ORIGINAL RESEARCH

Research on the Changes in Catecholamine and Other Indicators during Different Training Phases in Female Volleyball Players

Xi Wang, MD; Lin Huang, PhD; Defeng Zhao, PhD; Jun Qiu, PhD; Yanhong Ma, PhD

ABSTRACT

Objective • This study seeks to assess the functional status and central fatigue state of athletes in the Shanghai women's volleyball team during the training phase of the 2021 Shaanxi National Games. Employing a comprehensive methodology involving functional status assessment and catecholamine index analysis, the research aims to establish a scientific foundation for preparing for the 2025 National Games. Additionally, it aims to provide valuable insights for preventing excessive fatigue and promoting the rational elimination of fatigue.

Methods • (1) Participants: Twelve adult female volleyball players from Shanghai participated in the study. The average age of the participants was 26.23±3.39 years, and they had an average training period of 11.92±3.73 years. (2) Training Period: The study spanned a duration of 21 consecutive weeks, during which the training regimen was divided into eight distinct stages based on specific content and tasks. (3) Testing Procedures: Various tests were conducted at specific intervals throughout the training period. These included assessments performed at the conclusion of each upper training stage and the Metamorphosis stage. Additionally, comprehensive testing was carried out before and after both the preliminaries and championship matches of the National Games. Fasting elbow venous blood samples were collected for assessing functional status indicators, including Hemoglobin (HGB), Blood Urea Nitrogen (BUN), Creatine Kinase (CK), Serum Ferritin (SF), Testosterone (T), Cortisol (C), Testosterone/Cortisol ratio (T/C). Moreover, blood catecholamine indicators (Dopamine (DA), Norepinephrine (NE), Epinephrine (E) were analyzed before the National Games, at the end of Metamorphosis stage 2, and at the conclusion of upper phase 3. (4)Data Analysis: The collected data underwent rigorous statistical processing using SPSS 25.0 statistical software package and Microsoft Excel software. This comprehensive analysis was essential for deriving meaningful conclusions and identifying significant patterns in the athletes' functional status and central fatigue states.

Results • (1) HGB, T, and T/C showed the same trend throughout the whole period. The upper phase 1 drops significantly to the lowest value and the Metamorphosis stage increases. The training stage 2 fell again, but the decline was less than the training stage 1, and the Metamorphosis stage 2 increased significantly, and there was a significant difference between the basic value and the training stages ($P < .05$). Testosterone increased significantly to the maximum before the final of the National Games, and there was a significant difference between the baseline and the pre-match ($P < .05$). (2) At the end of the training stage, DA, and E decreased significantly, and there was no significant difference in NE decline. During the preliminaries of the National Games, DA, NE, and E all declined, but there was no significant difference. In the championship stage, DA, NE, and E both increased, but only NE was significantly different from the Metamorphosis stage and the championship ($P < .05$).

Conclusion • (1) Performance Enhancement: Recognizing and addressing performance dips in the training stage through targeted adjustments can optimize athlete performance. Athletes exhibit improved competitiveness during actual games, indicating the effectiveness of tailored interventions (2) Strategic Fatigue Management: Distinguishing between body and central fatigue is vital. Monitoring sensitive markers like blood dopamine and adrenaline in the training stage enables timely fatigue management. Understanding the relationship between blood testosterone and dopamine offers insights into energy levels and mental resilience, aiding in effective training strategies. (3) Efficient Evaluation Tools: Hemoglobin and blood testosterone serve as efficient markers for evaluating athletes' states. Regular assessment of these indicators allows for proactive adjustments in training, preventing excessive fatigue and promoting overall well-being. (*Altern Ther Health Med.* 2024;30(7):192-201).

Xi Wang, MD, Associate professor; School of Athletic Performance, Shanghai University of Sport, Shanghai, China; Physiological laboratory, Shanghai Research Institute of Sports Science (Shanghai Anti-Doping Agency), Shanghai, China. **Lin Huang,** PhD, Lecturer; Department of Physical Education, Shanghai Dianji University, Shanghai, China. **Defeng Zhao,** PhD, Professor; Physiological laboratory, Shanghai Research Institute of Sports Science (Shanghai Anti-Doping Agency), Shanghai, China. **Jun Qiu,** PhD, Professor; Shanghai Research Institute of Sports Science (Shanghai Anti-Doping Agency), Shanghai, China. **Yanhong Ma,** PhD, Professor; Department of Graduate, Shenyang Sport University, Shenyang, China.

Corresponding author: Yanhong Ma, PhD
E-mail: tyjwc@aliyun.com

INTRODUCTION

Volleyball is a complex sport that combines technique, tactics, and physical fitness.[1] Serving, receiving, setting, attacking, and blocking are typical game actions that determine the outcome of a match. In volleyball matches, athletes are required to maintain a high level of concentration and respond rapidly to stimuli. This keeps volleyball players in a state of heightened arousal and makes them prone to fatigue. In order to achieve victory, athletes must maintain a good state of physical and mental condition and minimize fatigue.[2] Like any sport, the success of volleyball largely depends on the arrangement of well-designed training cycles. These training cycles consist of training sessions, microcycles, and macrocycles. Periodized training is a structured training method primarily based on variations in training volume and intensity. By providing necessary physiological adaptation and recovery, periodized training enables athletes to reach their optimal performance at the appropriate time.[3] Women volleyball players face unique challenges in training and competition. They need to maintain concentration in the technically demanding sections while coping with the pressure of long competition cycles and fatigue accumulation. Maintaining a good state and mental quality is essential for success. Depending on the type of training and the objectives of the annual training cycle, carefully planned training loads induce favorable changes in athletes' morphological and physiological characteristics.[4] However, sustained high-intensity training may lead to changes in various biochemical indicators, such as cortisol, urea, iron, catecholamines, and blood cell counts.[5] These indicators are commonly used to monitor physiological and biochemical changes during training. For example, the level of skeletal muscle protein in the blood may serve as a signal of muscle damage.[6] Elevated levels of cortisol and urea are widely considered markers of increased protein metabolism,[7] while hemoglobin and iron levels are crucial for oxygen transport and utilization.[8] Changes in catecholamines usually indicate insufficient recovery after training.[5,6] To improve athletes' performance in competitions, the selection of training loads must be suitable for individual athletes' adaptive capacity.[9]

In domestic volleyball training, different training cycles include phases such as volume accumulation, adjustment, pre-competition, competition, league, and national games. The National Games, held every four years, is the most important domestic event in which coaches and athletes from various provinces and cities attach great importance to achieving outstanding results. Therefore, it is crucial to arrange training cycles properly in order to excel at the National Games. Each training cycle should not only improve athletes' skills but also enhance their endurance and adaptability to handle higher training and competition loads. However, overloading during long competition cycles can lead to severe fatigue, muscle injuries, and other damage. In the long run, this may reduce athletes' physical fitness and result in sports fatigue. The Australian National Volleyball Team, for instance, undergoes an average of 9-13 training sessions per week and participates in 15-40 matches during the international season.[10] Professional volleyball players in the Polish League spend approximately 800 hours per year playing volleyball and improving their physical health.[9] The application of training loads during competitions (typically over consecutive days) shortens the recovery time, which can accelerate athletes' fatigue and increase the risk of injury. However, the adaptive capacity acquired through training experience can help shorten recovery time. Most studies tend to assume that the primary factors influencing and altering athletes' metabolism during training are oxidative damage and the action of pro-inflammatory cytokines in damaged muscle fibers. Therefore, additional evaluation of changes in intracellular damage levels and antioxidant capacity induced by different training and competition cycles can significantly enhance athletes' efficiency.[11] Functional status indicators and central fatigue index are of great significance. Functional status indicators reflect physical conditions, such as muscle damage, protein metabolism, and oxygen transport capacity, helping to assess health status and adjust training programs. The central fatigue index indicates that the central nervous system is fatiguing and guides appropriate rest and recovery to maintain a competitive state.

Therefore, this study aims to: (1) Evaluate the impact of different training and competition cycles on the physical fitness of female volleyball players. (2) Investigate changes in endogenous stress markers and central fatigue levels during distinct training phases of the Shanghai women's volleyball team in preparation for the 2025 National Games. (3) Identify characteristic patterns in indicator changes throughout various training cycles, elucidating the nuances of physiological responses. (4) Provide a scientific foundation for National Games preparation, guiding coaches and athletes in optimizing training strategies and performance preparation. (5) Prevent excessive fatigue by delineating optimal training thresholds and recovery intervals, ensuring athletes' well-being and sustainable performance. (6)Offer valuable insights into effective strategies for fatigue elimination, contributing to athletes' long-term health, peak performance, and overall success in competitions.

PATIENTS AND METHODS

Study Participants

A total of 12 adult female volleyball players from the frontline team in Shanghai were included in this study. All participants were registered members of the Shanghai women's volleyball team for the 2021 Shaanxi National Games. The average age of the participants was 26.23±3.39 years, and they had an average training experience of 11.92±3.73 years. All participants had achieved a level of national athlete or higher. The basic characteristics of the participants are summarized in Table 1.

Table 1. Summary of Basic Characteristics of Study Participants

Age(Y)	Height (CM)	Weight (KG)	Training years (Y)	Sports level
26.23±3.39	182.92±6.68	73.77±8.32	11.92±3.73	National champion

Table 2. A detailed list of the different training stages

Phase division	Training phase 1			Metamorphosis stage 1	Training phase 2			Metamorphosis stage 2	Training phase 3			The pre-preliminary stage of the National Games		Preliminary competition of the National Games	After the preliminaries	The pre-championship phase	Championship game		Post-championship stage	Before the National Games	
Time (weeks)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Total training days	6	6	6	6	6	6	6	6	6	6	6	6	6	7	5	7	7	7	6	6	6
Training Course (session)	10	10	10	6	10	10	10	6	10	10	10	10	11	5	9	10	5	5	10	10	10
Total training Hours (h)	27	27	27	12	27	27	27	12	27	27	27	21	23	10	20	21	10	10	20	20	20
Specific Physical Training Duration (h)	7.5	7.5	7.5	4.5	7.5	7.5	7.5	4.5	7.5	7.5	7.5	7.5	7.5	3	6	6	3	3	6	6	6
Special Technical and Tactical Training (h)	19.5	19.5	19.5	4.5	19.5	29.5	19.5	4.5	19.5	19.5	19.5	13.5	13.5	7	14	14	7	7	14	14	14
Official competition (session)														5			5	5			

Research Methods

Division of Different Training Phases. Starting from the end of the 2020-2021 Women's Volleyball League and ending at the beginning of the final of the 2021 Shaanxi National Games, the training period was divided into eight phases over a continuous period of 21 weeks. These phases were categorized based on training content and tasks, including Training Phase 1 lasting for 3 weeks, followed by Metamorphosis Stage 1 lasting for 1 week, whose aim is to build basic endurance and physical fitness through extensive training This was followed by Training Phase 2, which spanned 3 weeks, and then Metamorphosis stage 2 which lasts for 1 week and provides the athlete with short-term recovery and adaptation. The training then continued with Training Phase 3, also lasting for 3 weeks and aiming to consolidate and improve physical fitness levels in preparation for the upcoming competition. As the competition approached, the athletes entered the National Games Preliminary Phase, consisting of 2 weeks before the competition, 1 week of competition, and 1 week after the competition. Subsequently, they entered the Championship Phase, which included 1 week before the competition, 2 weeks of competition, and 1 week after the competition. During this critical competition phase, the training content and objectives of the athletes will be more focused on the technical and tactical aspects to meet the high-intensity competition challenges. Finally, there was the National Games Pre-competition Phase lasting for 2 weeks. Table 2 provides an overview of the detailed information for each training phase. The detailed content of specialized physical fitness training and specialized technical and tactical training for each phase can be found in Table 3.

The choice of these eight training phases was based on optimizing the training program to meet the different training and competition needs of women's volleyball players over a duration of up to 21 weeks. Each phase has specific training content and objectives to ensure optimal physiological adaptation and competitive performance of athletes within the different phases.

Testing Indicators

Functional state indicators: Hemoglobin (HGB) (g/L), Blood Urea Nitrogen (BUN) (mmol/L), Creatine Kinase (CK) (u/L), Serum Ferritin (SF) (ug/dL), Testosterone (T) (ng/dL), Cortisol (C) (ng/dL), Testosterone/Cortisol ratio (T/C).Central fatigue indicators (Catecholamines): Dopamine (DA)(ng/L), Norepinephrine (NE)(ng/L), Epinephrine (E) (ng/L).

In different training stages, functional status indicators and central fatigue indicators showed the following characteristics, and the changes in each indicator reflected the changes in the physiological adaptation and competitive state of athletes: in the physical training stage, HGB decreased significantly, probably because the high intensity of training increased the demand for oxygen transport, leading to the loss of red blood cells. However, during the metabolic phase, HGB gradually rose, probably due to the reduced training intensity, which favored the regeneration of red blood cells and thus improved oxygen supply. BUN was elevated during the physical training phase, possibly due to increased nitrogen metabolism caused by high-intensity exercise, leading to increased urea production. During the metabolic phase, BUN may decrease, indicating decreased metabolism of nitrogen. CK was significantly elevated during the physical training phase, probably due to the muscle being damaged by training and releasing CK. However, during the metabolic phase, CK decreases, probably due to the muscle being restored and repaired. The apparent decrease in SF during the physical training phase may be due to the increased demand for iron due to training, leading to a decrease in iron stores in the body. During the metabolic phase, SF may rise, indicating that iron stores are replenished. T decreased during the physical training phase, probably because the high-intensity training induced endogenous stress, leading to a decrease in T levels. Before the race, T may rise, possibly due to an increased state of excitement. C may be elevated during the physical training phase because of the stress response induced by the training. Before the race, C may be lower, possibly due to an adaptive response. The T/C ratio may decrease during the physical training phase because C increases and T decreases. During the metabolic phase, the T/C ratio may rise, indicating an increased level of adaptation. DA, NE, and E are indicators of central fatigue, and their changes in different training stages may be related to exercise intensity and emotional state. During intense physical training, catecholamine hormones may be elevated, reflecting increased sympathetic activity. However, before the competition, these indicators may be elevated again, possibly because the athlete is in a tense competitive state.

Table 3. List of special physical training and technical and tactical training content

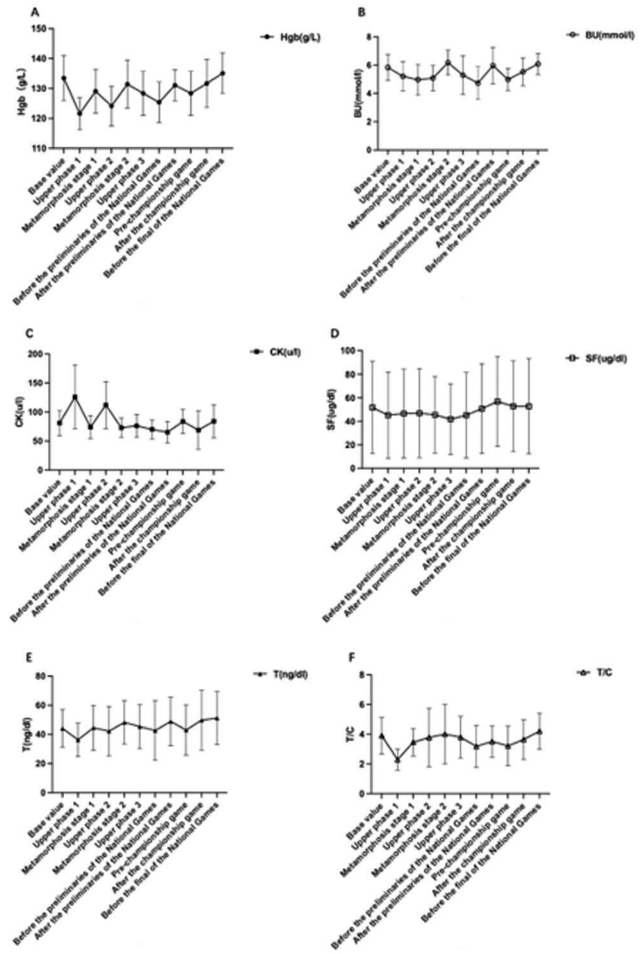
Special physical training	Special technical and tactical training
Strength Training (cycle)	Passing, receiving, attacking, blocking, defending, counterattacking
Aerobic training (cycle)	Offense + counterattack, small ball training
Aerobic Training (continuous)	Simulated competition against training, etc
Agility drill	

Table 4. Overview of Changes in Functional State Indicators in Different Training Phases

	Base value (T1)	Training phase 1 (T2)	Metamorphosis stage 1 (T3)	Training phase 2 (T4)	Metamorphosis stage 2 (T5)	Training phase 3 (T6)	Before the preliminaries of the National Games (T7)	After the preliminaries of the National Games (T8)	Pre-championship game (T9)	After the championship game (T10)	Before the final of the National Games (T11)
HGB (g/L)	133.46±7.55	121.62±5.39[a]	129.08±7.31[a,b]	124.15±6.59[a,b,c]	131.46±8.02[b,d]	128.38±7.33[a,b,d,e]	125.38±6.80[a,b,c,e,f]	131.08±5.22[b,d,f]	128.38±7.33[a,b,d,h]	131.69±8.04[b,c,d,f,g,i]	135.08±6.80[b,c,d,e,f,g,h,i,j]
BU (mmol/l)	5.84±0.91	5.21±1.03[a]	4.97±1.07[a]	5.08±0.89[a]	6.18±0.87[b,c,d]	5.30±1.36e	4.73±1.15[a,e]	5.97±1.29[d,f]	4.98±0.78[a,g,h]	5.53±0.99[c,e,g,i]	6.08±0.75[b,c,d,f,g,i,j]
CK (u/l)	81.08±21.70	126.08±54.99[a]	74.00±19.92[b]	112.08±40.56[b,c]	72.85±16.53[b,d]	76.23±19.76[b,d]	70.08±16.28[bd]	65.31±18.67[a,b,d]	83.77±21.09[b,d,g,h]	68.92±33.04[b,d]	84.08±28.28[b,d,g,h]
SF (ug/dl)	51.69±39.25	45.14±36.67[a]	46.53±37.64	46.90±37.74	45.48±32.51	41.70±29.87[a]	45.11±36.25[a]	50.67±38.19[f,g]	56.81±40.38[b,c,d,e,f,g,h]	52.73±38.46[b,e,f,g]	52.72±40.46[f,g]
T (ng/dl)	44.12±12.96	36.30±11.47[a]	44.54±15.45[b]	42.22±16.91	48.24±14.78[a,b,c,d]	45.34±15.11[b,e]	42.68±20.36[e]	48.91±16.68[b,d]	43.03±17.18[e]	49.77±20.53[g,i]	51.20±18.31[a,c,d,g,i]
T/C	3.90±1.22	2.29±0.71[a]	3.46±0.92[b]	3.78±1.96[b]	4.00±2.00[b]	3.80±1.40[b]	3.18±1.41[b]	3.51±1.05[b]	3.21±1.34[b,e]	3.64±1.33[b,i]	4.20±1.21[b,g,i]

Note: [a]$P < .05$ compared to T1, [b]$P < .05$ compared to T2, [c]$P < .05$ compared to T3, [d]$P < .05$ compared to T4, [e]$P < .05$ compared to T5, [f]$P < .05$ compared to T6, [g]$P < .05$ compared to T7, [h]$P < .05$ compared to T8, [i]$P < .05$ compared to T9, and [j]$P < .05$ compared to T10.

Figure 1. Overview of Changes in Functional State Indicators in Different Training Phases. (A) Hbg (g/L); (B) BU (mmol/L); (C) CK (u/L); (D) SF (ug/dL); (E) T (ng/dL); (F) T/C



Testing Time

The testing time varies depending on the actual training situation and the different testing indicators.

Functional state indicators: Baseline testing is conducted before the start of the training phase. Subsequent tests are performed at the end of each training phase and Metamorphosis stage. In addition, testing is conducted before and after the National Games Preliminary Phase, Championship Phase, and National Games Pre-competition Phase, for a total of 11 tests. These tests are conducted on Monday mornings, in a fasting state, to collect venous blood from the elbow for the measurement of relevant biochemical indicators.

Catecholamine indicators: Testing is conducted at the end of Metamorphosis Stage 2, Training Phase 3, before and after the National Games Preliminary Phase, Championship Phase, and National Games Pre-competition Phase, for a total of 7 tests. These tests are conducted on Monday mornings, in a fasting state, by collecting 2ml of venous blood from the elbow. The plasma concentration of catecholamines is measured using a high-performance liquid chromatography-electrochemical detection method.

Testing Methods

After each blood collection, 100 μl of Ethylenediaminetetraacetic Acid (EDTA) anticoagulated blood is used for complete blood count analysis using an automated hematology analyzer. 2ml of whole blood is centrifuged and the serum is used for the measurement of BUN, CK, T, and C. 2 ml of EDTA anticoagulated blood is centrifuged and the plasma is used for the measurement of catecholamines.[5]

Statistical analysis

In this study, repeated measures analysis of variance (ANOVA) was chosen as the appropriate statistical method for comparing data from different phases. Repeated measures ANOVA is specifically designed for situations where the same subjects are used for each treatment or condition. Unlike traditional ANOVA, repeated measures ANOVA takes into account the correlated nature of the data, which arises when measurements are taken on the same subjects over multiple time points or under different conditions.

In our study, using repeated measures ANOVA was crucial because it allowed us to assess the effects of different phases while considering the within-subject variability, which is particularly relevant when studying changes over time within the same group of participants. By employing this method, we were able to analyze the variations within individuals across the different phases of the study, providing a more robust and accurate evaluation of the observed differences. The significance level was set at $P < 0.05$, indicating that any observed differences with a probability of occurrence less than 5% were considered statistically significant, ensuring a rigorous approach to data interpretation.

RESULTS

Characteristics of Functional State Indicators in Different Training Phases

Table 4 and Figure 1 reveal distinct patterns in functional state indicators across training phases. HGB consistently

Table 5. Summary of catecholamine changes in different training stages

	Metamorphosis stage 2 (T5)	Training phase 3 (T6)	Before the preliminaries of the National Games (T7)	After the preliminaries of the National Games (T8)	Pre-championship game (T9)	After the championship game (T10)	Before the final of the National Games (T11)
DA(ng/L)	34.04±7.72	27.82±2.02[a]	31.05±2.72[b]	30.33±2.72[b]	31.12±1.90[b]	31.26±2.65[b]	29.83±2.80[b]
NE(ng/L)	251.08±78.29	201.00±92.59	375.85±111.11[a,b]	339.69±127.075[b]	311.23±134.68[b]	391.31±100.68[a,b,d]	356.38±98.41[a,b]
E(ng/L)	36.38±8.34	31.08±4.88[a]	32.05±4.36	29.85±2.07[a]	32.25±3.46	32.55±2.88[c]	33.68±4.35[c]

Note: [a]$P < .05$ compared to T5, [b]$P < .05$ compared to T6, [c]$P < .05$ compared to T8, and [d]$P < .05$ compared to T9.

decreases during training phases but recovers in the Metamorphosis stages, with significant differences between testing points ($P < .05$). T, T/C, and HGB show similar trends, with significant decreases in Training Phase 1, followed by increases in Metamorphosis stage 1 ($P < .05$). Training Phase 2 witnesses a smaller decrease than Phase 1, followed by a significant increase in Metamorphosis stage 2 ($P < .05$), showing deviations from the baseline and other phases. T levels enhance during competition phases, particularly post-competition, and significantly rise before the National Games finals.BU shows different changes during the training phases. It significantly decreases during Training Phase 1 ($P < .05$), starts to increase during Training Phase 2, reaches its peak during Metamorphosis stage 2, and decreases significantly again during Training Phase 3 ($P < .05$). The trend during the competition phases is similar, with significant increases post-competition compared to pre-competition ($P < .05$). CK exhibits an opposite pattern to HGB, with a significant increase during the training phases and a significant decrease during the Metamorphosis stages ($P < .05$). During the competition phases, CK decreases, and there is no significant difference post-competition compared to pre-competition ($P < .05$). However, it increases again before the National Games finals, without a significant difference from the baseline ($P < .05$). SF significantly decreases after the start of Training Phase 1 and reaches its lowest point during Training Phase 3, showing significant differences from the baseline ($P < .05$). It starts to increase during the competition phases and reaches its peak before the championship phase, with significant differences from the training and Metamorphosis stages ($P < .05$).

Change characteristics of catecholamines in different training stages

Table 5 and Figure 2 show that at the end of Training Phase 3, DA and E significantly decreased ($P < .05$), while NE showed no significant difference in decline ($P < .05$). During the National Games Preliminary Phase, DA, NE, and E all decreased, but without significant differences compared to before the preliminary phase ($P < .05$). In the Championship Phase, DA, NE, and E all increased, but only NE showed significant differences compared to the Metamorphosis stage and before the championship ($P < .05$). Prior to the National Games Final, NE was significantly higher than the level during the Metamorphosis stage, and although DA and E decreased compared to the Metamorphosis stage, there were no significant differences ($P < .05$). Throughout the entire training phase, DA and NE during the competition stages were significantly higher than the levels at the end of the Training Phase ($P < .05$).

Figure 2. Summary of catecholamine changes in different training stages. (A) DA (ng/L); (B) NE(ng/L); (C) E(ng/L)

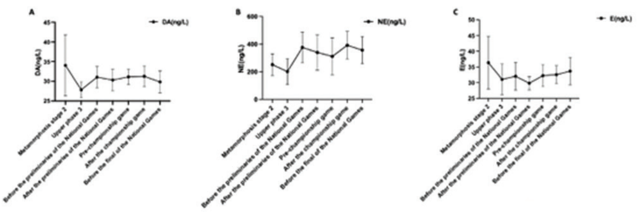


Table 6. List of correlation coefficients between DA and functional monitoring indicators

	HGB	BU	CK	SF	T	T/C
Metamorphosis stage 2	-0.601[a]	-0.008	0.165	-0.193	-0.573[a]	0.374
Training phase 3	-0.043	-0.308	-0.241	0.199	0.111	0.286
Pre-heat	-0.402	-0.241	-0.476	0.008	0.688[a]	-0.706[b]
After the heats	0.196	0.034	-0.112	-0.010	-0.211	-0.002
Pre-championship game	-0.183	-0.045	-0.042	0.107	0.565[a]	0.437
Post-championship game	0.132	-0.002	-0.560[a]	-0.284	-0.144	-0.059
Before the National Games	-0.197	0.145	0.309	0.438	0.505	0.324

Note: [a]$P < .05$, [b]$P < .01$

Table 7. List of correlation coefficients between NE and functional state indicators

	HGB	BU	CK	SF	T	T/C
Metamorphosis stage 2	-0.244	-0.104	-0.165	-0.154	-0.226	0.043
Training phase 3	0.063	-0.087	-0.556[a]	-0.165	-0.211	-0.449
Pre-heat	-0.333	0.000	0.084	0.187	0.136	0.372
After the heats	0.305	-0.182	-0.261	-0.052	0.564[a]	0.534
Pre-championship game	-0.175	0.017	0.114	-0.201	-0.088	-0.062
Post-championship game	-0.122	-0.147	0.405	-0.186	-0.436	-0.132
Before the National Games	0.197	0.037	0.046	0.470	0.113	0.079

Note: [a]$P < .05$.

Table 8. E List of correlation coefficients with functional status indicators

	HGB	BU	CK	SF	T	T/C
Metamorphosis stage 2	-0.337	-0.351	0.501	-0.138	-0.316	0.308
Training phase 3	-0.175	-0.395	-0.443	0.201	-0.062	-0.369
Pre-heat	-0.675[a]	-0.020	0.278	0.228	-0.251	-0.129
After the heats	-0.175	-0.351	-0.189	-0.354	0.156	0.429
Pre-championship game	-0.311	-0.089	-0.183	0.095	-0.254	-0.523
Post-championship game	0.096	0.508	-0.045	-0.186	0.340	0.275
Before the National Games	-0.266	0.124	0.027	-0.635[a]	-0.240	-0.031

Note: [a]$P < .05$.

Correlation between different training stages of catecholamines and functional state indicators

From Tables 6-8 it can be observed that the correlations between DA, NE, E, and HGB vary in different training stages, and even positive and negative correlations may simultaneously appear in one indicator. DA showed significant correlations with T in multiple stages ($P < .05$).

DISCUSSION

Analysis of the change characteristics of functional state indexes in different training stages

In volleyball, different training stages include the training stage, competition stage, league stage, and national games stage. The different training phases should not only improve the athletes' skills but also increase their athletic endurance and adaptability to adapt to higher training and competition loads. Excessive training loads can lead to severe fatigue, muscle damage, and other injuries that can degrade an athlete's physical fitness in the long run.

Monitoring the changes in hemoglobin during training can understand the adaptability of athletes to the training load. Yu Liang et al. found that training based on endurance will increase the hemoglobin of athletes, while training based on speed will decrease the hemoglobin of athletes. In this study, it was found that hemoglobin decreased significantly at the end of the first training phase and recovered slightly after 1 week of adjustment. The decline occurred again in the two subsequent periods. It is the stress response of the body to the increase in training load. However, in the two subsequent stages, although hemoglobin decreased, the decrease was smaller than that in the first stage, which is also a manifestation of the body gradually adapting to the training load. Guo Li et al.[12] believe that hemoglobin is an effective indicator to reflect the change of athletes' physical function. At the beginning of the large exercise training, the metabolism level of the body is enhanced, and the consumption increases significantly, which is easy to leads to a significant decrease in the hemoglobin level in the body. After an upper stage of training, the body gradually adapts to the amount of exercise, the functional state begins to improve, and the hemoglobin level begins to rise, which is the performance of improved athletic ability and improved functional state. In the competition stage, it can be seen that the hemoglobin increased during the competition, indicating that the adjustment training before the competition made the body function gradually reach the best level. Finally, the hemoglobin increased steadily from the championship competition to the pre-National Games stage and reached the best level of function when starting before the National Games. Zhao Guangsheng et al.[13] tracked and monitored the athletes preparing for the 13th Asian Games and found that after the Metamorphosis stage, the athletes' functional state rose to a better level before the game. Therefore, through this tracking and monitoring, the athlete's functional status can be understood, so that the coach can adjust the training plan at any time so that the athlete's physical function topic can reach the best level before the competition.

In this study, CK will significantly increase in the upper dose stage, decrease in the Metamorphosis stage, and then increase again in the upper dose stage, but the increased amplitude is smaller than that in the previous upper dose stage. Therefore, the increase of CK gradually decreased in the three consecutive loading stages. High CK activity in the blood is often used as an indirect indicator of muscle cell membrane damage. Studies have shown that CK activity in tissues is higher than normal, which can increase the availability of cell energy and improve the contraction response of muscle fibers. Therefore, high levels of serum CK, in the absence of muscle injury or other pathological conditions, may reflect the level of individual enzyme tissue activity.[14] In the Metamorphosis stage after each exercise training stage, CK shows a significant decline and such training arrangement is very necessary, because exercise-induced muscle mechanical and metabolic micro-tears, if not compensated by proper recovery, may lead to higher risks of injury or overtraining, long-term decline in athletic ability and physical fitness.[15] Exercise-induced free radical compounds, along with pro-inflammatory and anti-inflammatory cytokines, are crucial during routine training. They cause metabolic changes that allow athletes to adapt to higher training loads and competitions. In the competition stage, CK was higher than CK after the competition due to training, but there was no significant difference. The average exercise intensity of volleyball games is moderate, as most of the body load comes from frequently repeated jumps,[16] but due to the centrifugal muscle contractions during the landing phase of many jumps by nature, these fast and violent movements lead to muscle damage and inflammatory responses.[17] Exercise-induced muscle damage is also included in complex interactions. Muscle rupture due to muscle fiber damage, impaired excitation-contraction coupling associated with local ATP depletion, changes in intracellular calcium homeostasis, oxidative stress, and inflammatory responses, etc. On the other hand, the products of reactive oxygen and nitrogen accumulation and cytokines stimulate changes in cell signal transduction and gene expression, which may lead to adaptive response contraction activities, changes in stress protein expression, and some antioxidant enzymes, DNA repair proteins, and mitochondrial electron transfer proteins are related.[8]

The trace element iron improves athletic performance by increasing the ability to transport oxygen.[18] Due to increased mechanical hemolysis, gastrointestinal problems, and iron loss due to sweating, elite athletes are at increased risk of iron deficiency and iron deficiency anemia.[19] In fact, reduced iron reserves can seriously affect aerobic capacity, strength, muscle fatigue, and skeletal muscle recovery in elite athletes.[20] Foreign studies[21] suggest that the iron blood indexes of female athletes should be monitored especially, because female volleyball players have certain negative effects on iron metabolism. In this study, we observed that the ferritin was in a continuous downward trend during the three training phases, and the ferritin began to rise after the athletes entered the competition stage. We speculate that the increase in ferritin during this period may be due to a decrease in hepcilin due to low iron reserves, which leads to an increase in iron intake at the gut level.[22] The decrease in strength during the race produces inflammatory cytokines that interfere with iron supply, ultimately leading to a negative effect of iron metabolism on red blood cell production of hemoglobin and iron content.[23] According to foreign research reports,[24] during the competition period, the iron reserve of female volleyball players dropped to the lowest level in the 18 weeks after iron supplementation was stopped. In a highly competitive season, any advantage brought by iron supplementation would disappear in the 18 weeks after iron supplementation was stopped. In the case of no iron supplement, the iron reserve of female volleyball players will be reduced in more than 11 weeks. The results of this study were similar, and the serum ferritin reached its lowest level in the upper dose stage 3, i.e., week 12.

In the past few years, new discoveries have revealed the existence of a neurophysiological basis for regulating physical activity. Exercise is a mature dimorphic behavior in rodents in response to male hormones and estrogen.[25] Testosterone is a hormone known to be involved in skeletal muscle anabolic processes, while cortisol has catabolic functions.[26] Previous studies have shown that by monitoring blood levels of the hormones cortisol and testosterone, it is possible to determine the level of physical stress caused by the training load during a specific training or competition.[27] This study found that for volleyball players, T and T/C showed the same change rule. In the adjustment week after the end of the first training stage, athletes' T increased, but T/C did not return to the basic level, which may be due to the stress effect of the body on the heavy load of exercise in the training phase. The one-week adjustment did not make the body recover well but did not cause fatigue accumulation. Subsequently, after the body adapted to the training rhythm, the change amplitude of T and T/C tended to be consistent, showing the same change law, and showed an upward trend during the preliminary competition and the championship, which also showed that the functional state and competitive state maintained a better level during the competition. Finally, before the final of the National Games, T and T/C reached the highest value of the year. Mazon[28] studied the effects of the training cycle on cardiac autonomic nerve regulation and endogenous stress markers of volleyball players and found that T and T/C were significantly increased after the match, which was consistent with the results of this study. Both T and T/C were significantly increased, indicating the state of anabolic metabolism, especially T/C, as a powerful endocrine biomarker of "anabolic catabolic balance", is a diagnostic tool for evaluating overtraining of athletes.[29] The significant increase in T/C during the competition indicates an anabolic trend that is beneficial to competition performance. Although it is clear that sex hormones are important neuroendocrine regulators of physical activity behavior, their mechanisms of action are still poorly understood. Most simply assess the relationship between sex hormones and human physical activity through objective indicators.

It is worth noting that in this study, HGB, T, and T/C commonly used functional status monitoring indicators showed the same change trend in different training cycles (Training and Metamorphosis stages) and competition cycles, and finally reached the highest value before the National Games. In combination with the good results of the Shanghai Women's Volleyball team in the final of the National Games as a dark horse, it shows that the training load in the 21 weeks of preparation and competition stage has a positive impact on the body of the athletes, so that the athletes finally reach the best functional state and competitive state of the year before the National Games, and achieve excellent results.

Analysis of catecholamine change characteristics at different training stages

Catecholamines play a crucial role in the regulation of physiological processes during exercise. During high-intensity exercise, the plasma catecholamine concentration increases rapidly, resulting in increased cardiac output, contraction of non-contractile muscle blood vessels, increased stimulation of sweat glands, delivery of oxygen and energy substrates, and increased contractile force of skeletal muscle.[30] Studies have shown that training regulates autonomic activity. Adaptations associated with long-term training include a decrease in sympathetic nerve activity and an increase in parasympathetic activity.[31] Studies have found that after long-term training, the level of adrenaline in the human body is low in the quiet state.[29] In these studies, the decrease in adrenaline seemed to have more to do with exercise intensity than with duration. In the study of 21 consecutive weeks of incremental load training for swimmers, there were significant differences in the response of catecholamine to training, and adrenaline and dopamine were negatively correlated with training intensity and load.[32] The results of this study were similar, with the same changes in dopamine and adrenaline, which decreased in the Training phase and increased in the Metamorphosis stage, and were negatively correlated with the training load. Foreign studies have found a pathway for DA to produce trace amounts of NO through endogenous morphine and mu3 receptors on endothelial cells. Exercise upregulates DA and this pathway. However, over exercise, the negative feedback of NO down-regulates DA.[33] Dopamine in the brain is a neurotransmitter associated with fatigue, a feeling that results in reduced or interrupted exercise intensity, which regulates athletic performance. Fatigue has recently been proposed as a defense mechanism rather than a physiological malfunction in the context of prolonged aerobic exercise. Experiments in rodents clearly show that increased dopamine is associated with improved performance.

In physical training, high training load and frequent competition may trigger a physical stress response, leading to changes in neurotransmitters. The high load during the training phase may lead to elevated dopamine levels, which helps to improve the attention and excitement state of the athlete, which is beneficial for the training of technique and skill. However, excessive training load and lack of adequate recovery may trigger the release of norepinephrine and epinephrine, which may lead to increased fatigue, tension, and stress responses. The release of dopamine may be further increased during the competition phase, especially the intense competition phase, helping athletes to maintain a high level of concentration and positive mood to cope with competitive stress.

In addition, the frequency of training and competition may also influence changes in neurotransmitters. Frequent high-intensity training and competition may result in the cumulative release of dopamine, norepinephrine, and epinephrine, thereby increasing the risk of central fatigue to some extent. Different phases may differ in training intensity and recovery time, thus affecting the balance of neurotransmitters. For example, higher training intensity and shorter recovery times may lead to rapid increases in dopamine levels, whereas prolonged high-load training may lead to sustained increases in norepinephrine and epinephrine.

In the preliminary and championship stages of the National Games, the changes in catecholamine indexes were completely opposite. In the qualifying stage of the National Games, DA, E, and NE showed a downward trend, while in the championship stage two weeks later, DA, E, and NE showed an upward trend. The reason may be related to the difference in the training load before the two competitions and the different durations of the two competitions. The National Games qualifiers enter the pre-competition preparation stage immediately after the end of the third phase, the adjustment time is short and the body load is large. The significant increase of DA and NE during competition reflects the positive adaptation to the training load during competition. At the same time, E showed an increasing trend after the qualifiers until the National Games, and the increase of E was related to receptor adaptation, which occurred under a load of training and competition. This may reflect a phenomenon known as "motor adrenal", which is characterized by increased adrenaline-secreting capacity due to changes in the density and sensitivity of adrenergic receptors in well-trained subjects.[34] High-performing skiers have a high functional reserve of the adrenaline system, which is 10 times higher during a long race than during rest.[35] Foreign studies have found that before and after 12 weeks of consecutive league games, plasma epinephrine increased significantly, while total catecholamine and norepinephrine levels decreased. This is not the same as the results of our study in the competition stage. Analysis of the reason, the foreign study of the competition stage is the 12-week league, the average weekly game 1-2 games, while our competition stage is 7-10 days, 1 game a day. The difference in race density and race interval leads to the difference in the load of the race stage, so the results of the study are different.

In the competition stage, DA synthesis and metabolism tend to balance, and metabolism is relatively reduced. Wang Chen et al.[36] believe that catecholamines can be used to assess athletes' functional status in training monitoring, and athletes' load adaptation can be judged according to the content of dopamine in the recovery period, and then the training load can be adjusted. DA results of volleyball players show that with the continuous increase of sports training time, the sympathetic nervous system activity is enhanced, and the catecholamine output will also increase. It is suggested to establish a catecholamine index database for longitudinal training monitoring of athletes, and indirectly assess the physical function status of athletes by comparing the catecholamine output of athletes in different training periods, so as to guide coaches to adjust the training plan and content arrangement of athletes in time. Only by paying attention to the monitoring of athletes' routine training, especially the monitoring of training in different periods before the competition, doing scientific preparation, and ensuring that athletes are in the best competitive state and give full play to their due level, can satisfactory competition results be achieved.

Correlation between catecholamines and functional status indicators at different training stages

"Endogenous stress markers" refer to the changes in a series of biochemical indexes or physiological parameters that occur when the body is exposed to different types of stress (such as sports training, competition, tension, etc.). "Central fatigue" refers to the state of the central nervous system after prolonged high-intensity activity, which is manifested as decreased attention, decision-making ability, and reaction speed, as well as increased emotional fluctuations and fatigue. This fatigue is not only reflected in muscle fatigue but also in the nervous system, that is, central fatigue. Central fatigue may be caused by a variety of factors, including changes in neurotransmitters, increased brain energy expenditure, and so on. The appearance of central fatigue may affect the performance and competitive performance of athletes. Therefore, studying the changes of endogenous stress markers and central fatigue will help to better understand the physiological and psychological state of athletes during training and competition, as well as how to effectively manage the load of training and competition, so as to improve competitive performance and prevent excessive fatigue.

The inhibitory process produced by the central nervous system after exercise does not occur in the whole brain but shows an inhibitory effect in the hypothalamus and an excitatory effect in the midbrain and striatum. The research shows that the high content of DA in the brain is conducive to the maintenance of athletic ability and the delay of athletic fatigue. When DA is discharged too much, the athletic endurance will decrease significantly, which will affect the athletes' performance.[37] The results of this study showed that DA, NE, and E decreased significantly after the end of the Training Phase 3, suggesting obvious central fatigue in the training stage. Combined with the functional state indexes HGB and T decreased significantly in the Training Phase 3, it indicates that the fatigue caused by the more training load stage is a double fatigue of physical fatigue and mental fatigue. The decrease of DA content in the brain, the weakening of activity, and the decrease of concentration will cause nerve disorders and the reduction of muscle coordination, which will lead to the decline of motor ability. Foreign studies have proved that excessive exercise load can reduce DA and other levels by NO negative feedback, while DA does not significantly reduce under appropriate exercise load.[38] Therefore, T is still in a downward trend in the pre-preliminary stage, but DA, NE, and E begin to rise, and DA and NE are significantly higher than the pre-preliminary level. These results indicate that catecholamines are more sensitive to training load than HGB and T. Because the training load in the pre-competition stage is smaller than that in the upper stage, but larger than that in the Metamorphosis stage, HGB and T are still in a downward trend at this time, but the situation that catecholamine has begun to rise may indicate that the physical state may not be adjusted to the best level, but the mental state has reached a better level.

The correlation between T and DA may indicate the reciprocal relationship between the physiological and mental states of athletes during different training phases. T (testosterone) is a male hormone that is closely related to physiological processes such as muscle growth, strength, and endurance. DA (dopamine) is a neurotransmitter involved in mental processes such as mood, motivation, and attention. During the high-intensity physical training phase, athletes may experience physical stress, leading to a decrease in T levels, and at the same time DA levels may increase, reflecting psychological excitement and increased alertness. This opposite change between T and DA may be a physiological and psychological response of athletes to adapt to high-intensity training.

After the preliminary stage and the pre-championship Metamorphosis stage, T and catecholamine were on the rise at the same time in the championship stage, indicating that the physical function state and endogenous neural markers had recovered to a better level to cope with the competition stage. Foreign studies have found that the sex hormone is an important neuroendocrine regulator of physical activity behavior, but its mechanism is still poorly understood.[39] The brain dopamine system is considered to be the ultimate common pathway of a complex network of neuromediators that helps regulate physical activity, especially the activation of striatal DA receptor DR1 and DR2 neurons with selective control over movement.[40] Animal models are an indispensable tool for understanding how T affects motor behavior. Mice with testicular removal and androgen receptor knockout had late cancellation of wheel movement, both replicating several behavioral features of human sex hormone deficiency.[40] This study shows that there is a significant correlation between T and DA at multiple stages. Some experts have found that testosterone primarily acts on brain circuits that stimulate movement, and autonomic movement may be regulated by a complex network of neurotransmitters, but the dopamine system is thought to be the ultimate common pathway. Other experts believe that testosterone acts on the dopamine system by regulating the availability of extracellular dopamine.[41] Orchiectomy affects not only the basal concentration of extracellular DA, but also its release during stimulation. Testosterone may inhibit the enhanced release of dopamine in the brain of ORX rats after amphetamine stimulation, thus weakening the resulting motor response. Testosterone deficiency significantly impaired physical activity in male mice, and the effect was a purely central phenomenon rather than the result of muscle dysfunction. Testosterone regulates the brain's control of movement primarily through a dopamine-dependent pathway. Although studies have shown that testosterone plays a role in dopamine synthesis, release, and metabolism, the results are mostly inconsistent.[42-44] Therefore, in order to better understand the neuroendocrine interaction between male hormones and the central dopamine system, further research is necessary.

This study, while providing valuable insights into the functional status and central fatigue state of athletes, has certain limitations. Firstly, the research focused specifically on the Shanghai women's volleyball team, limiting the generalizability of the findings to other sports or athlete populations. Secondly, the study duration of 21 weeks might not capture long-term changes or seasonal variations that could affect athletes' functional status differently. Additionally, although comprehensive tests were conducted, other potential influencing factors like nutrition, sleep patterns, and psychological stressors were not extensively explored, which could have provided a more holistic understanding of the athletes' overall well-being. Furthermore, the study did not consider individual differences among athletes, such as their training responses and recovery rates, which could impact the observed results. Lastly, while the study emphasized the importance of different indicators, it did not delve into specific interventions or strategies to mitigate central fatigue, leaving room for further research in the field of sports science and performance optimization.

In summary, this study underscores the intricate interplay between hormones and neurotransmitters in athletes' performance. Future research should focus on unraveling the specific molecular mechanisms governing these interactions. Longitudinal studies considering diverse training phases and individual differences are essential. Investigating the impact of nutrition and psychological factors, coupled with advanced technologies, can provide real-time insights. Interdisciplinary collaborations are crucial for a holistic approach. These efforts are pivotal in developing personalized interventions and advancing our understanding of athlete well-being and performance.

CONCLUSION

Functional State Index Changes: The study reveals diverse trends in the functional state index across training stages. Notably, the index declines during the more training load stage but rebounds post-adjustment, with a notably slower decline after the second dose. The competition stage exhibits a significant improvement, indicating enhanced functional and competitive states among athletes.

Types of Fatigue: Fatigue during the Training Phase encompasses both body and central fatigue. Blood dopamine and epinephrine prove particularly sensitive during the the more training load stage, while norepinephrine maintains high levels during competition. Moreover, a strong correlation exists between serum testosterone and dopamine levels.

Effective Indicators: Hemoglobin and blood testosterone emerge as effective markers for evaluating volleyball players' functional status across different training stages, providing valuable insights into athletes' physiological responses.

Practical Implications:

Understanding these nuanced changes in functional state indices is pivotal for sports practitioners. Coaches, trainers, and athletes can utilize this knowledge to optimize training regimens and enhance performance strategies. For instance, recognizing the specific stages where functional states decline enables targeted adjustments in training intensity and recovery protocols. Coaches can tailor training schedules, focusing on periods of optimal functional states, thus maximizing athletes' readiness for competitions. Moreover, the observed correlation between serum testosterone and dopamine suggests a potential avenue for intervention. Coaches and trainers could explore strategies to modulate these hormones, possibly through targeted nutrition or psychological interventions, to manage fatigue and optimize performance.

By integrating these practical implications into training programs, sports professionals can proactively enhance athletes' functional states, ensuring peak performance and promoting overall well-being.

ETHICAL COMPLIANCE

This study was approved by the ethics committee of Shanghai Research Institute of Sports Science (Shanghai Anti-Doping Agency). Signed written informed consents were obtained from the participants.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

AUTHOR CONTRIBUTIONS

XW and DZ designed the study and performed the experiments, XW and LH collected and analyzed the data, XW and YM prepared the manuscript. All authors read and approved the final manuscript.

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