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Effect of *Lycium barbarum* on Immune Function of Plateau Training Plain Middle and Long Distance Runners

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Abstract

During the altitude training at 2366 m altitude, the immunoglobulin and IL-6 of male athletes of middle and long distance running in the plain were measured. Taking Qinghai *Lycium barbarum* can increase immunoglobulin, CD8+ T lymphocyte and NK cell, reduce the decline of IL-6, CD3+, CD4+ T lymphocyte and CD4+/CD8+, which is beneficial to improve the immune ability of athletes and ensure the effect of altitude training.

Keywords

Lycium barbarum, Altitude Training, Athletes, Immune Function

1. Introduction

The influence of exercise on immune system function is restricted by many external factors. Heavy load and high-intensity sports training can inhibit immune function and improve the body's susceptibility to diseases, thus affecting the health level and sports ability of athletes (Wang & Gao, 2019). Hypoxic environment can cause the disorder and decline of immune function (Li et al., 2017; Pu et al., 2018), and the damage of immune function under hypoxic condition is more serious than that of pure hypoxic environment or simple exercise training (Jin, Hu, & Jin, 2015), while the dual stimulation of hypoxic environment and exercise load during high altitude training will cause more complex impact on immune system (Gao et al., 2018). Therefore, it is very important to take effective measures to prevent the decline of athletes' immune function during altitude training to ensure the training effect.

Lycium barbarum not only has rich biological activities, but also has no toxic and side effects. Its pharmacological effect is to affect the immune function of human body. It has the effects of hematopoiesis, enhancing immunity, antioxidant, anti hypoxia, anti-fatigue and so on (Wang et al., 2017). Studies have confirmed that *Lycium barbarum* polysaccharide can effectively enhance the humoral immune function and anti-fatigue ability of sub-health mice (Hao et al., 2015), *Lycium barbarum* can significantly improve the anti-hypoxia and anti-fatigue ability of mice (Zhang, 2020), and wolfberry juice has a very obvious role in improving the body's adaptability to exercise load, delaying the generation of fatigue and accelerating the elimination of fatigue (Hu et al., 2008). However, there are few reports on the effects of *Lycium barbarum* juice on the immune function of human body, especially athletes.

Thanks to the unique climate conditions of Qinghai Tibet Plateau, Qinghai *Lycium barbarum* fresh fruit has rich nutritional value (Yang et al., 2012), rich in vitamins and amino acids, especially in the medical and health care functions of *Lycium barbarum* polysaccharide content as high as 8.3%, and is the best quality *Lycium barbarum* in China (Cui et al., 2020). *Lycium barbarum* juice is the juice product obtained by physical methods such as squeezing, centrifugation and extraction with fresh *Lycium barbarum* fruit as raw material. Pure *Lycium barbarum* juice is a new type of zero addition, non-concentrated and reduced *Lycium barbarum* juice, which fully retains the main functional components and nutritional elements of fresh *Lycium barbarum* (Weng et al., 2016). This study was conducted to observe and summarize the effects of *Lycium barbarum* L. on the immunity of athletes during altitude training in plain men's middle-distance runner, thus, the research and application of altitude adaptation and altitude training can be referred to *Lycium barbarum*.

2. Research Methods

2.1. Research Object

According to the matching principle of age and sports level, 18 elite male middle and long distance runners were selected and divided into the experimental group and the control group according to the matching principle of age and sports level. Among them, the experimental group had 10 athletes, including 3 elite athletes, 4 first-class athletes and 3 second-class athletes, with an average age of 21.00 ± 3.71 years, an average of 116.0 ± 225.6 m in the birthplace, and 5.20 ± 4.49 years of professional training The average age of the control group was 19.38 ± 3.11 years, the average altitude of birth was 143.8 ± 173.8 m, and the professional training period was 5.50 ± 4.34 years.

2.2. Research Plan

The control group and the experimental group received altitude training for 30 days from the plain to the altitude of 2366 m, during which the two groups received the same content and load intensity of exercise training. The experimental group took 2 pieces of pure *Lycium barbarum* juice before going to bed every night, while the control group did not drink it. The results of pony test showed that every 100 ml of *Lycium barbarum* juice contained 360 - 440 mg of LBP, and it did not contain any stimulants as determined by China Anti Doping center.

2.3. Research Indicators and Methods

The number of immune cells and lymphocytes plays an important role in monitoring the changes of immune function under hypoxia training (Li & Lin, 2018). In this study, the indexes of immunoglobulin (Ig), interleukin (IL), T lymphocyte subsets and natural kill cell (NK) in blood were detected and compared.

On the first day (basic value), the 14th day (intermediate value) and the 28th day (end value) of altitude training, 3 ml of elbow venous blood was collected for detection of immunoglobulin (IGA, IgG, IgM) and interleukin-6 (IL-6) by EDTA-K2 anticoagulant, and 4 ml of elbow vein blood was taken from common non-anticoagulant blood collection vessel, centrifuged at 3000 r/min for 15 min T lymphocyte subsets (CD3+, CD4+, CD8+) and NK cell subsets (CD3 - CD16+ or CD56+) were measured. Immunoglobulin was detected by turbidimetric method in Cobas 8000 c702, IL-6 in Siemens IMMULITE 1000 chemiluminescence analyzer, serum T lymphocyte subsets and NK lymphocytes were detected by flow cytometry in American Backman cyto flex flow cytometry. Spss20.0 was used to analyze the test data. Paired t-test, independent sample t-test and one-way ANOVA were used to analyze the changes of each index in different stages of the same group and between groups.

3. Research Results

3.1. Immunoglobulin

In the IgA experimental group, the intermediate value increased by 13.8%, and the end value was still higher than the basic value by 5.7%, while the intermediate value of the control group decreased by 22.4%, and the end value returned to the basic level; the intermediate value of the IgG experimental group increased by 3.6%, and the end value was close to the basic value, while the intermediate value of the control group decreased by 14.9%, and the end value was still lower than the basic value by 9%; after the intermediate value of IgM experimental group decreased (8.7%), the end value returned to the basic level The control group showed a continuous decrease (6.1% - 10.5%) during altitude training (**Table 1**).

3.2. Interleukin 6

During altitude training, the serum IL-6 of the two groups showed a downward trend. Compared with the basic value, the mid-term value and the end value of the experimental group decreased by 17%, while the control group decreased by 32% - 37% (**Table 1**).

Table 1. Statistical results of immunoglobulin and interleukin-6 of plain athletes during altitude training (mean ± SD).

Classification	Index	Grouping	Base value	Intermediate value	Terminal value
Immunoglobulin	IgA (g/L)	experience group	1.98 ± 0.53	2.26 ± 0.85	2.10 ± 0.73
		control group	1.76 ± 0.86	1.37 ± 0.41	1.76 ± 0.99
	IgG (g/L)	experience group	9.02 ± 1.69	9.35 ± 1.99	8.99 ± 2.36
		control group	9.02 ± 1.70	7.68 ± 0.99	8.17 ± 1.77
	IgM (g/L)	experience group	0.96 ± 0.35	0.87 ± 0.13	0.96 ± 0.23
		control group	1.09 ± 0.22	1.02 ± 0.22	0.98 ± 0.07
Interleukin	IL-6 (pg/mL)	experience group	1.52 ± 1.02	1.25 ± 0.82	1.26 ± 0.54
		control group	1.87 ± 1.42	1.17 ± 0.82	1.26 ± 0.24

3.3. T Lymphocytes

During high altitude training, except for the experimental group, the medium-term value of CD8+ increased by 11.9%, and the later end value decreased to the basic value in the experimental group, the mid-term value of CD3+ decreased by 3.7%, the terminal value decreased by 9.4%, the mid-term and terminal value of CD4+ decreased by 17% - 19%, the mid-term value of CD4+/CD8+ decreased by 15.5% ($P < 0.05$), and the end-stage value decreased by 6.8%; in the control group, the mid-term value of CD3+ decreased by 13.6%, the end-stage value decreased by 7.9%, CD4+ decreased by 13% - 18%, CD8+ by 5% - 11%, and the median CD4+/CD8+ value decreased by 12.9% and the end-stage value decreased by 10.7% (Table 2). Compared with the control group, the decrease of CD3+ and CD4+/CD8+ in the experimental group was lower, and CD8+ showed an increasing trend.

3.4. NK Cells

NK cells in the experimental group showed a continuously increasing trend (Table 2). Compared with the basic value, the end-stage value increased by 49.8% ($P < 0.05$), while that in the control group increased by 22.3%, which was lower than that in the experimental group.

4. Analysis and Discussion

The effects of different exercise intensities on immunoglobulin are different. Moderate and regular exercise can increase the contents of IgA, IgM and IgG, and enhance the immune function of the body. However, the contents of IgA, IgM and IgG significantly decrease during long-term and high-intensity exercise, resulting in the decrease of immune function of the body (Xing & Li, 2007). During winter training, the humoral immune indexes such as serum immunoglobulin of high-level kayak athletes are relatively lower, and their immune ability is faced with great risk. Rhinitis, influenza and other diseases without obvious

Table 2. Statistical results of T lymphocyte and NK cell of plain athletes during altitude training (mean ± SD).

Classification	Index	Grouping	Base value	Intermediate value	Terminal value
T lymphocyte	CD3+ (%)	Experience group	65.10 ± 12.97	62.67 ± 10.21	59.00 ± 9.94
		control group	64.13 ± 8.98	55.40 ± 9.81	59.00 ± 10.43
	CD4+ (%)	experience group	33.20 ± 7.58	27.50 ± 3.89	26.89 ± 4.17*
		control group	36.25 ± 7.49	29.60 ± 5.03	31.50 ± 4.76
	CD8+ (%)	experience group	29.5 ± 7.82	33.00 ± 8.46	29.67 ± 8.28
		control group	24.63 ± 5.04	22.00 ± 6.82	23.50 ± 6.83
	CD4+/CD8+	experience group	1.03 ± 0.15	0.87 ± 0.23*	0.96 ± 0.23
		control group	1.40 ± 0.40	1.22 ± 0.33	1.25 ± 0.28
NK cells	CD3 - CD16+ or CD56+ (%)	experience group	20.40 ± 11.38	27.33 ± 8.19	30.56 ± 8.69*
		control group	19.63 ± 8.83	27.75 ± 6.90	24.00 ± 5.00

Note: compared with the basic value, * means significant difference ($P < 0.05$), and ** means very significant difference ($P < 0.01$).

pathogenic factors occur frequently (Li & Zhang, 2014). High altitude or hypoxia training can have a certain impact on plasma immunoglobulin (Tiolier et al., 2005). Hypoxia can cause salivary IgA to decrease. Gao Binghong et al. found that living at high altitude and training low oxygen for 4 weeks can inhibit the immune function of elite female rowers (Gao & Chen, 2014). Reasonable supplement of nutrients is conducive to promoting the recovery of immune function. The supplementation of LBP in high-intensity endurance exercise can effectively prevent the decline of immune function and help maintain the immune system (Liu, Pan, & Wang, 2011). During Qinghai plateau training, IgA, IgG and IgM of the control group showed different degrees of decline, which indicated that the immune function of plain athletes decreased due to the dual stimulation of exercise training load and hypoxia in high altitude environment, while the immunoglobulin IgA, IgG and IgM in the experimental group increased slightly, and the immune function of the body was improved to some extent In order to improve the athletes' immunoglobulin function, effectively alleviate the exercise-induced immunosuppression.

The main role of IL-6 is to promote the proliferation and differentiation of B cells and secrete antibodies, which is conducive to the body to resist pathogenic microorganisms, and has obvious anti-inflammatory effect (Zhao, 2005). Studies have found that IL-6 plays an important regulatory role in skeletal muscle metabolism (Pedersen, Steensberg, & Schjerling, 2001). When skeletal muscle intake of glucose increases over a long period of time, IL-6 in the body can play an important role in regulating liver metabolism, so as to maintain the stability of blood glucose level during exercise; at the same time, IL-6 also participates in promoting the decomposition of fat and other metabolic pathways (Stouthard et al., 1996), which can promote the decomposition of fatty acids and improve the level of fatty acids The amount of free fatty acids available to meet the energy needs of exercise muscles. In addition, IL-6 plays an important role in promoting angiogenesis, inducing and regulating erythropoietin (EPO) production and inducing vascular endothelial growth factor (VEGF) (Ma, 2012; Zhu, Wu, & Geng, 2004). Compared with the control group, the decrease of IL-6 in the experimental group was lower than that in the control group, which suggested that during the plateau training, taking *Lycium barbarum* juice could alleviate the decline of IL-6 in plain athletes, which was beneficial to maintain blood glucose level and promote fat catabolism, as well as the formation of VEGF and EPO regulatory factors, so as to improve sports endurance and anti hypoxia ability.

The research confirmed that (Gao, Wu, & Li, 2011) after long-term heavy load training, the content of CD3+, CD4+, CD8+ decreased, and the ratio of CD4+/CD8+ decreased, which led to the disorder of immune regulation function. Hypoxic environment exposure or hypoxic training can lead to changes in T lymphocytes. After acute and chronic hypoxia exposure at 5050 m altitude, CD3+ and CD4+ T lymphocytes decreased (Facco et al., 2005), resulting in the damage of THL/Th2 immune balance homeostasis regulation and increased risk of infection. During hypoxic training, the level of CD3+ and CD4+/CD8+ decreased by 4.13% and 2.4%, respectively (Wang & Gao, 2014). During altitude training, the combined effect of hypoxia and exercise can reduce the immune indexes of athletes. CD8+ is more sensitive to low-pressure hypoxia double stimulation (Wang & Gao, 2019). Generally, the decrease of CD4+/CD8+ ratio of athletes is regarded as a sign of low immune function.

In this study, the levels of CD3+, CD4+, CD8+ and CD4+/CD8+ in the experimental group and the control group showed a downward trend, in which CD3+ decreased by 3.7% - 13.6%, CD4+ decreased by 13% - 18%, and CD4+/CD8+ decreased by 6.8% - 15.5%. However, the decrease of CD3+ and CD4+/CD8+ in the experimental group was lower than that in the control group, and CD8+ showed an increasing trend. The experiment shows that (Li, 2008) LBP supplementation can significantly increase the CD3+, CD4+, CD4+/CD8+ ratio, IgA, IgG, cellular immunity and humoral immunity of volleyball players who have been engaged in intensive training for a long time. In this study, athletes taking pure *Lycium barbarum* juice during high altitude training can effectively alleviate the imbalance of immune function caused by exercise and hypoxia, reduce the decrease range of CD3+, increase CD8+, and appropriately reduce the imbalance of CD4+/CD8+, which is beneficial to the middle and long term of plain Runners can improve the immune function and the ability to adapt to hypoxia.

In the process of exercise, NK cells are the most significantly changed lymphocytes. Various acute exercises can increase the number and activity of NK cells, while long-term and high-intensity exercise will cause the decrease of NK cell count and function (Li, 2016). In addition, hypoxia can affect NK cells. Gao found that after 4 weeks of HiLo hypoxia training (Gao, Wu, & Sheng, 2014), the NK cells of female rowers decreased significantly and lasted for a long time. Studies have confirmed that (Gao, Wu, & Li, 2011), heavy load training can cause the number of NK cells of wrestlers to decrease, acupuncture pretreatment can improve NK cell activity, thus changing the immune function disorder of the body, and has positive significance in fighting fatigue. Liu (Liu, Pan, & Wang, 2011) and other experiments showed that LBP supplementation in high-intensity endurance exercise can increase the number of NK cells and effectively prevent the decline of immune function. Peng (Peng et al., 2019) found that after drinking pure *Lycium barbarum* juice for 32 days, Th cells, NK cells and serum immunoglobulin IgA and IgG in adult men of different age groups were increased. It is believed that drinking pure *Lycium barbarum* juice has a good positive regulatory effect on immune function of the body. In this study, the increase of NK cells in the experimental group of athletes taking medlar juice during altitude training was greater than that of the control group ($P < 0.05$). It is suggested that *Lycium barbarum* juice can play the role of immune enhancer. By mobilizing the reserve function of thymus of central immune organs, more mature NK lymphocytes are released to the peripheral to make up for the deficiency and play a role in hypoxia exposure, which can fully regulate the motivation The body adapts to the sudden change of oxygen concentration as soon as possible, so as to minimize the influence and damage of hypoxia on the body, so as to realize the protective effect on the body (Tian et al., 2010).

5. Research Conclusion

During the altitude training at 2366 m altitude, the level of immunoglobulin IgA, IgG, IgM, IL-6, T lymphoid CD3+, CD4+ and CD4+/CD8+ decreased in varying degrees. The NK cells increased. Taking *Lycium barbarum* juice can help to increase IgA, IgG, CD8+, NK, and reduce the levels of IL-6, CD3+, CD4+/CD8+ in plain male middle- and long-distance runners, which is conducive to improving the athletes' immune ability.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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