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The Effect of Nanoselenium Consumption during High Intensity Interval Training on IL-4 and IFN-γ Gene Expression in Thymus Organ of Dexamethasone-Induced Immunosuppressive Rats

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ABSTRACT

Introduction: The thymus is a specialized lymphatic organ in the immune system and plays a vital role in the normal functioning of the immune system. Observed 24 and 48 hours after dexamethasone, thymocytes are reduced by 55% and 84%, respectively, which can be associated with a decrease in anti-inflammatory cytokines such as IL4 and IFN-γ. The aim of present study was to evaluate the The effect of nanoselenium consumption during high intensity interval training on IL-4 and IFN-γ gene expression in thymus organ of dexamethasone-induced immunosuppressive rats.

Methods: The study samples in the present study consisted of 40 male Wistar rats that were randomly divided into 5 groups: healthy control group (CON), immunosuppression group (DEX), immunosuppression + exercise group (DEX + TRA), immunosuppression group + nanosillenium (DEX + SEL), immunosuppression group + nanoselnium + training (DEX + TRA + SEL) were divided. Suppression of the immune system of the samples was performed by injecting of 0.4 mg / kg per day dexamethasone for three days. The training program included 4 weeks of intense intermittent training (HIIT) in the DEX + TRA and DEX + TRA + SEL groups, and supplementation with 100 mg / kg selenium nanoparticles in the DEX + SEL and DEX + TRA + SEL groups. Data were analyzed using one way ANOVA in SPSS 26 software at significance level of α <0.05.

Results: The data showed that IFN-γ gene expression decreased in all groups compared to the control group (p = 0.0001). The difference between DEX + SEL and DEX + SEL + TRA groups was less than other groups. Also, IL4 gene expression in thymus tissue was significantly reduced in DEX and DEX + TRA groups compared to healthy controls (p = 0.048 and p = 0.013, respectively).

Conclusion: In the present study, it was found that intense exercise activity in high-intensity interval training (HIIT) may inhibit immune reactions and anti-inflammatory cytokines in the thymus tissue of rats whose immune system was suppressed by DEX. Therefore, exercise for strengthening the immune system in these people should be done with more caution.

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Introduction

Weaknesses in the immune system can be caused by two primary and secondary sources. The disease has a genetic origin of weakness of the primary immune system and a person suffers from it from birth. Secondary immune system deficiencies can also be caused by diseases such as AIDS and cancer, malnutrition, chemotherapy, or the use of corticosteroids such as dexamethasone. Today, dexamethasone is used in many inflammatory, autoimmune, and organ transplant diseases to suppress the immune system. In addition, many athletes use dexamethasone to reduce inflammation due to sports injuries, reduce pain, and improve athletic performance, which can lead to impaired immune function (Vernec et al, 2020).

The thymus is a lymphatic organ specialized in the immune system and plays a vital role in the normal functioning of the immune system. Once formed in the bone marrow, T lymphocytes travel through the bloodstream to the thymus and mature by it (Wang et al, 2020). Observed 24 and 48 hours after taking dexamethasone, thymocytes are reduced by 55% and 84%, respectively, which can be associated with impaired immune function (Ansar Ahmed et al,

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1994). In addition, dexamethasone has been shown to reduce T lymphocytes (Chen et al, 2018).

Interleukin 4 (IL-4) is a cytokine that differentiates simple helper T cells (Th0 cells) from Th2 cells. Th2 is activated by IL-4, which subsequently produces additional IL-4 in a positive feedback cycle. IL-4 is mainly produced by mast cells, Th2 cells, eosinophils and basophils. Stimulation of B and T cell proliferation is an important IL-4 task that plays a crucial role in the adaptive immune system (Yang et al, 2017). On the other hand, interferon-gamma (IFN-γ), which is secreted mainly by activated lymphocytes such as CD4 T type 1 helper cells (Th1) and cytotoxic CD8 T cells, coordinates the signaling pathway of several biological responses. These responses are primarily involved in host defense and immune monitoring, but are also effective in building adaptive immunity and in regulating inflammation, apoptosis, and the cell cycle. IFN-γ inhibits Th2 cell differentiation, and thus IL-4 production. This regulation involves inhibition of the IL-4 / STAT6 pathway required for Th2 cell differentiation, and is mediated at least by IFN-γ-induced SOCS1, which inhibits IL-4 receptor signaling (Castro et al, 2018).

Exercise as an interfering factor can play an effective role in improving immune system function. The volume and intensity of exercise are two factors that determine the rate of response and adaptation to exercise. Regarding the effect of high-intensity interval and endurance exercises, most researches shows that the negative effect of these types of exercises. But further investigation shows that intense interval training that has been going on for a long time in each position has a negative effect on immune system indices (Ito et al, 2019).

Contrary to previous reports of high-intensity interval exercise suppression, it has been reported that 6 weeks of high-intensity interval training has no adverse effect on the immune system and when combined with zinc supplementation improves immune system-related indicators in athletes (Saeedy et al, 2018). However, the results of research in the field are contradictory and more studies are needed. It seems that when intense interval training combined with the use of an antioxidant is evaluated, it has a significant impact on improving immune function (Saeedy et al, 2018; Khorasani et al, 2019). It is possible that antioxidants in foods with the help of antioxidant enzymes can improve the function of the immune system. Selenium (Se), on the other hand, is a rare mineral that is essential for human and other animal health. Many studies have shown that Se improves the immune function of cells (Radomska et al., Kurana et al., 2019). Se has been shown to increase the production of IL-2 and M and G immunoglobulins (Avery et al, 2018). Se has also been shown to play an important role in protecting T and B lymphocytes against certain toxins (Salimian et al, 2014). Taken together, these data suggest that Se could be a potential treatment for preventing immunological damage from the effects of dexamethasone or strenuous exercise. However, there is little information in this area and therefore the aim of the present study was to investigate the effect of 4 weeks of high intensity interval training along with nanoselnium supplementation on IL-4 and IFN-γ gene expression in thymus tissue of dexamethasone-induced immunosuppression rats.

Materials and Methods

Research Methods and Samples

The present study was experimental and the samples consisted of 40 male Wistar rats (180 to 220 g). Sampling was done randomly and the samples were stored in the standard laboratory environment of Baqiyatallah University of Medical Sciences after purchase from Pasteur Institute of Iran. The animals were kept in polycarbonate cages at 22 ± 4.1 ° C and the light cycle in the dark for 12:12 h and a humidity of 55.6 4 4.6%.

Methods

After transferring the samples to the laboratory environment, rats were exposed to the environment for 1 week. The rats were then introduced to the training protocol for one week and randomly divided into 5 groups: healthy control group (CON), immunosuppression control group (DEX), immunosuppression + exercise group (DEX + TRA), immunosuppression + Nanosilnium group (DEX + SEL), immunosuppression + nanosilnium + exercise group (DEX + TRA + SEL) were divided.

Suppression of the Immune System

In the present study, suppression of the immune system in samples was done by injecting 0.4 mg

/ kg / d dexamethasone (made by Osweh Iran) for three days intraperitoneally (Dehghani et al, 2021). Then Immune system attenuation samples were performed in 4 groups of immunosuppression control group (DEX), immunosuppression + exercise group (DEX + TRA), immunosuppression + Nanosilnium group (DEX + SEL), immunosuppression + nanosilnium + exercise group (DEX + TRA + SEL). In the control group, the same amount of normal saline (NS) solution was injected over three days.

Training Protocol

The training program performed in the present study included a 4-week Intense Interval Training Program (HIIT) involving running on a rodent treadmill at a speed of 24 to 34 meters per minute, equivalent to 85 to 100% of the maximum oxygen consumption of the samples. Exercises were performed six days a week (Little et al, 2011). In each session, 8-12 repetitions of 1 minute with an intensity of 24 to 34 meters per minute with active rest intervals of 75 seconds. The principle of incremental overload was implemented by increasing the speed of the treadmill and the number of repetitions per week of the training program.

Receiving Nanoselnium Supplement: To prepare selenium nanoparticles, first a 2.5 mM solution of selenium dioxide was prepared and added to a 2.5 mM solution of ascorbic acid being mixed. The resulting mixture was centrifuged and washed using filter paper (Yazdi et al, 2015). The stock solution of selenium nanoparticles was prepared and 100 mg in 250 nm size was given to mice by gavage and every other day. At the same time, normal saline solution was given by gavage in a healthy control group.

Table 1. IL-4 primer sequence, IFN-γ Genes name Primer sequences

	Genes name	Primer sequences
1	IFN-γ	Forward: CCCACAGATCCAGCACAAAG Reverse: TCTACCCCAGAATCAGCACC
2	IL-4	Forward: CAAGGAACACCACGGAGAAC Reverse: TCTTCAAGCACGGAGGTACA
3	GAPDH	Forward: CAAGTTCAAGGGCACAGTCA Reverse: CCCCATTTGATGTTAGCGGG

The maximum oxygen consumption (VO2max) of rats was assessed using a sloping treadmill (5 lanes) with a positive slope of 25 degrees (Radomska et al, 2021). After applying the independent variable, all samples with completely similar conditions and in basic conditions (48 hours after the last training session and 12 to 14 hours of fasting) by intraperitoneal injection of a combination of ketamine (60 mg / kg) and xylazine (mg / kg 5) they fainted. Then the split chest and thymus tissue were immediately divided into two parts after separation and washing and transferred to liquid nitrogen in formalin (for the tissue process of measuring gene expression). They were then stored in the refrigerator at -80 ° C until measurement.

Measurement of variables: In vitro analysis of IL-4, IFN-γ gene expression levels in rat thymus tissue was determined using special commercial kits by real-time PCR with primer sequence of Table 1.

Statistical methods: After confirming the normality of the data using Shapiro-Wilkes test, data analysis was performed using one way ANOVA and Tukey post hoc test to compare the two groups. Was used. All calculations were performed using SPSS software version 24 at a significance level of 0.05.

Table 2. Results of one-way analysis of variance in comparison of IL-4 and IFN-γ gene expression in research groups

	Sum of Squares	df	Mean Square	F	Sig.
IL-4	1.376	4	.344	4.147	.007*
INFgama	3.483	4	.871	44.599	.000*

*: Significant difference at the level of α ≤0.05

Results

The results of data analysis using one-way analysis of variance (ANOVA) are shown in Table 2. The results show that there is a significant difference between the groups in the expression of IL-4 and IFN-γ genes in the thymus tissue of the samples in different groups (P = 0.0001 and P = 0.007, respectively).

Figure 1 shows a comparison of IFN-γ gene expression in thymus tissue of different research groups. The results show that the expression of

this gene in DEX group is different from healthy control groups (p = 0.0001) and DEX + SEL (p = 0.0001). Also, DEX + SEL group showed a significant difference with control groups (p = 0.001) and DEX + TRA (p = 0.0001). IFN-γ gene expression in DEX + TRA group was significantly different from control group (p = 0.0001) and DEX + SEL + TRA group (p = 0.0001).

Figure 2 shows a comparison of IL4 gene expression in thymus tissue of different research groups. The results show that the expression of this gene in the healthy control group is significantly different from the DEX (p = 0.048) and DEX + TRA groups (p = 0.013).

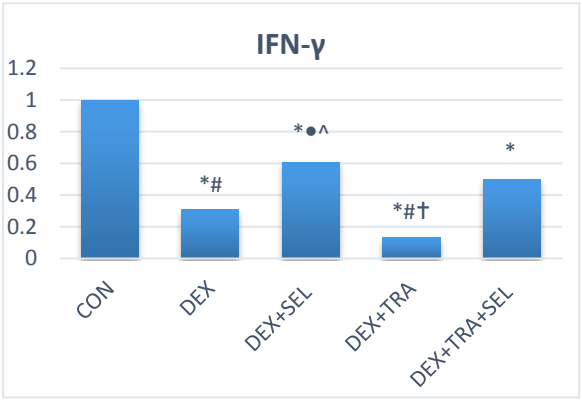

Figure 1. Tukey test results and comparison of IL-4 gene expression in different research groups

*: Significant difference with healthy control group at the level of α ≤0.05
●: Significant difference with the DEX group at the level of α ≤0.05
^: Significant difference with the DEX+TRA at the level of α ≤0.05
#: Significant difference with DEX+SEL consumption group at the level of α≥0.05
†: Significant difference with DEX +TRA+SEL at the level of α ≤0.05

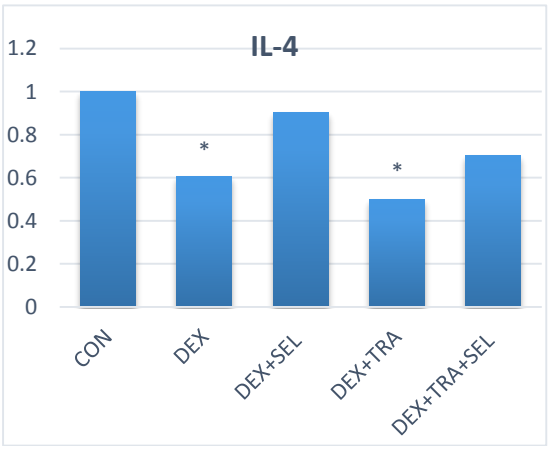

Figure 2. Tukey test results and comparison of IL-4 gene expression in different research groups
*: Significant difference with healthy control group at the level of α ≤0.05

Discussion

The results of the present study showed that weakening the immune system using dexamethasone reduces the expression of IFN-γ and IL-4 genes in rat thymus tissue. The thymus gland, as seen in mouse models, is a vital regulator for the development of various branches of lymphocytes called CD4 cells, which are necessary to ensure the formation of helper T cells. Thymocytes, positive selection, regardless of MHC specificity, lead them to become CD4 (van Vliet et al, 2017; Mitevska et al, 2015). Given the role of the thymus in the maturation of infocytes, it seems that the decrease in the expression of

these cytokines in thymus tissue leads to a decrease in the function of this tissue in the immune system's response to pathogens. On the other hand, studies have shown that dexamethasone leads to atrophy and reduction of thymus volume (Dehghani et al, 2021). Thymus atrophy in DEX-treated mice may be due to a decrease in the number of immature T cells in its cortex. The thymus has two parts, the cortex and the medulla. The cortex is made up of lymphocytes and thymocytes, which are supported by retinal epithelial cells. But there are fewer lymphocytes and more epithelial cells in the medulla (Jeklova et al, 2007). The results of studies show that most of the decrease in thymus volume is related to the volume of the thymus cortex, which has a greater role in the maturation of lymphocyte cells. The cortex and medulla contain immature and mature T cells, respectively (Pearse et al, 2006). Hussar et al. Found that DEX induced immature T cell apoptosis in the thymus cortex, while the change in lymphocyte count in the medulla was less pronounced. It is estimated that approximately 90% of immature T cells are naturally removed by intrinsic agents, but DEX plays a major role in the development of the apoptotic pathway. Sensitivity to glucocorticoids in immature T cell apoptosis has been associated with markers of oxidative stress and mitochondrial dysfunction in previous studies. Immature T cells in the thymus cortex are thought to be more sensitive to oxidative stress than lymphocytes in the medulla (Dehghani et al, 2021). They are effective in reducing IL-4 and IFN-γ (Nunes-Cabacet al, 2022).

Another result of the present study was that selenium supplementation in mice whose immune systems were weakened significantly compensated for the decrease in IL-4 gene expression. In the case of IFN-γ, although selenium intake reduced the expression of this gene slightly, its expression was still lower than in the healthy control group. These results are consistent with the findings of a study showing that selenium administration to DEX-treated mice increased total thymus volume by 37.6% and cortex by 80.5% compared with the DEX group (Dehghani et al, 2021). However, these findings emphasize that the volume of the thymus medulla remained unchanged in selenium-receiving mice. In fact, the findings show that DEX reduces the volume of all areas associated with the thymus, spleen, and lymph nodes, but selenium, as an immunomodulator, improves changes in the thymus cortex, white spleen pulp, and outer cortex of the lymph nodes. (Dehghani et al., 2021).

Selenium may exert its effect through a mechanism involving free thiols, reducing oxidative stress conditions, and increasing lymphocyte proliferation (Hoffmann et al, 2010). Hawkes et al. Also showed that selenium promotes the proliferation of B lymphocytes and possibly T cells. Kiremidjian-Schumacher et al. Also stated that selenium proliferates cytotoxic progenitor cells. Cheng et al. Reported that selenium increased the number of B and T cells. The antioxidant properties of selenium appear to cause the proliferation of immature T cells in the thymus cortex but have no effect on mature T cells in the thymus medulla. We found that selenium stimulates B cell proliferation more than the growth of mature T cells in the spleen and lymph nodes. In this study, selenium administration alone had no effect on the different structures of lymphatic organs in healthy mice. Selenium is likely to inhibit atrophic lymph nodes in immunocompromised conditions, but has no effect on the natural structure of these tissues.

Interleukin-4 (IL-4) is a peptide consisting of four short helices that is produced as a pleiotropic cytokine mainly by lymphocytes and thymocytes. IL-4 regulates various processes in cell types. In addition to its role in B cell differentiation, it enhances Th2 by inhibiting the fate of Th1 and Th17 in T cells (Nunes-Cabacet al, 2022). IL-4 is also involved in the function of T cells called CD8 because it increases their proliferation and cytotoxic activity (Oliver et al, 2012) and can also act as a negative regulator of CD8 T cell responses. (Wijesundara et al, 2013). In the thymus, IL-4 is required for the growth of CD8 cells (Jameson et al, 2015). CD8 T cells, after activation, are rapidly producing IFN-γ (Jameson et al., 2015). Mice and CD4 T cells are MHC class II dependent in humans (Nunes-Cabacet al, 2022). Importantly, the response to IL-4 during mouse CD8 T cell development and homeostasis may alter their functional response and response to pathogens (Kabaku et al., 2022).

Intensity of activity is one of the most important variables affecting the effects of exercise on the immune system. Most studies have shown that exercise leads to the renewal and improvement

of immune function (Tylutka, 2021; Papp et al, 2021). However, the mechanisms of such effects are still highly unclear. Improving exercise-induced immunity can be associated with reduced inflammation, maintenance of thymus mass, changes in memory composition and simple T lymphocytes, or increased immune monitoring. Indeed, physical activity is a powerful intervention that has great potential for improving the immune system and health outcomes in the elderly, obese, and patients with cancer and chronic viral infections (Simpson et al, 2012). However, in the present study, it was found that intense exercise activity in high-intensity intermittent exercise (HIIT) may inhibit immune responses and anti-inflammatory cytokines by increasing the production and secretion of inflammatory and proinflammatory cytokines that promote lymphocyte differentiation. They create thymus in the tissue. This was especially true in samples whose immune systems were weakened by DEX administration, and therefore exercise should be done with greater caution in order to strengthen the immune system in these individuals. Because the effect of such activities may be synergistic with the pharmacological effects of glucocorticoids in weakening the immune system and lead to a double weakening of the function of this system.

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