

Original Article

The effect of pre-competition training on biochemical indices and immune function of volleyball players

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Received July 17, 2013; Accepted August 18, 2013; Epub September 1, 2013; Published September 15, 2013

Abstract: Objective: Pre-competition sports training can have varying effects on an athlete's immune function, including causing reduced resistance. The aim of this study was to explore effects of pre-competition training on some biochemical indices and immunologic functions in top-level volleyball athletes to determine whether training should be modified for optimal health. Methods: Biochemical indices (Hb, BUN, CK, LDH) and immunologic function (IgA, IgG, IgM, CD3⁺, CD4⁺, CD8⁺) were detected by semiautomatic biochemistry analyzer, light scattering photometer, or flow cytometry in sera from 24 top-level volleyball athletes and compared before and after pre-competition training. Results: After training, the levels of Hb, IgA, IgG, IgM, and CD4⁺ and the CD4⁺/CD8⁺ ratio decreased significantly, while BUN, CK, LDH, and CD8⁺ increased significantly ($P < 0.05$). Further, the decrease in Hb levels in female athletes was more significant than that in male athletes ($P < 0.05$). Conclusions: These results indicate that pre-competition training affects biochemical indices and immunologic function in this group of athletes. Additionally, more dramatic changes in Hb in female athletes may indicate a need for adapted training loads and rest periods for females.

Keywords: First-grade volleyball athlete, biochemical indexes, immunologic function

Introduction

Pre-competition training is commonly used among athletes to improve technique and enhance skill. Recent focus has shifted to the effects of the training level on the physical function of athletes. Under a low pre-competition training load, athletic ability does not improve significantly; however, an extremely high pre-competition training load not only does not improve athletic ability, but is detrimental to health. Indeed, changes that have been noted include immune dysfunction, upper respiratory tract infection, and decline in resistance [1-3]. These effects have led to the implementation of monitoring of biochemical indices and immune functions during the training period to evaluate the functional state of the athletes [4]. Here, biochemical indices and immune functions were assessed in level-1 university volleyball players, defined by General Administration of Sport of China, both before and after pre-competition training. The goal was to provide a comprehensive assessment

of the players' physical functions, establishing an objective foundation by which coaches can understand players' adaptation to training load and can regulate their training loads for optimal performance.

Subjects and methods

Subjects

Twenty-four level-1 volleyball players from our university were selected as study subjects; the group included 12 males (mean age 21.6 ± 0.9 years) and 12 females (mean age 21.5 ± 1.0 years). All the subjects were healthy, with normal eating habits and no history of major diseases.

Sports training program

The total period of pre-competition training was 4 weeks, with 6 days of training and one day of rest each week. Training sessions occurred twice daily, once in the morning and once in the afternoon, for 2 hours each. Blood samples

Table 1. Comparison of biochemical indices before and after pre-competition training ($\bar{X} \pm s$)

Sex	Time	n	Hb (g/L)	CK (U/L)	LDH (U/L)	BUN (mmol/L)
Male	Before training	12	148.5 \pm 4.4	102.8 \pm 14.0	162.6 \pm 8.6	5.40 \pm 0.33
	After training	12	139.3 \pm 4.8	140.9 \pm 27.3	192.7 \pm 9.3	6.20 \pm 0.22
	F		27.058	22.701	80.350	74.181
	P		0.001	0.001	0.001	0.001
Female	Before training	12	131.7 \pm 6.8	101.3 \pm 16.7	163.3 \pm 9.7	5.27 \pm 0.24
	After training	12	121.0 \pm 7.0	137.7 \pm 27.3	197.6 \pm 7.0	6.38 \pm 0.28
	F		35.610	11.718	160.311	178.399
	P		0.001	0.006	0.001	0.001
F			73.331	0.144	1.014	0.073
P			0.001	0.708	0.325	0.789

Table 2. Comparison of IgA, IgG, and IgM levels before and after pre-competition training ($\bar{X} \pm s$)

Sex	Time	n	IgA (g/L)	IgG (g/L)	IgM (g/L)
Male	Before training	12	1.71 \pm 0.16	11.71 \pm 1.00	1.79 \pm 0.10
	After training	12	1.39 \pm 0.07	10.18 \pm 0.40	1.01 \pm 0.06
	F		30.881	23.101	550.481
	P		0.001	0.001	0.001
Female	Before training	12	1.76 \pm 0.14	11.57 \pm 0.92	1.71 \pm 0.14
	After training	12	1.38 \pm 0.08	10.12 \pm 0.47	1.01 \pm 0.06
	F		56.739	28.551	329.165
	P		0.001	0.001	0.001
F			0.424	0.202	1.428
P			0.522	0.658	0.245

were taken from the cubital vein between 07:00 and 08:00 one day before training began; samples were collected again 2 days after the 4-week training period ended. Samples were collected into heparin-coated tubes.

Biochemical and immune function analyses

Biochemical parameters (hemoglobin (Hb), creatine kinase (CK), lactate dehydrogenase (LDH), and blood urea nitrogen (BUN)) were detected by a semi-automatic biochemical analyzer (Hitachi Science and Systems, Japan); levels of CD3⁺, CD4⁺, and CD8⁺ were detected by a flow cytometer (BD Biosciences, USA); and serum IgA, IgG, and IgM levels were determined by a light scattering photometer (Binding Site, UK).

Statistical methods

SPSS17.0 statistical software was used for data processing. Measurement data are expressed as mean \pm standard deviation ($\bar{X} \pm s$). Repeated measures ANOVA was applied to compare biochemical indices and immune function between the two groups. Tests were

two-sided, with α level of 0.05, and $P < 0.05$ was considered as statistically significant.

Results

Comparison of biochemical indices before and after pre-competition training

Compared with values obtained before pre-competition training, Hb levels significantly declined in the volleyball players following training; in contrast, CK, LDH, and BUN levels significantly increased ($P < 0.05$) (Table 1). Interestingly, Hb levels decreased more in female players than male players ($P < 0.05$), but no statistically significant difference was observed in the variation trend between female and male players ($P > 0.05$).

Comparison of immune function before and after pre-competition training

Compared with values before pre-competition training, IgA, IgG, IgM (Table 2), and CD4⁺ levels as well as the CD4⁺/CD8⁺ ratio (Table 3) significantly decreased after pre-competition training. The CD8⁺ level significantly increased ($P < 0.05$), but no statistically significant difference was observed in for CD3⁺ levels ($P > 0.05$). Additionally, no statistically significant difference was observed in the variation trend of the above immune function indices between female and male players ($P > 0.05$).

Discussion

Hb plays a critical role as the transporter of O₂ and CO₂ in the body. Hb from red blood cells

Table 3. Comparison of CD3⁺, CD4⁺, and CD8⁺ levels and the CD4⁺/CD8⁺ ratio before and after pre-competition training ($\bar{X} \pm s$)

Sex	Time	<i>n</i>	CD3 ⁺ (%)	CD4 ⁺ (%)	CD8 ⁺ (%)	CD4 ⁺ /CD8 ⁺
Male	Before training	12	60.0 ± 3.3	35.5 ± 1.7	22.5 ± 3.9	1.63 ± 0.36
	After training	12	58.0 ± 3.5	31.0 ± 1.6	25.5 ± 4.0	1.25 ± 0.25
	<i>F</i>		3.264	40.628	5.806	11.846
	<i>P</i>		0.098	0.001	0.035	0.006
Female	Before training	12	59.3±3.9	35.4 ± 2.2	21.9 ± 5.7	1.76 ± 0.60
	After training	12	56.6±2.8	30.4 ± 1.7	25.8 ± 3.3	1.20 ± 0.22
	<i>F</i>		3.400	45.982	5.128	10.837
	<i>P</i>		0.092	0.001	0.045	0.007
<i>F</i>			0.950	0.487	0.085	0.011
<i>P</i>			0.340	0.493	0.773	0.916

participates in synthesis of muscle protein and red blood cell regeneration. Hb levels can be significantly decreased when athletes are overtired, perhaps resulting from accelerated red blood cell destruction during intense exercise; therefore, changes in Hb level can be used as an indicator to determine whether players are overtired [5]. Another marker, CK, is a key enzyme in energy metabolism of the skeletal muscle. Strenuous exercise can lead to an increase in muscle membrane permeability or muscle fiber damage, which can cause CK and its isoenzymes to escape from muscle cells, and thereby further increasing serum CK concentration. Some studies have shown that elevated serum CK is related mainly to exercise intensity and duration [6, 7]. Similarly, the enzyme LDH can be used as an indicator of the ability of skeletal muscle to withstand a load; a relative insufficiency of motor muscle groups during strenuous exercise can cause increases in LDH [8]. The last biochemical index, BUN, reflects the level of collective fatigue and can be used to evaluate the functional status of athletes. All exercise uses protein as a fuel, causing a significant increase in BUN generation, which keeps increasing as the exercise load does. Therefore, BUN can be used as an indicator to determine the exercise load and whether the athletes are fatigued [9]. Under long-duration, high-intensity exercise, the muscle energy balance has been damaged, catabolism of protein and amino acid is enhanced, and urea is increasingly generated, thus causing increased content of urea in the blood [10]. Results of this study have shown that, after 4 weeks of pre-competition training, the Hb level of volleyball players is significantly decreased, while CK, LDH, and BUM levels are sig-

nificantly increased, indicating that pre-competition training alters biochemical indices, which can be used to reflect intensity of exercise training. Interestingly, female players had a more evident decline in Hb, indicating they may be more prone to fatigue than male players. Thus, training intensity for female players should be reduced, and the rest period should be extended, to accommodate this difference.

Immunoglobulin (Ig) has antibody activity or a chemical structure similar to an antibody and is able to exert immune defense, immune surveillance, and immune homeostasis functions. It not only can resist the corresponding pathogen, microorganisms, and toxins directly, but also can induce a variety of other effects, such as complement activation, phagocytosis, etc. Studies have shown that, after high-load sports training, the Ig content of the players is significantly decreased [11, 12]. Our results indicate significant decreases in IgA, IgG, and IgM levels of level-1 volleyball players, indicating decreased immunity of the players and inhibition on the immune system by strenuous exercise, likely increasing disease susceptibility. Decreased Ig may result from consumption of immunoglobulin as an energy source during long-term strenuous exercise [13].

T lymphocytes act during cellular immune response and have both immune effector functions and immunomodulatory functions: T lymphocytes are the material basis for cellular immunity. T lymphocytes subsets can be mutually restraint and assistant. The ratio of T-cell subsets plays an important role in maintaining the body's normal immune function, and any imbalance will lead to a variety of immune injuries or diseases [14]. It is generally believed that CD3⁺ cells represent T lymphocytes, mainly divided into two subsets, CD4⁺ and CD8⁺. CD4⁺ cells are helper T lymphocytes, promoting transformation into effector cells, helping B cells produce antibodies, and promoting macrophage activation. In contrast, CD8⁺ cells are

cytotoxic T lymphocytes, having opposite effects with CD4⁺ cells. These CD8⁺ and CD4⁺ cells must occur in proportion [15]. High-load training significantly increased the level of CD4⁺ cells in male tennis players, but no obvious changes were observed on CD8⁺ cells; this led to an imbalance that could influence immune function (8). Here, after 4-week pre-competition training of volleyball players, CD4⁺ levels significantly decreased, while CD8⁺ levels significantly increased, causing a decrease in the ratio of CD4⁺/CD8⁺. Sports training may therefore impose an immunoregulatory function disorder with long-term exercise leading to a reduced multiplication capacity of lymphocytes, further inhibiting the immune function.

In summary, 4-week pre-competition training can cause significant changes in biochemical indices (Hb, CK, BUN, LDH) and immune function (IgA, IgG, IgM, CD4⁺, CD4⁺/CD8⁺) of volleyball players. These indices can be used to evaluate the exercise load of similar athletes. These indicators are fluctuating within the normal ranges, suggesting that such fluctuations are only physiological changes or transient, and proper rest may return them to pre-training levels. Interestingly, the load level of this pre-competition training is moderate, and appears to do no long-term harm to body function. Adequate rest should be provided during training, especially for female players, who may be more prone to fatigue and therefore need appropriately extended rest time.

Disclosure of conflict of interest

None.

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References

- [1] Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 2000; 80: 1055-1081.
- [2] Pedersen BK, Steensberg A. Exercise and hypoxia: effects on leukocytes and interleukin-6-shared mechanisms? *Med Sci Sports Exerc* 2002; 34: 2004-2013.
- [3] Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleschner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, Simon P. Position statement. Part one: Immune function and exercise. *Exerc Immunol Rev* 2011; 17: 6-63.
- [4] Gleeson M, Bishop NC. The T cell and NK cell immune response to exercise. *Ann Transplant* 2005; 10: 43-48.
- [5] Umeda T, Takahashi I, Danjo K, Matsuzaka M, Nakaji S. Changes in neutrophil immune functions under different exercise stresses. *Nihon Eiseigaku Zasshi* 2011; 66: 533-542.
- [6] Nicholson GA, McLeod JG, Morgan G, Meerkin M, Cowan J, Bretag A, Graham D, Hill G, Robertson E, Sheffield L. Variable distributions of serum creatine kinase reference values. Relationship to exercise activity. *J Neurol Sci* 1985; 71: 233-245.
- [7] Aspenes ST, Karlsen T. Exercise-training intervention studies in competitive swimming. *Sports Med* 2012; 42: 527-543.
- [8] Lawton TW, Cronin JB, McGuigan MR. Strength testing and training of rowers: a review. *Sports Med* 2011; 41: 413-432.
- [9] Gleeson M, McDonald WA, Cripps AW, Pyne DB, Clancy RL, Fricker PA. The effect on immunity of long-term intensive training in elite swimmers. *Clin Exp Immunol* 1995; 102: 210-216.
- [10] Shibata S, Levine BD. Effect of exercise training on biologic vascular age in healthy seniors. *Am J Physiol Heart Circ Physiol* 2012; 302: 340-346.
- [11] Weinstock C, König D, Harnischmacher R, Keul J, Berg A, Northoff H. Effect of exhaustive exercise stress on the cytokine response. *Med Sci Sports Exerc* 1997; 29: 345-354.
- [12] Nieman DC, Pedersen BK. Exercise and immune function. Recent developments. *Sports Med* 1999; 27: 73-80.
- [13] Nieman DC, Henson DA. Role of endurance exercise in immune senescence. *Med Sci Sports Exerc* 1994; 26: 172-181.
- [14] Wilson LD, Zaldivar FP, Schwindt CD, Wang-Rodriguez J, Cooper DM. Circulating T-regulatory cells, exercise and the elite adolescent swimmer. *Pediatr Exerc Sci* 2009; 21: 305-317.
- [15] Dong J, Tian YP, Gao YH, Li LQ. Exercise-induced changes of T lymphocytes subgroups and immune factors. *Nan Fang Yi Ke Da Xue Xue Bao* 2010; 30: 2277-2280.