

## **Change in the red blood cell immunity function and T-lymphocyte and its subpopulations before and after acute incremental load exercise.**

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### **Abstract**

**Objective:** This paper discusses the changes in the Red Blood Cells (RBC) immunity function and T-lymphocyte and its sub-populations before and after acute incremental load exercise.

**Methods:** A total of 112 patients in our hospital from January 2015 to May 2016 were selected as research objects. Blood samples at four stages (before the exercise (T1), 1 min (T2), 0.5 h (T3) and 24 h (T4) after the acute incremental load exercise) were collected from antecubital veins. Changes in RBC-C3bRR, RBC-ICR, CD3+, CD4+, CD8+ and CD4+/CD8+ before and after the exercise were compared.

**Results:** The RBC-C3bRR values at T2 and T3 were  $6.22 \pm 0.94\%$  and  $6.95 \pm 0.79\%$ , respectively, which were significantly lower than the value before the exercise ( $8.32 \pm 1.03\%$ ) ( $P < 0.05$ ). The RBC-ICR values at T2 and T3 were  $16.97 \pm 1.37\%$  and  $16.53 \pm 1.44\%$ , respectively, which were significantly higher than the value before the exercise ( $15.32 \pm 1.65\%$ ) ( $P < 0.05$ ). However, these two indexes recovered to the normal state at T4 but were slightly lower than the value before the exercise. No significant differences were observed between the two groups ( $P > 0.05$ ). CD3+, CD4+, CD8+ and CD4+/CD8+ at T3 decreased continuously and recovered to the normal conditions at T4. T-lymphocyte and its subpopulations changed slightly before and after the acute incremental load exercise. The difference between groups had no statistical significance ( $P > 0.05$ ).

**Conclusions:** After the acute incremental load exercise, RBC-C3bRR in human blood decreased significantly, whereas RBC-ICR increased dramatically, indicating potential risks of secondary erythrocyte immunodeficiency. Erythrocyte immunosuppression persisted until 24 h after the exercise. The T-lymphocyte level and CD4+/CD8+ decreased continuously after the exercise and recovered to normal levels 24 h later. No significant fluctuations were observed in T-lymphocyte and its subpopulations.

**Keywords:** Acute incremental load exercise, RBC immunity function, T-lymphocyte subpopulation.

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### **Introduction**

As societies develop, exercise regimens enjoy popular support. People are concerned about exercises and the importance of self-adjustment in terms of health under the premise of improved living quality. The Red Blood Cell (RBC) adherent immunity function plays an important role in disease and tumor resistance [1,2]. Depression of the RBC adherent immunity function causes various diseases, such as rheumatoid arthritis, autoimmune hepatitis, angitis, systemic lupus erythematosus, nephritis, and malignant tumors [3]. Exercise can enhance the transportation of RBC immune complex receptor rosettes, and appropriate physical exercise can improve the immunity of human bodies [4]. To explore changes in the RBC immunity function and T-lymphocyte and

its subpopulations before and after acute incremental load exercise, 122 patients in our hospital from January 2015 to May 2016 were selected as research objects. Blood samples were collected at four stages before and after the exercise. Changes in RBC-C3bRR, RBC-ICR, CD3+, CD4+, CD8+ and CD4+/CD8+ before and after the exercise were compared.

### **Data and Methods**

#### **General information**

The 122 subjects did not have immune system or endocrine diseases, mental disorders, expression problems or dyskinesia. They had not participated in any vigorous exercises and had normal diets scheduled 72 h before the test. All subjects

volunteered to participate in this study and signed an informed consent form. The subjects comprised 83 males and 39 females aged 16-33 y ( $22.7 \pm 3.1$  on an average); the average weight and height were  $62.4 \pm 5.2$  kg and  $173.2 \pm 3.4$  cm, respectively.

## Methods

### Exercise methods

All subjects were at calm state and subsequently asked to perform acute incremental load exercise on an indoor exercise bike. All respondents had 1 min of warm up, and the initial power of the exercise bike was 25 W. The power of the exercise bike in the official experiment was maintained at 80 W and 60 rpm/min until the subjects were exhausted.

### Research methods

For the blood sample collection, venous blood samples were collected from antecubital veins before and after (1 min, 0.5 h and 24 h) the acute incremental load exercise. Changes in RBC-C3bRR and RBC-ICR were tested through Wright's staining method. The expression rates of T-lymphocyte subpopulations CD3+, CD4+, and CD8+ were tested through conventional direct immunofluorescent labelling.

### Judgment standard of exhaustion

Five standards were established as follows: HR  $\geq 180$ /min, Respiratory Quotient (RQ) $>1.2$ , BLA  $\geq 9$  mmol/L, maximum oxygen uptake occurs, and akinesia of the subjects. If three of these five standards are met by a subject, then the subject is deemed exhausted.

### Statistical method

All data were processed with SPSS12.0, expressed as ( $\bar{x} \pm s$ ), and tested through a t-test.  $P < 0.05$  denotes a statistically significant difference.

## Results

### Changes in RBC-C3bRR and RBC-ICR before and after the acute incremental load exercise

RBC-C3bRR and RBC-ICR at T2 and T3 were compared with those at T1, and significant differences ( $P < 0.05$ ) were observed. RBC-C3bRR and RBC-ICR recovered to normal levels at T4 and were slightly lower than those before the exercise. No significant difference was observed between the two groups ( $P > 0.05$ , Table 1).

**Table 1.** Changes in RBC-C3bRR and RBC-ICR ( $\bar{x} \pm s$ , %).

	RBC-C3bRR	RBC-ICR
Before	$8.32 \pm 1.03$	$15.32 \pm 1.65$
1 min after	$6.22 \pm 0.94$	$16.97 \pm 1.37$
0.5 h after	$6.95 \pm 0.79$	$16.53 \pm 1.44$

24 h after	$8.24 \pm 1.02$	$15.12 \pm 1.38$
t <sub>1</sub> , P <sub>1</sub>	16.6339, <0.05	8.4979, <0.05
t <sub>2</sub> , P <sub>2</sub>	11.6573, <0.05	6.1026, <0.05
t <sub>3</sub> , P <sub>3</sub>	0.6096, >0.05	1.0270, >0.05

### Changes in T-lymphocyte and its subpopulations before and after the acute incremental load exercise

The proportions of CD3+, CD4+, CD8+, and CD4+/CD8+ decreased continuously at T3 and recovered to normal levels before the exercise at T4. T-lymphocyte and its subpopulations changed slightly before and after the acute incremental load exercise. No statistically significant difference was found between the two groups ( $P > 0.05$ , Table 2).

**Table 2.** Changes in T-lymphocyte and its subpopulations ( $\bar{x} \pm s$ ) ( $n=122$ ).

	CD3+ (%)	CD4+ (%)	CD8+ (%)	CD4+/CD8+
Before	$76.32 \pm 8.77$	$43.26 \pm 9.34$	$38.60 \pm 9.58$	$1.33 \pm 0.36$
1 min after	$74.36 \pm 8.01$	$41.21 \pm 9.77$	$37.98 \pm 7.54$	$1.30 \pm 0.32$
0.5 h after	$72.96 \pm 16.78$	40.81 10.73	$\pm 36.58 \pm 6.29$	$1.26 \pm 0.27$
24 h after	$77.27 \pm 8.03$	$41.99 \pm 8.49$	$38.95 \pm 9.78$	$1.22 \pm 0.40$
t <sub>1</sub> , P <sub>1</sub>	1.8227, >0.05	1.6752, >0.05	0.5617, >0.05	0.6880, >0.05
t <sub>2</sub> , P <sub>2</sub>	1.9601, >0.05	1.9023, >0.05	1.9468, >0.05	1.7182, >0.05
t <sub>3</sub> , P <sub>3</sub>	0.8825, >0.05	1.1114, >0.05	0.2824, >0.05	0.8209, >0.05

Note: In Tables 1 and 2, t<sub>1</sub> and P<sub>1</sub> are comparisons between T1 and T2, t<sub>2</sub> and P<sub>2</sub> are comparisons between T1 and T3, and t<sub>3</sub> and P<sub>3</sub> are comparisons between T1 and T4.

**Table 3.** Changes in immune factors before and after the exercise.

Exercise time	IFN- $\gamma$ (pg/mL)	IL-4 (pg/mL)	Th-1/Th-2
Before	$25.33 \pm 1.84$	$2.11 \pm 14.29$	$18.57 \pm 5.11$
0.5 h after	$34.65 \pm 1.9^a$	$2.81 \pm 11.06^c$	$23.02 \pm 4.73$
24 h after	$39.29 \pm 1.54^b$	$2.65 \pm 12.76^d$	$11.27 \pm 3.58$

Note: <sup>a,b</sup>Comparison results with those before the exercise,  $P < 0.05$ ; <sup>c,d</sup>Comparison results with those before the exercise,  $P > 0.05$ . Th-1/Th-2 is IFN- $\gamma$ /IL-4.

### Changes in FN- $\gamma$ and IL-4 before and after the acute incremental load exercise

Analysis of the data in Table 3 revealed that IFN- $\gamma$  and Th-1/Th-2 at T4 decreased significantly compared with those at T1 ( $P < 0.05$ ).

IL-4 showed no statistically significant difference before and after the exercise ( $P > 0.05$ , Table 3).

## **Discussion**

A protein called Complement Receptor (CR1) exists on the surface of RBCs. It can stick to the immune complex and is the main carrier of the Circulating Immune Complex (CIC) in human bodies. RBC immune complexes are supplemented through the cell surface receptor and subsequently carried to the liver and spleen and digested by macrophage, thereby eliminating “wastes” of CIC in blood vessels [5]. RBCs that are supplemented by cell surface receptor proteins can support the circulation of viruses and bacteria in the blood, which makes them easier to be digested by macrophage, thus increasing the immunity of the human body. Antigens recognized and carried by the RBC immune system eliminate CIC, increase immune responses of T-lymphocytes, and promote macrophage phagocytosis [6]. Peroxidase on the RBC cytomembrane influences immune complex macrophages and produces large amounts of RBCs (the amount of RBCs is 1000 times higher than that of leukocytes). Exercise can enhance the transportation of RBC-ICR, and appropriate physical exercise can enhance the immunity of human bodies.

Incremental load exercise or process load exercise involves the gradual application of loads. According to relevant studies, exercise can change the immune functions of human bodies. After acute incremental load exercise or exhausting exercise, the immune functions of athletes decrease and susceptibility increases, which is damaging for physical health [7]. Clinical studies have revealed the presence of several cytokine profiles in human bodies. Different types of cell molecules have different secretion objects. The secretion object of IFN- $\gamma$  is Th-1, and the secretion object of IL-4 is Th-2. Th-1/Th-2 is often balanced in human bodies at calm states, indicating good immune functions under this circumstance. However, Th-1/Th-2 imbalance enhances or weakens the immune functions of human bodies [8]. Appropriate physical exercise can improve these functions because exercise can stimulate the immune system to respond, which further stimulates a series of immune reactions by perceiving internal environmental changes with its complicated recognition system; the reactions include producing a specific antibody, enhancing the activity of K cells, increasing leukocytes and sensitized achrocytosis, releasing cytokines (e.g., immunomodulatory factors IL-1, IL-2, and IL-6 as well as tumor necrosis factor) and maintaining new internal environmental stability within the body. Long-term repetition of appropriate exercise loads can maintain the immunity of bodies at a high level [9]. Research has proven that one appropriate aerobic exercise can increase leukocytes and immune globulin significantly; physical exercise can even improve disease resistance. Generally, acute exercise exerts only a temporary impact on the immune system, and only long-term physical exercises can exert a durable effect on the immune system, thus enhancing human immunity and preventing diseases [10].

To discuss the changes in the RBC immunity function and T-lymphocyte and its subpopulations before and after the acute incremental load exercise, 112 patients were selected as research objects in this study. Blood samples were collected at

four stages (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub>) and tested. Changes in RBC-C3bRR, RBC-ICR, CD3+, CD4+, CD8+, and CD4+/CD8+ before and after the exercise were compared. RBC-C3bRR and RBC-ICR at T<sub>2</sub> and T<sub>3</sub> exhibited statistically significant differences with those at T<sub>1</sub> (P<0.05). RBC-C3bRR and RBC-ICR recovered to normal levels at T<sub>4</sub>. These changes in RBC-C3bRR and RBC-ICR indicate that RBC immune functions were inhibited gradually after the acute incremental load exercise until 24 h later. Furthermore, the proportions of CD3+, CD4+, CD8+, and CD4+/CD8+ decreased continuously at T<sub>3</sub> and gradually recovered to normal levels before the exercise at T<sub>4</sub>. T-lymphocyte and its subpopulations changed slightly before and after the acute incremental load exercise and showed no statistically significant intergroup differences, indicating that T-lymphocyte level remained basically the same after the acute incremental load exercise; its immune response is insensitive to RBC.

## **Conclusion**

RBC-C3bRR decreased sharply, whereas RBC-ICR increased dramatically after the acute incremental load exercise. They showed potential risks of secondary erythrocyte immunodeficiency. Erythrocyte immunosuppression remained until 24 h after the exercise. T-lymphocyte level and CD4+/CD8+ decreased continuously after the exercise and recovered to normal levels 24 h later. No significant fluctuations were observed in T-lymphocyte and its subpopulations.

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