JOMH
Journal of Men's Health

Original Article
DOI: 10.22374/1875-6859.14.3.1

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

By Eun-Ju Choi[1], Chang-Jin Lee[2], Hyo-Hyun Park[3], Wi-Young So[4]

[1]Assistant Professor, Department of Physical Education, Daegu Catholic University, Gyeongsan, Korea.

[2]Professor, Division of Sports and Health Science, Konkuk University, Chungju-si, Korea

[3]Senior Researcher, Korean Oriental Medicine Technique Headquarters, National Development Institute of Korean Medicine, Gyeongsan, Korea.

[4]Associate Professor, Sports and Health Care Major, College of Humanities and Arts, Korea National University of Transportation, Chungju-si, Korea.

Corresponding Author Wi-Young So, PhD: wowso@ut.ac.kr

Submitted: December 20, 2017. Accepted: May 30, 2018. Published: June 9, 2018.

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-γ), and interleukin-4 (IL-4).[8] Cytokines of both T helper 1 (Th1) cells, such as IL-2 and INF-γ 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-γ is expressed by activated T cells after stimulation by antigens or mitogens and functions as a cytokine in immune reactions.[15] INF-γ 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]

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′-agc gtg tgt tgg att tga ctc-3′; R: 5′-atg atg ctt tga cag atg gcta-3′), *INF*-γ (F: 5′-tca agt ggc ata gat gtg gaa gaa-3′; R: 5′-tgg ctc tgc agg att ttc atg-3′), *IL-4* (F: 5′-aca gga gaa ggg acg ccat-3′; R: 5′-gaa gcc gta cag acg agc tca-3′), and *GAPDH* (F: 5′-cac ccg cga gta caa cct tc-3′; R: 5′-ccc ata ccc acc atc aca 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 = $2^{-\Delta\Delta CT}$, 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 $\alpha = 0.05$.

RESULTS

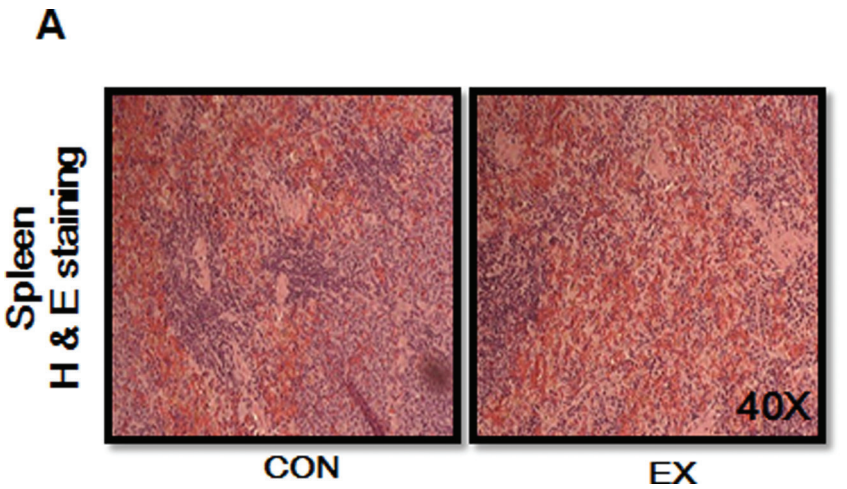
Change in Spleen Tissue Shape after Low-Intensity Exercise

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

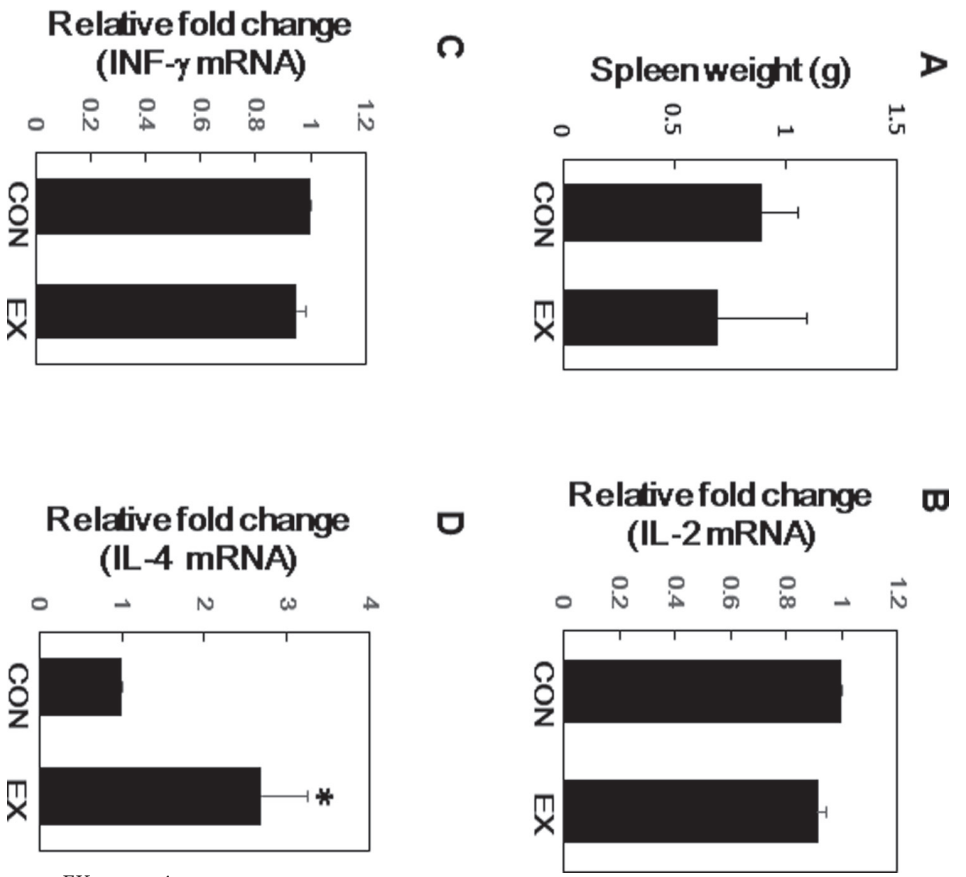
The spleen tissue weight was lower in the exercise group than in the control group, but this difference was not significant (Figure 2A). The real-time PCR results for *IL-2*, *INF*-γ, and *IL-4* expression in spleen tissue are summarized in Figure 2B, 2C, and 2D, respectively. The *IL-2* and *INF*-γ 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

2C) ($p > 0.05$). 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-γ 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-γ* 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-γ expression.[22] Representative pro-inflammatory cytokines, such as IL-2 and INF-γ 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-γ 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-γ) in the activated spleen.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (NRF-2017S1A5A8019407).

REFERENCES

1. Simpson AL, Ray AD, Lundgren CE, et al. Energy cost of breathing at depth: effect of respiratory muscle training. Undersea Hyperbar Med Soc 2012;39(4):829–36.
2. Caris AV, Lira FS, De Mello MT, et al. Carbohydrate and glutamine supplementation modulates the Th1/Th2 balance after exercise performed at a simulated altitude of 4500m. Nutrition 2014;30(11–12):1331–6.
3. Walsh NP, Gleeson M, Shephard RJ, et al. Position statement. Part one: Immune function and exercise. Exercise Immunol Rev 2011;17:6–63.
4. Ryan AS, Nicklas BJ, Berman DM, et al. Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women. Int J Obes Relat Meab Disord 2003;27(9):1066–71.
5. Passos GS, Poyares D, Santana MG, et al. Exercise improves immune funtion, antidepressive response, and spleep quality in patients with chronic primary insomnia. BioMed Res Internat 2014;2014:498961.
6. Balkwill F. Cytokines in health and disease. Immunol Today 1993;14(4):149–50.

7. Tanaka S, Inoue S, Isoda F, et al. Impaired immunity in obesity: suppressed but reversible lymphocyte responsiveness. Int J Obesity Relat Metabol Disord 1993;17(11):631–6.
8. Filipov NM, Thompson FN, Sharma RP, et al. Increased proinflammatory cytokine production by ergotamine in male BALB/c mice. J Toxicol Environment Health Part A 1999;58(3):145–55.
9. Gleeson M, Bishop NC, Stensel DJ, et al. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nature Rev Immunol 2011;11(9):607–15.
10. Collisson E, Griggs L, Drechsler Y. Macrophages from disease resistance B2 haplotype chickens activate T lymphocytes more effectively than macrophages from disease susceptitible B19 birds. Development Comparat Immunol 2017;67:249–56.
11. Mahajan SG, Mehta AA. Suppression of ovalbumin-induced Th2-driven airway inflammation by beta-sitosterol in a guinea pig model of asthma. Eur J Phamacol 2011;650(1):458–64.
12. Li J, Wang J, Shao JQ, et al. Effect of schisandra chinensis on interleukins, glucose metabolism, and pituitary-adrenal and gonadal axis in rats under strenuous swimming exercise. Chinese J Integr Med 2015;21(1):43–8.
13. Kaya O. Effect of a four-week exercise program on the secretion of INF-γ, TNF-γ, IL-2 and IL-6 cytokines in elite Taekwondo athletes. Biosci Rep 2016;5(3):367–70.
14. Ho IC, Miaw SC. Regulation of IL-4 expression in immunity and diseases. Adv Experiment Med Biol 2016;941:31–77.
15. Modabbernia A, Taslimi S, Brietzke E, et al. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. Biol Psychiatr 2013;74(1):15–25.
16. Lee H, Baek S, Joe SJ, et al. Modulation of INF-gamma production by TNF-alpha in macrophages from the tumor environment: significance as angiogenic swith. Int Immunopharmacol 2006;6(1):71–8.
17. Cao Y, Hu Y, Zhao HX, et al. Effects of a Chinese traditional formula Kai Xin San (KXS) on chronic fatigue syndrome mice induced by forced wheel running. J Ethnopharmacol 2012;139(1):19–25.
18. Bedford TG, Tipton CM, Wilson NC, et al. Maximum oxygen consumption of rats and its changes with various experimental procedures. J Appl Physiol Respir Environ Exercise Physiol 1979;47(6):1278–83.
19. Choi EJ, Lee S, Chae JR, et al. Eupatilin inhibits lipopolysaccharide-induced expression of inflammatory mediators in macrophages. Life Sci 2011;88(25-26):1121–6.
20. Lee HS, Choi EJ, Lee KS, et al. Oral administration of p-Hydroxycinnamic acid attenuates atopic dermatitis by down regulating Th1 and Th2 cytokine production and keratinocyte activation. PLoS One 2016;11(3):e0150952.
21. Lu YM, Zhang H. Effects of electroacupunture on T-lymphocytes, spleen index, thymus index and lymphopoiesis levels in strenuous exercise-induced stress rats. Zhen Ci Yan Jin 2012;37(2):136–9.
22. Haahr PM, Pedersen BK, Fomsqaard A, et al. Effect of physical exercise on in vitro production of interleukin 1, interleukin 6, tumour necrosis factor-alpha, interleukin 2 and interferon-gamma. Int J Sports Med 1991;12(2):223–7.
23. Kobayashi Y, Nakatsuji A, Aoi W, et al. Intense exercise increases protein oxidation in spleen and liver of mice. Nutr Metab Insights 2014;13(7):1–6.
24. Brubdaker JO, Thompson CM, Morrison LA, et al. Th1-associated immune responses to beta-galactosidase expressed by replication-defective herpes simplex virus. J Immunol 1996;157(4):1598–604.
25. Pisa P, Halapi E, Pisa EK, et al. Selective expresssion of interleukin 10, interferon gamma and granulocyte-macrophage colony-stimulating factor in ovarian cancer biopsies. Proc Nat Acad Sci 1992;89(16):7708–12.
26. Sypek JP, Chung CL, Mayer SH, et al. Resolution for cutaneous leishmaniasis: Interleukin 12 initates a protective T helper type 1 immune response. J Experiment Med 1993;177(6):1797–802.
27. Guo J, Liu C, Wang Y, et al. Role of T hepler lymphokines in the immune-inflammatory pathophysiology of schizophrenia: Systematic review and meta-analysis. Nord J Psychiatr 2015;69(5):364–72.
28. Clerici M, Ferrario E, Trabattoni D, et al. Multiple defects of T helper cell funtion in newly diagnosd patients with Hodgkin's disease. Eur J Cancer 1994;30(10):1464–70.