EFFECT OF 12-WEEK LOW-INTENSITY EXERCISE ON INTERLEUKIN-2, INTERFERON-GAMMA AND INTERLEUKIN-4 CYTOKINE PRODUCTION IN RAT SPLEENS

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ABSTRACT

Background and Objective
High-intensity exercise has been linked to immunity; however, the relationship between low-intensity exercise and the immune system is unclear. In this study, the effects of exercise on cytokine production in T helper 1 (interleukin-2 [IL-2] and interferon-gamma [INF-γ]) and T helper 2 cells (interleukin-4 [IL-4]) in spleens were investigated.

Material and Methods
Sprague–Dawley male rats were divided into a control group (CON, n = 10) and a low-intensity exercise group (EX, n = 10). EX rats were trained on a treadmill (8 m/min, 50 min/day, 5 times over 12 weeks). Spleen tissues were analyzed by hematoxylin-eosin staining and real-time PCR to quantify IL-4, INF-γ, and IL-2 expression.

Results
IL-4 expression was significantly higher in the EX group than in the CON group (p < 0.05). However, IL-2 and INF-γ expression did not differ between groups (p > 0.05).

Conclusion
These results suggest that exercise in rats enhances immune function by regulating cytokine production in T helper type 2 (IL-4) cells, but not in T helper type 1 (IL-2 and IFN-γ) cells of the activated spleen.
Exercise is known to affect the immune system.\(^1\) Recently, exercise immunology, a sub-area of exercise physiology, has gained attention owing to studies demonstrating positive and negative effects of exercise on immune function.\(^2\) Proper exercise can have a positive effect by stimulating the immune system.\(^3\) Additionally, regular exercise can improve the regenerative ability of the body as well as activate immune functions of cells; however, a lack of exercise is linked to immune-related diseases.\(^4\) Exercise increases the production of the immune-related cytokine CD8+ and spleen cells as well as the response to antigens.\(^5\) Lymphocytes (T cells and B cells), macrophages, dendritic cells, and NK cells play important roles in the immune system, and cytokines produced by these cells play roles in immune function control and signal transduction.\(^6,7\)

The spleen is involved in protective immune reactions against antigens in the blood and is an important lymphoid organ in which lymphocyte differentiation, T and B lymphocyte maturation, and antigen stimulation occur. Representative cytokines expressed by spleen cells with immune system functions are interleukin-2 (IL-2), interferon-gamma (INF-\(\gamma\)), and interleukin-4 (IL-4).\(^8\) Cytokines of both T helper 1 (Th1) cells, such as IL-2 and INF-\(\gamma\) and of T helper 2 (Th2) cells, such as IL-4, are closely related to exercise.\(^9\) Cytokines that are expressed by T lymphocytes are involved in nonspecific immune reactions, anti-virus action, and host defense at the early immune reaction phase; they are strong macrophage activators that induce cell-mediated immunity, suggesting the importance of T lymphocytes in the immune system.\(^10\)

IL-2 is expressed by Th1 cells and is affected by IL-4, which is expressed by Th2 cells.\(^11\) IL-2 expression is affected by exercise.\(^12,13\) IL-4 is involved in T cell proliferation and controls B cell, mast cell, and basophil reactions.\(^14\) INF-\(\gamma\) is expressed by activated T cells after stimulation by antigens or mitogens and functions as a cytokine in immune reactions.\(^15\) INF-\(\gamma\) activates antioncogenes, such as it is directly related to oncocytes. It stimulates toxic activity and plays an indirect role in cancer prevention by macrophages.\(^16\) Many studies have examined the effects of high-intensity exercise on Th1 and Th2 cytokines in the spleen, but the effects of low-intensity exercise are unclear.\(^17\) Additional studies are needed to determine whether low-intensity exercise affects the spleen, including histological properties and gene expression. Thus, in this study, the effects of low-intensity exercise on histological properties and immune-related cytokines were investigated in the spleens of albino rats.

**METHODS**

**Animals**

This study included 20 randomly selected 8-week-old Sprague–Dawley albino rats (200 ± 15 g) with similar times of birth. The 20 experimental animals were subjected to pre-exercise to adjust to the environment, and then the rats were randomly divided into two groups (10 rats per group), a control group (CON) and a low-intensity exercise group (EX). Rats were provided Samtako feed (protein 22.5%, fat 3.5%, low-fiber 7.0%, ash 9.0%, calcium 0.7%, and phosphorus 0.5%) and free access to water during the experimental period. Lights were turned on and off at 12-hour intervals, and rats were raised in a breeding room at the Gwangju Institute of Science and Technology at 22 ± 2°C and 70–80% humidity. The study was approved by the animal ethics board of the Gwangju Institute of Science and Technology.

**Experimental Design and Exercise Methods**

The endurance exercise was a low-intensity exercise on a treadmill for small animals with a graded load. The intensity for the graded exercise was established following the methods of Bedford et al.\(^18\) The experiment was conducted for 12 weeks (5 days/weeks) with 60 min of running consisting of 3 m/min for 5 minutes at a 0% grade, 5 m/min for 5 minutes, and 8 m/min for 50 minutes. The spleen tissue was extracted 48 hours after the last treadmill exercise, and samples were stored in a liquid nitrogen tank at −80°C immediately after extraction.

**Histological Examination**

The spleen tissues of rats were fixed in normal buffered formalin solution to observe histological changes. After fixation, samples were demineralized using Calci-clear Rapid (National Diagnostics, Atlanta, GA, USA), washed by normal methods, and embedded in paraffin after a dewatering process. Then, 3-μm fragments were stained with hematoxylin-eosin.\(^19\)
**Effects of Exercise on Cytokine Production**

**RNA Isolation**

For RNA extraction, each spleen tissue of less than 50 mg was extracted using surgical equipment without RNase in a Petri dish, and 0.5 ml of TRIzol reagent (JBI, Seoul, Korea) was added to a 1.5-mL Eppendorf tube, pulverized using a homogenizer, supplemented with chloroform, and centrifuged for 15 minutes at 13,000 rpm, 4°C, followed by 20–30 seconds of vortexing. The solution was divided into three parts, and isopropyl alcohol containing RNA on the top layer was kept for 10 minutes at room temperature, followed by 15 min of centrifugation at 4°C and 15,000 rpm. Samples were centrifuged for 5 minutes at 4°C and 13,000 rpm, and the supernatant was removed and washed with 75% EtOH (in DEPC water). The pellet was dried for more than 1 h after the supernatant was removed again, and the pellet was then dissolved in DEPC water. To measure the total RNA level, absorbance was measured at 260 nm/280 nm using a UV spectrophotometer after dissolving samples in RNase-treated 0.1% DEPC water. In the experiment, 5 of 7 mice that exercised effectively were randomly chosen. High-quality RNA samples from 3 mice were used for RT-PCR analysis.19

**RT-PCR (Reverse Transcription-Polymerase Chain Reaction) and Real-Time PCR**

PCR products were quantified by semi-quantitative RT-PCR after spleen tissues were isolated from each experimental animal. For RT-PCR, extracted total RNA (1 μg) and Oligonucleotide dT primers (100 pmol) were mixed in Accupower® RT PreMix (Bioneer Co., Daejeon, Korea) and preprocessed for 5 min at 70°C in a total volume of 20 μl. cDNA synthesis was performed for 1 h at 45°C, and heat treatment for 5 min at 95°C was used to deactivate the reverse transcriptase.

For the real-time PCR analysis, PCR amplification was conducted using the DyNAmo SYBR Green qPCR Kit and DNA Engine Opticon (MJ Research, Waltham, MA, USA). The cDNA mold and each specific primer were added to 2× PCR Master Mix, and the reaction was conducted in PCR conditions. Primers were as follows: *IL-2* (F: 5′-age tgt tgt tgg att tga ctc-3′; R: 5′-atg agt ctt tga cag ata gct-3′), *INF-γ* (F: 5′-tca agt ggc ata gat gtg gaa gaa-3′; R: 5′-tgg ctc tgc agg att-3′), *IL-4* (F: 5′-aca gga gaa ggg acg ccat-3′; R: 5′-gaa gcc gta cag acg cca tc-3′), and *GAPDH* (F: 5′-cac ccg cga cta cca cct gc-3′; R: 5′-ccc ata ccc acc atc aa cc-3′).20 Expression levels were quantified relative to *GAPDH* expression, and the degree of increase in gene expression was determined by comparisons between the treatment group and control group according to the following equation: fold change = 2<sup>ΔΔCT</sup>, where ΔΔCT = (CT(Target) – CT(GAPDH)) at time x – (CT(Target) – CT(GAPDH)) at time 0; time x is a random time and time 0 is the time when quantified value relative to *GAPDH* for the interested becomes 1 for the control group without any treatment (CON).

**Statistical Analysis**

The results are expressed as means and standard deviation, as determined using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Kolmogorov–Smirnov one-sample tests were used to test for normal distributions because the sample size (i.e., the number of rats) was small; these tests indicated that data were normally distributed. Independent *t*-tests were conducted to examine differences in mean values between groups. Significance was set at α = 0.05.

**RESULTS**

**Change in Spleen Tissue Shape after Low-Intensity Exercise**

Changes in spleen tissue shape and cell shape in tissue samples after low-intensity exercise are shown in Figure 1. Based on hematoxylin-eosin staining, the low-intensity exercise group showed significantly fewer mature cells showing a darker color than that observed in the control group. However, no specific inflammation or tissue necrosis was observed in the low-intensity exercise group compared with that in the control group (<40).

**IL-2, INF-γ and IL-4 mRNA Expression Analysis**

The spleen tissue weight was lower in the exercise group than in the control group, but this difference was not significant (Figure 2A). The real-time PCR results for *IL-2*, *INF-γ*, and *IL-4* expression in spleen tissue are summarized in Figure 2B, 2C, and 2D, respectively. The *IL-2* and *INF-γ* levels in spleen tissue did not differ significantly between the control group and the low-intensity exercise group (Figure 2B and
FIG. 1 Change in spleen tissue condition after low-intensity exercise.

CON = control group; EX = exercise group.
*Significantly different from levels in the control group at p < 0.05.

FIG. 2 Weight of spleen tissues and the expression of IL-2 (B), INF-γ (C), and IL-4 (D) mRNA determined by real time-PCR in the spleen.

CON = control group; EX = exercise group
However, the low-intensity exercise group showed a higher IL-4 expression level in the spleen compared with that of the control group, and this difference was significant (Figure 2D) ($!p! < 0.05$).

**DISCUSSION**

In this study, the effects of exercise on the immune system in the spleen were examined to improve our understanding of immunity. The spleen plays an important role in immune defense, and exercise is closely related to macrophage and lymphocyte properties.$^{21}$ Proper and regular exercise improves the adaptive immune response, such as the antibody-mediated immune response that induces the activation of leukocytes, macrophages, and lymphocytes.$^{22}$ Many recent studies have examined exercise and the spleen, but most have focused on high-intensity exercise.$^{23}$

We did not observe inflammation and tissue necrosis in either group (i.e., the control or low-intensity exercise group), according to the results of hematoxylin-eosin staining. These results indicate that low-intensity exercise does not directly affect the shape of the spleen. For more detailed analyses of the shape of the spleen, inflammatory cytokines should be estimated by immunohistochemistry in future studies.

IL-2 is a pro-inflammatory cytokine of Th1 cells and a marker of immune activity.$^{24}$ INF-$\gamma$ is another pro-inflammatory cytokine produced in Th1 cells; it suppresses the production of IL-4, a Th2 anti-inflammatory cytokine.$^{25,26}$ The low-intensity exercise group showed decreased levels of IL-2 and INF-$\gamma$ compared with those of the control group in spleen tissues, but these differences were not significant (see Figure 2B and C). The low-intensity exercise group showed higher IL-4 levels in the spleen compared with those of the control group, and this difference was significant ($!p! < 0.05$; Figure 2D). Haahr et al. reported that bicycle ergometer exercise does not affect IL-2 and INF-$\gamma$ expression.$^{22}$ Representative pro-inflammatory cytokines, such as IL-2 and INF-$\gamma$ are secreted at an early stage to prevent the aggravation of tissue damage. If these cytokines cannot function in host defense, it may lead to excessive secretion, which may negatively impact immune function by disrupting the balance of anti-inflammatory cytokines, such as IL-4.$^{27}$ Furthermore, if pro-inflammatory cytokines, such as IL-2 and INF-$\gamma$ are excessively secreted, disrupting the balance in anti-inflammatory cytokines, host viability may be impacted.$^{28}$ Our results indicated that low-intensity treadmill exercise has the potential to improve immune function by maintaining a balance between pro-inflammatory and anti-inflammatory cytokines.

This study has a few limitations. First, owing to the menstrual cycle, avoiding hormone disturbance was difficult and exercise performance was limited in females; hence, a similar study should be specifically designed for female rats. In addition, further studies are required to compare the effect of exercise on changes in the production of interleukin-2, interferon-gamma, and interleukin-4 between males and females.

**CONCLUSION**

The results of this study suggest that exercise in rats enhances immune function by regulating cytokine production by Th2 cells (IL-4), but not by Th1 cells (IL-2 and IFN-$\gamma$) in the activated spleen.

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**REFERENCES**


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