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Full Length Research Paper

Immune function changes of rats in incremental graded exercise test

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This study evaluated the effect of incremental graded exercise on immune function of spleen and thymus in rats. The animals were divided into 6 groups, with 32 animals in per group: no exercise (W0), exercise for 1 week (W1), exercise for 2 weeks (W2), exercise for 3 weeks (W3), exercise for 4 weeks (W4) and exercise for 5 weeks (W5). Each group was further divided into 4 groups, with 8 animals in per group: sedentary (S), no recovery after exercise (AE0), recovery for 3 h after exercise (AE3), and recovery for 24 h after exercise (AE24). Incremental graded exercise test was performed on a rodent treadmill (gradient of 0°), which lasted for 5 weeks. The rats were motivated to exercise for 30 min a day for 6 days a week and weekend breaks. Speed in the treadmill gradually increased to 5 (W0), 20 (W1), 25 (W2), 30 (W3), 35 (W4) and 40 m/min (W5). At the end of exercise, the rats were killed, and thymus and spleen tissue were collected and weighed. The results showed that incremental exercise affected the immune function of spleen and thymus. With the incremental implementation and extension of exercise time, the effect of exercise on spleen index and thymus index became increasingly greater.

Key words: Incremental graded exercise, immune function, spleen index, thymus index rats.

INTRODUCTION

Over the past two decades, the response of the immune system to exercise and sport has evolved into a topic of significant interest to both health and sport professionals (Radons and Multhoff, 2005). A large number of studies suggest that the immune function is likely to change with effects of exercises, and the changes may have different performances with different forms, intensity and amount exercises. Body resistance to disease, that is, immune function, can be improved through long-term regular exercises, while excessive exercises can suppress immune function, or even damage the immune system. Athletes' resistance to disease training decreases after high intensity training , which is shown as being more easily sensitive to infectious diseases (Braun and Duvillard, 2004; Bishop, 2005; Nieman, 2007; Senchina et al., 2009).

Spleen is the body's largest lymphoid organ, and the thymus is also an important immune organ in vivo (Nahrevanian and Dascombe, 2006; Renner et al., 2009). Spleen index was the ratio of spleen weight (mg) and body weight (g); and thymus index was the ratio of thymus weight (mg) and body weight (g) (Liu et al., 2008; Tian et al., 2010). Changes in spleen index and thymus index can reflect changes in immune function, which can provide experimental basis for study of the mechanism of immune function's change after exercises (Chen and Wang, 2007). In the present study, we investigated the effect of incremental graded exercise on immune function in rats through determined spleen index and thymus index.

MATERIALS AND METHODS

Experimental animals and their management

The protocol of the study was reviewed and approved by the local ethics committee. A total of 192 inbred adult male Spargue-Dawley rats weighing between 130 and 150 g were obtained from the

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Table 1. Animals grouping.

Exercise time	First grouping	Second grouping
0 week	No exercise (W0)	Sedentary (S-W0) No recovery after exercise (AE0-W0) Recovery for 3 h after exercise(AE3-W0) Recovery for 24 h after exercise(AE24-W0)
1 week	Exercise for 1 week (W1)	Sedentary (S-W1) No recovery after exercise (AE0-W1) Recovery for 3 h after exercise(AE3-W1) Recovery for 24 h after exercise(AE24-W1)
2 weeks	Exercise for 2 weeks (W2)	Sedentary (S-W2) No recovery after exercise (AE0-W2) Recovery for 3 h after exercise(AE3-W2) Recovery for 24 h after exercise(AE24-W2)
3 weeks	Exercise for 3 weeks (W3)	Sedentary (S-W3) No recovery after exercise (AE0-W3) Recovery for 3 h after exercise(AE3-W3) Recovery for 24 h after exercise(AE24-W3)
4 weeks	Exercise for 4 weeks (W4)	Sedentary (S-W4) No recovery after exercise (AE0-W4) Recovery for 3 h after exercise(AE3-W4) Recovery for 24 h after exercise(AE24-W4)
5 weeks	Exercise for 5 weeks (W5)	Sedentary (S-W5) No recovery after exercise (AE0-W5) Recovery for 3 h after exercise(AE3-W5) Recovery for 24 h after exercise(AE24-W5)

Experimental Animal Center of Hunan Province (Certificate No. 20071348). The rats were housed in metal cages in the metabolic laboratory with uniform temperature of 23 to 25°C, 12 h/12 h light/dark cycle and maintained with free access to standard rat chows and water made available ad libitum.

Exercise training protocol

After adapting to the lighting conditions for 1 week, rats were divided into 6 groups, with 32 animals in per group: no exercise (W0), exercise for 1 week (W1), exercise for 2 weeks (W2), exercise for 3 weeks (W3), exercise for 4 weeks (W4), exercise for 5 weeks (W5). As shown in Table 1, each group was further divided into 4 groups, with 8 animals in per group: sedentary (S), no recovery after exercise (AE0), recovery for 3 h after exercise (AE3) and recovery for 24 h after exercise (AE24).

Incremental graded exercise test was performed on a rodent treadmill (gradient of 0°) lasted for 5 weeks. The rats were motivated to exercise for 30 min a day for 6 days a week and weekend breaks. As can be seen from Figure 1, a gradual increase in the treadmill speed to 5 (W0), 20 (W1), 25 (W2), 30 (W3), 35 (W4) and 40 m/min (W5) (Chen and Wang, 2007). To avoid circadian variations in physical activity, exercise was performed between 8:00 and 10:00.

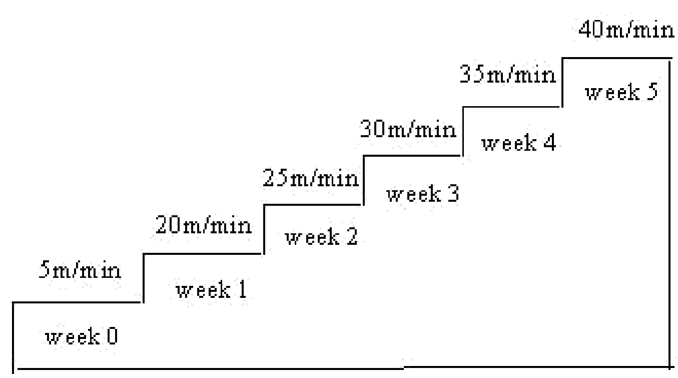

Figure 1. Exercise protocol.

Tissue preparation

At the end of exercise, the rats were anaesthetized with ether (an

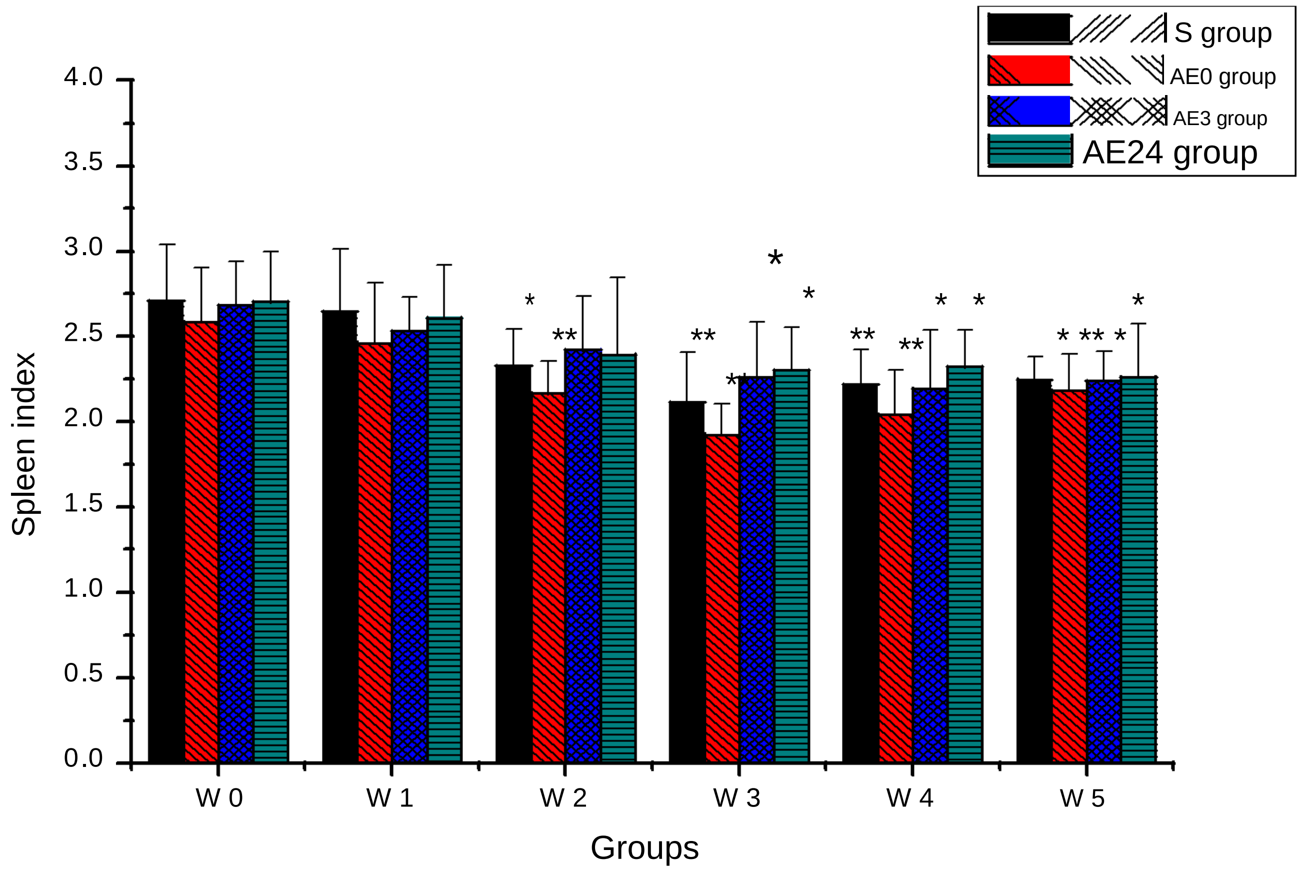

Figure 2. Effect of Incremental Exercise on spleen index of rats.
*** very highly significant (P<0.01),** highly significant (P<0.01), * significant (P<0.05), compared with the S-W0 group.

anesthesia chamber was utilized as the induction method of delivering volatile anaesthetic agent to the rats); the ether was volatilized by placing it on cotton balls in the bottom of the jar. Then all the rats were killed by decapitation, then thymus and spleen tissue were collected and weighed.

Evaluation of the immune function in rats

Immune functions in rats were evaluated through determined spleen index and thymus index. The spleen index and thymus index of rats were assayed according to the method (Gao et al., 2005) and calculated according to the following formula:

$$Spleen \quad index = \frac{Weight \quad of \quad spleen(mg)}{Body \quad weight \quad (g)}$$

$$Thymus \quad index = \frac{Weight \quad ofthymus(mg)}{Body \quad weight \quad (g)}$$

Statistical evaluation

Statistical analysis was performed using Student's t-test and one way analysis of variance (one way-ANOVA). The accepted level of significance was preset as P< 0.05. Data are represented as means ±SEM.

RESULTS

Effect of incremental exercise on spleen index of rats

Effect of incremental exercise on spleen index of rats is given in Figure 2. The spleen index of rats in W1 groups was not significantly different from that in the S-W0 group (P>0.05). The spleen index of rats in S-W2, AE3-W3, AE24-W3, AE3-W4, AE24-W4, S-W5, AE3-W5, and AE24-W5 groups were significantly lower than that of S-W0 group (P<0.05). The spleen index of rats in AE0-W2, S-W3, AE0-W3, S-W4, AE0-W4, and AE0-W5 groups were significantly lower than that of S-W0 group (P<0.01).

Effect of Incremental exercise on thymus index of rats

Effect of Incremental Exercise on thymus index of rats is given in Figure 3. The thymus index of rats in W1 groups was not significantly different from that in the S-W0 group (P>0.05). The thymus index of rats in S-W2, AE3-W2, AE24-W2, and AE3-W3 groups were significantly lower than that of S-W0 group (P<0.05). The thymus index of

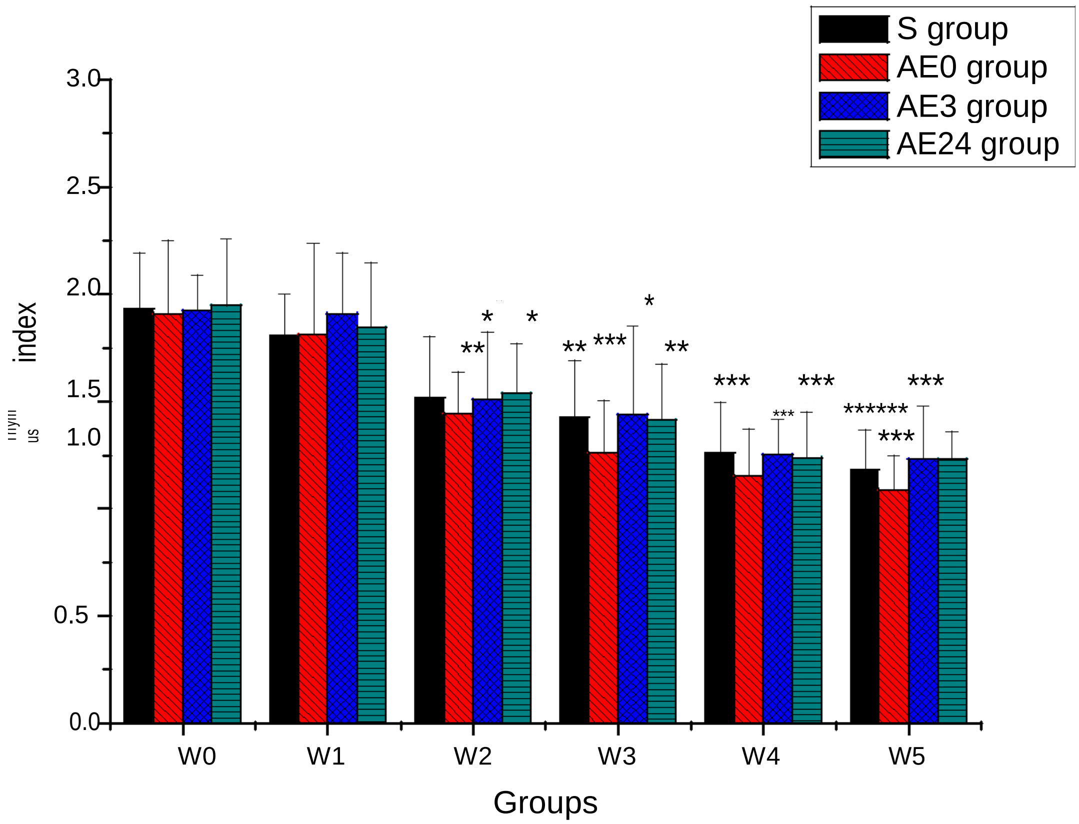

Figure 3. Effect of Incremental Exercise on thymus index of rats.
*** very highly significant (P<0.001),** highly significant (P<0.01), * significant (P<0.05), compared with the S-W0 group.

rats in AE0-W2, S-W3, and AE24-W3 groups were significantly lower than that of S-W0 group (P<0.01). The thymus index of rats in AE0-W3, S-W4, AE0-W4, AE3-W4, AE24-W4, S-W5, AE0-W5, AE3-W5, and AE24-W5 groups were significantly lower than that of S-W0 group (P<0.001).

DISCUSSION

In recent years, it has been demonstrated that physical activity provokes changes in the immune system. Moderate bouts of exercise have been shown to enhance immunity (Nieman, 1994; Pershin et al., 2002). However, intense exercise depresses the immune system (Brown et al., 2006). More specifically, during moderate and intense bouts of exercise there are transient increases in circulating pro- and anti- inflammatory cytokine levels, concentration of lymphocytes and lymphocyte sub-sets, and macrophage activity (McCarthy and Dale, 1988; Ostrowski et al., 1999; Romeo et al., 2010).

The spleen is the largest lymphoid organ in vivo. It is where lymphocytes stay, proliferate and produce immune response (Dos Santos Cunha et al., 2004). It's also where tuftsin, properdin factor and some complement components are synthesized and secreted (Harrus et al., 1998). Spleen is rich in macrophages, which can remove degenerative cells, various pathogens and other foreign particles in blood. External stimulation to the spleen will affect the immune function (Diamantstein and Odenwald, 1974). The data from this study demonstrate that the spleen index of rats in S-W2, AE3-W3, AE24-W3, AE3-W4, AE24-W4, S-W5, AE3-W5, and AE24-W5 groups were significantly lower than that of S-W0 group (P<0.05). The spleen index of rats in AE0-W2, S-W3, AE0-W3, S-W4, AE0-W4, and AE0-W5 groups were highly significantly lower than that of S-W0 group (P<0.01). This indicated that incremental graded exercise affected the immune function of spleen, naturally it also affected organism's immune function so as to lower immune function. With the incremental implementation and extension of exercise time, the effect of exercise on spleen index became increasingly greater, that is, spleen index was gradually decreased. This is similar to the results of previous studies (Li et al., 2004). In a state of exercise, neuroendocrine system and body's internal environment will have different effects on immune system, which itself will make corresponding changes to resist and adapt to exercise stress.

Thymus is an important immune organ *in vivo*.

Degradation and dysfunction of thymus is one of the reasons for decreased immune function (Zhou et al., 2006). Thymus is the central lymphoid organs, which produces immune function, and it is where immune cells are developed and differentiated. Thymus plays a leading role in organism's stability and immune surveillance, and its main function is to produce T cells and secrete factor secretion, bringing into play immune function (Huang et al., 2008). The data from this study demonstrate that the thymus index of rats in W1 groups was not significantly different from that in the S-W0 group (P>0.05). The thymus index of rats in S-W2, AE3-W2, AE24-W2, and AE3-W3 groups were significantly lower than that of S-W0 group (P<0.05). The thymus index of rats in AE0-W2, S-W3, and AE24-W3 groups were significantly lower than that of S-W0 group (P<0.01). The thymus index of rats in AE0-W3, S-W4, AE0-W4, AE3-W4, AE24-W4, S-W5, AE0-W5, AE3-W5, and AE24-W5 groups were significantly lower than that of S-W0 group (P<0.001). This indicated that incremental graded exercise affected the immune function of thymus. With the incremental implementation and extension of exercise time, the effect of exercise on thymus index became increasingly greater, that is, thymus index was gradually decreased. This is similar to the results of previous studies (Li et al., 2004). In a state of exercise, neuroendocrine system and body's internal environment will have different effects on immune system, and the hypothalamus - pituitary and thymus circle will have a direct effect on thymus index. Hypothalamus secretes growth hormone releasing hormone (GHRE), prolactin releasing factor (PRF) and thyrotropin releasing hormone (TRH), and it affects anterior pituitary, stimulates secretion of growth hormone (GH) and prolactin (PRL) which affect the development of thymus, thymus cells' function and hormone synthesis. As exercise stimulation produces abnormal secretion of much hormone, it affects the immune function of the thymus and results in suppression of organism's immune function.

Conclusion

In conclusion, this study demonstrates that incremental graded exercise affected the immune function of spleen and thymus. With the incremental implementation and extension of exercise time, the effect of exercise on spleen index and thymus index became increasingly greater. Future studies are required to elucidate the molecular and cellular mechanisms of the effect on immune function.

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