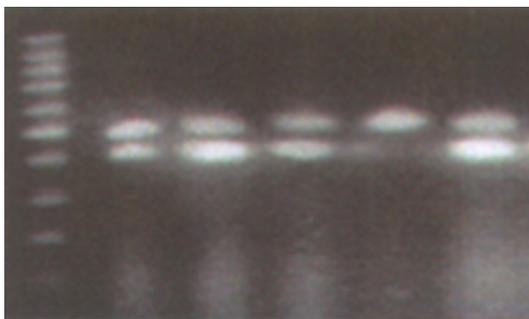


Figure 2. IL-1 β genetic expression of livers of the groups of rats.

difference from the quiet control group ($P < 0.05$). Positive expression of the overtraining group is strong but its expression of haematococcus pluvialis intervention group is reduced in comparison with it ($P < 0.05$).

IL-18 protein level: **Table 6** shows that IL-18 protein level of the renal tissue of the rats in the quiet control group and the general training group is only mildly expressed and there is no significant difference between the groups ($P > 0.05$). The other two groups show significant difference from the quiet control group ($P < 0.05$). Positive expression of the overtraining group is strong but its expression of haematococcus pluvialis intervention group is reduced in comparison with it ($P < 0.05$).

Renal tissue IL-1 β mRNA, IL-6 mRNA, TNF- α mRNA and IL-18 mRNA relative expression level

Table 7 and **Figures 2-5** show that renal tissue IL-1 β mRNA, IL-6 mRNA, TNF- α mRNA and IL-18 mRNA expression levels have no significant dif-

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Table 7. Comparison of renal tissue IL-1 β mRNA, IL-6, TNF- α mRNA and IL-18 mRNA relative expression levels

Group	n	IL-1 β (GAPDH)	IL-6 (GAPDH)	TNF- α (GAPDH)	IL-18 (GAPDH)
Ctrol	12	0.206 \pm 0.061	0.317 \pm 0.061	0.231 \pm 0.079	0.354 \pm 0.079
General training	12	0.241 \pm 0.053	0.431 \pm 0.041	0.253 \pm 0.068	0.401 \pm 0.049
Overtraining	11	0.845 \pm 0.128 ²⁾	0.969 \pm 0.192 ²⁾	0.945 \pm 0.257 ²⁾	0.947 \pm 0.192 ²⁾
Haematococcus pluvialis+Overtraining	14	0.537 \pm 0.121 ^{2,3)}	0.671 \pm 0.178 ^{2,3)}	0.671 \pm 0.219 ^{2,3)}	0.691 \pm 0.151 ^{2,3)}

Note: ¹⁾ $P < 0.05$, ²⁾ $P < 0.01$, compare with control group, ³⁾ $P < 0.05$, ⁴⁾ $P < 0.01$, compare with overtraining group.

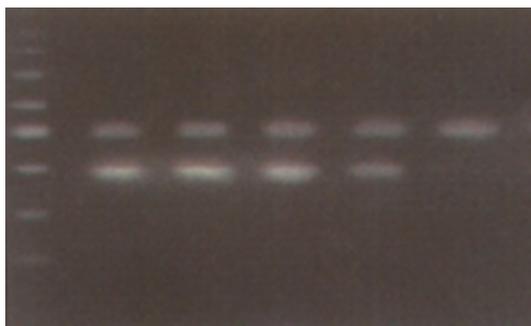


Figure 3. IL-6 genetic expression of livers of the groups of rats.

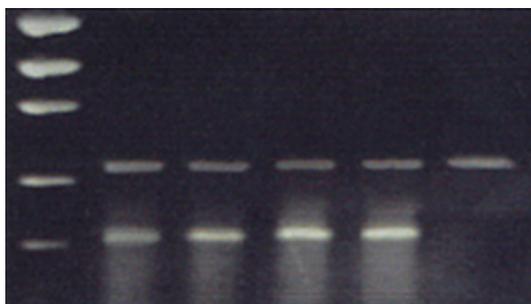


Figure 4. TNF- α genetic expression of livers of the groups of rats.



Figure 5. IL-18 genetic expression of livers of the groups of rats.

ference between the quiet control group and the general training group ($P > 0.05$). The other

two groups show significant difference from the quiet control group ($P < 0.05$). The expression of haematococcus pluvialis intervention group is reduced in comparison with the overtraining group ($P < 0.05$).

Relative expression level of TGF- β 1 mRNA in renal tissue

Table 8 and **Figure 6** show kidney tissue TGF- β 1 mRNA expression level. There is no significant difference between the quiet control group and the general training group ($P > 0.05$). The other two groups are significantly higher than the quiet control group (respectively, $P < 0.05$, $P < 0.01$). Haematococcus pluvialis intervention group is significantly lower than the overtraining group ($P < 0.05$).

Discussion

Glomerular ECM is a highly ordered dynamic network structure formed by various components, which is composed of collagen, elastin, polysaccharide and glycoprotein. ECM is mainly distributed around the cells so as to support and connect the cells, meanwhile, it plays an important role in maintaining cell nutrition, morphology, adhesion, proliferation, differentiation and normal organizational structure and function. As a component of the glomerular basement membrane, ECM plays an important role in ensuring the integrity of the glomerular filtration barrier. At the same time, it has a major part to play in protecting the structure and function of renal tubular epithelial cells and developing renal tubular interstitial fibrosis. Under normal condition, it faces the dynamic equilibrium between metabolic renewal and degradation remodeling from time to time. But in some pathological conditions, ECM quantitative and qualitative change can lead to micro environment change surrounding cells and directly affect cell function and organ morphology [8]. The mechanism and deposition pro-

Table 8. Comparison of renal tissue TGF-β1 mRNA relative expression level

Group	n	TGF-β1 mRNA (GAPDH)
Ctrol	12	0.821 ± 0.053
General training	12	0.921 ± 0.037
Overtraining	11	1.341 ± 0.015 ²⁾
Haematococcus pluvialis+Overtraining	14	1.069 ± 0.116 ^{1,3)}

Note: ¹⁾P < 0.05, ²⁾P < 0.01, compare with control group, ³⁾P < 0.05, ⁴⁾P < 0.01, compare with overtraining group.

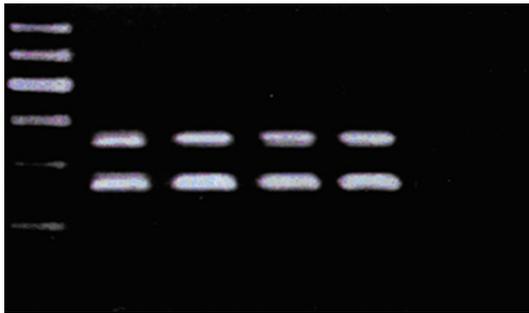


Figure 6. TGF-β1 genetic expression of livers of the groups of rats.

cess of ECM in the glomerular are complex, but the main mechanism is that ECM in the glomerular increases or (and) ECM degradation decreases [9].

As a multifunctional cytokine, TGF-β can regulate cell proliferation, differentiation and apoptosis through complex receptor signal transduction pathway on the cell surface in auto-crine and paracrine manner and it plays an important role in the development and formation of many tissues. Especially, TGF-β1 of three isomers is strongly expressed in the renal tissues. Studies show that as a ECM regulator, TGF-β1 controls ECM synthesis and degradation, expression of adhesion molecule receptors and integrin, thus it affects ECM reconstruction and its expression increase coincides with ECM deposition increase [10]. Currently, it is known that ECM content increase, induced by TGF-β1, can be observed in different renal cells, including glomerular epithelial cells, kidney tubular epithelial cells, mesangial cells and normal renal interstitial fibroblasts [11]. TGF-β1 can promote the increase of ECM content through three major pathways [12, 13]: First, promote the collagen synthesis increase by improving the expression of ECM components such as

Col-I, Col-III, Col-IV and FN; second, promote protease inhibitory factor expression (such as plasminogen activator plasminogen activator inhibitory factor and metalloproteinase inhibitory factor) through inhibiting ECM degradation, including inhibition of enzymes synthesis that can degrade ECM components. Third, promote integrin expression and then it can impact ECM expression. Also TGF-β1 has an important regulatory role in immune response. Compared with other factors, it has a stronger immune inhibition, that is, it can inhibit proliferation and differentiation of the immune active cell as well as generation of cytokine and antibody [14].

It has been studied that the immune response of rats could be induced after a long time and high-intensity training and then the inflammatory effect cells of kidney could be activated, as a result, it may release the inflammatory mediators and disorder the extracellular matrix metabolism. At the same time, inflammatory mediators and extracellular matrix may also react to the effect cells. The interaction response effect among inflammatory cells, inflammatory mediators and extracellular matrix results in and aggravates occurrence and development of renal ischemic reperfusion injury, consequently, the kidney tissue micro-structure changes [12, 13]. When rats suffer from renal ischemia reperfusion injury because of a long time and the great intensity training, the renal tissue TNF-α, IL-1β, IL-6, IL-18 levels is a positive correlation with serum creatinine and blood urea nitrogen, which can reflect the severity of the exercise-related renal ischemia reperfusion injury [1-3]. The study results show that: 8 weeks' incremental load training has resulted in the excessive exercise and the exercise-related renal ischemia reperfusion as well as increase of serum creatinine and blood urea nitrogen levels; increase of renal tissue inflammatory factor gene expression may cause the increase of protein expression. These changes aggravate the inflammatory response of renal tissue in renal ischemia reperfusion injury, resulting in the increase of TGF-β1 gene expression and ECM metabolic imbalance. The related indexes of the general training group are not significantly changed, which indicates that the moderate intensity training doesn't damage kidney function. In Haematococcus pluvialis

intervention group, the relevant inflammatory factor protein and gene expression of the rat renal tissue ($P < 0.05$) and TGF- β 1 gene expression ($P < 0.05$) are lower than those of the overtraining group rats; serum creatinine and blood urea nitrogen levels are decreased ($P < 0.05$) compared with those of the overtraining group; glomerular ECM deposition is significantly lower than that of the overtraining group rats ($P < 0.05$). It is supplemented that *Haematococcus pluvialis* can effectively inhibit renal tissue inflammatory cytokine expression induced by the overtraining-related renal ischemia reperfusion from the gene to the protein level, so as to decrease renal tissue TGF- β 1 expression, maintain the balance of the ECM metabolism, prevent and delay occurrence of renal dysfunction. The mechanism may be described as follows: (1) the oxygen free radical is closely related with inflammation and it is one of the important pathological mechanisms in occurrence and development of inflammation. When renal ischemia reperfusion occurs, xanthine dehydrogenase (XD) is changed to xanthine oxidase (XO), which leads to the excessive production of superoxide anion free radical. Owing to the shortage of metabolic substrates in endogenous free radical scavenging system, it may result in reducing free radical scavenging capacity and then increasing oxygen free radicals in the tissue cells and causing kidney tissue peroxidation injury. *Haematococcus pluvialis* contains rich natural astaxanthin. On the one hand, because astaxanthin structure end contains a long conjugated double bonds and its tail end contains unsaturated hydroxyl ketone, it can be combined with target cells in the kidney. The unpaired electrons at the end are combined with free radicals or reactive oxygen ion to accelerate clearance and quenching of free radicals and reactive oxygen induced by the renal ischemia reperfusion [15]. On the other hand, it can effectively improve the antioxidant ability of the body by enhancing the activity of antioxidant enzymes in the tissues. By the joint effect, it can effectively improve the balance adjustment ability of the oxidative/antioxidation system, maintain the dynamic balance of free oxygen, reduce lipid peroxidation, protect the integrity of the cell membrane, enhance defensive capability of enzymatic system and non-enzymatic system, thereby inhibit renal tissue cells to secrete a large number of inflammatory factors, which is induced by the overtraining-related oxidative stress increase. [16]

(2) As a potent immune promoting agent, astaxanthin can significantly enhance the body's local and systemic immune function, promote the generation of human immunoglobulin, enhance B cell vitality expression in the immune system, effectively reduce the DNA damage and enhance the body to destroy the invasion of exogenous pathogen so as to reduce renal ischemia and reperfusion inflammatory injury [17]. (3) Additionally, astaxanthin can effectively increase the anti-apoptotic gene bcl-2 expression and inhibit Pro-apoptotic gene Bax expression so that Bax/Bcl-2 expression tends to balance, resulting in inhibition of Caspase 3 activation [18]. (4) Matrix metalloproteinases (MMPs) and serine protease system/plasminogen activator (PAs) play an important role in ECM degradation. TGF- β 1 can effectively inhibit MMPs transcription [20]. Astaxanthin can effectively inhibit TGF- β 1 expression and then ensure that MMPs activity and accelerate ECM degradation.

By supplementing *Haematococcus pluvialis*, it can effectively inhibit inflammatory factor expression in the renal tissue so as to reduce the renal tissue TGF- β 1 expression in case of the exercise-related renal ischemia reperfusion induced by the overtraining, maintain ECM homeostasis, delay or avoid the damage to the kidney.

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Disclosure of conflict of interest

None.

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