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Effect of 12 Weeks of Regular Exercise with Vitamin C Supplementation on the Salivary IgA of Male High School Students

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ABSTRACT

Introduction: Immunoglobulins are humoral elements, which indicate the function of the immune system. Studies have shown that exercise and vitamin C supplementation could increase immunoglobulin levels. The present study aimed to evaluate the effect of 12 weeks of regular exercise with vitamin C supplementation on the salivary IgA of male high school students.

Methods: This clinical trial was conducted on 40 male high school students who were randomly assigned to four groups of 10, including exercise with vitamin C supplementation, exercise, vitamin C supplementation, and control group. Groups one and two performed the selected exercises for three sessions, and groups one and three consumed three vitamin C tablets (250 mg) per week. Saliva samples were collected 24 hours before the first training session (pretest) and 48 hours after the last training session (posttest). Data analysis was performed in SPSS version 22 using t-test, one-way ANOVA, and Tukey's post-hoc test at the significance level of P≤0.05.

Results: Salivary IgA was significantly affected by 12 weeks of regular exercise and vitamin C supplementation. The differences between the groups indicated that the IgA levels in the exercise with vitamin C group (P=0.001) and exercise group (P=0.001) were significantly higher compared to the control group. However, no significant difference was observed between the control and vitamin C groups in this regard (P=0.49).

Conclusion: According to the results, regular exercise and vitamin C supplementation could increase the salivary IgA levels of the male high school students.

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Introduction

The immune system is a collection of cells and molecules that respond to foreign substances and infectious microbes. Innate immunity is the primary defense provided by the immune system, followed by acquired immunity as secondary defense mechanisms. Acquired immunity involves lymphocytes and immunoglobulins (1). Immunoglobulins are generated and secreted by B lymphocytes and plasma cells. These substances are classified into five categories of IgG, IgA, IgM, IgD, and IgE. IgA is the predominant immunoglobulin in the mucosal, gastrointestinal, respiratory, tear, and milk fluids, as well as an important defense mechanism factor against the germs that enter the body through mucosal surfaces (2). Antibodies are involved in the identification of antigens and developing the immunological memory of antigens. The function of antibodies is to bind to antigens, so that they could not reach the cells and macrophages and other killer cells would be stimulated for the elimination of microbes (2). Factors such as physical activity, stress, nutrition, vitamin supplementation, and diseases may alter the quantitative and qualitative capacity of the immune system (3), and the role of minerals is considered pivotal in this regard (4).

According to a study, the intake of vitamin A, iron, folic acid, vitamin D, calcium, zinc, and vitamin C and their combined consumption could significantly affect the immune function (5). Vitamin C is the most important water-soluble antioxidant in the body, which reduces fat peroxidation through the inhibition of free radicals (6). According to the literature, vitamin C has remarkable antioxidant properties that may be dose-dependent to help improve the immune function and reduce respiratory infections (7). In addition, longitudinal research confirms the effects of vitamins (especially

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vitamin C) on the function and modulation of immunoglobulins, particularly IgA and IgG, which could prevent and enhance the symptoms of colds (7). The same study also demonstrated that vitamin C has modulatory effects on cytokines, as well as tonic effects on other immune system factors, such as immunoglobulins in acute respiratory distress syndrome (7).

Moderate exercise increases the respiratory capacity and resistance against infections (e.g., colds), while strenuous exercise is associated with the increased risk of upper respiratory tract infections (8). Regular and long-term aerobic exercise in elderly men has been reported to increase the number of $CD25^+$ cells, interleukin-2 (IL-2), and salivary IgA (9). Moreover, moderate exercise has been shown to reduce the incidence of upper respiratory tract infections by 29%, while prolonged, high-intensity physical exercise is associated with a higher risk of these infections (10).

Evidence suggests that 12 weeks of exercise combined with breathing exercises could improve the pulmonary function and maximize oxygen consumption, while also decreasing the risk of depression in women with mild-to-moderate asthma (11). Similarly, eight weeks of modified Pilates exercise has been reported to enhance the pulmonary function and quality of life of chemical warfare victims (12). On the other hand, the results of a study indicated that breathing exercises had no significant effect on the pulmonary function of patients with chronic bronchitis (13).

In another research, the effects of two consecutive weekly training sessions were investigated on the levels of IgA and cortisol 20 in professional male gymnastics. The obtained results indicated no significant change in IgA, while the cortisol levels were observed to increase in the subjects. In the mentioned study, no significant correlation was denoted between IgA and cortisol (14). In a similar study, the effects of a soccer simulation training session were evaluated on salivary IgA, IgG, IgM and cortisol concentrations, and the researchers concluded that the exercises may cause the temporary weakening of IgA in the athletes possibly due to their duration and intensity (15). Given the discrepancy in the proposed findings in sport science studies, it seems that the effects of factors such as exercise, sleep, rest, nutrition, and vitamins on the immune function should be further explored. The research of the World Health Organization (WHO) has highlighted the role of factors such as an active lifestyle and nutrition in this regard (7).

The present study aimed to investigate the effect of 12 weeks of regular exercise with vitamin C supplementation in winter on the rate of salivary IgA in male high school students in District 17 of Tehran, Iran.

Materials and Methods

This clinical trial was conducted with a pretest-posttest design and a control group. Initially, the authors announced the conduction of the research to all the high schools of second period in District 17 of Tehran, and two high schools were selected via cluster sampling. At the next stage, the research objectives, benefits, and limitations were explained to the participants. In total, 40 students aged 15-18 years with the height of 160-175 centimeters, weight of 62-70 kilograms, and no history of cardiovascular diseases, acute pulmonary diseases, and other diseases affecting the research process were randomly selected as the sample population. Written informed consent was obtained from the subjects and their parents prior to enrollment.

The participants were randomly divided into four groups of 10, including exercise with vitamin C supplementation, exercise, vitamin C supplementation, and control. Data were collected using a researcher-made checklist to assess the level of salivary IgA at the pretest and posttest.

Exercise Protocol

The exercise groups performed the selected exercises for 12 weeks three sessions per week (total: 36 sessions, 90 minutes each). The exercise protocol consisted of general aerobic and stretching warm-ups and special combination exercises (10 minutes), skills training or review of the techniques and tactics (20 minutes), handball and futsal exercises (50 minutes), and aerobic and stretching exercises for cool-down (10 minutes) (16). The vitamin C supplementation groups received three vitamin C tablets (250 mg each) weekly during the study period (18).

The study protocol was approved by the Ethics Committee of Tehran Islamic Azad University of

Medical Sciences (code: IR.IAU.TMU.REC.1399.109).

Variable Measurement

Saliva samples of the subjects were collected 24 hours before the first training session (pretest) and 48 hours after the last training session(posttest) by a laboratory specialist and the researcher early in the day using DEXK276 laboratory kit (made in Germany), with the accuracy of microgram per microliter. Initially, each subject rinsed their mouths with water for one minute and rested for one minute. Following that, saliva samples were collected from their mouths within 60 seconds.

The samples were transferred to the laboratory for measurements. Salivary IgA sample levels were determined using the mentioned kits in the ELISA reader device (18). Data were recorded as the pretest and posttest of the study groups. Data analysis was performed in SPSS version 22 using t-test, one-way analysis of variance (ANOVA), and Tukey's post-hoc test at the significance level of P≤0.05. In addition, Shapiro-Wilk test was applied to assess the normal distribution of the data, and t-test was used to evaluate the differences between the pretest and posttest of the study groups. The differences between the groups were also assessed using one-way ANOVA and Tukey's post-hoc test.

Results

Table 1 shows the mean demographic variables of the study groups. Table 2 shows the mean salivary IgA levels of the study groups at the pretest and posttest. Figure 1 depicts the results of Tukey's post-hoc test regarding the differences between the study groups.

Table 1. The mean demographic variables of the study groups

Experimental Groups	Age (year)	Height (cm)	Weight (kg)
Exercise with Vitamin C	1.10±16.01	7.95±168.85	6.80±69.00
Exercise	0.84±16.28	8.81±168.40	8.07±67.80
Vitamin C	0.99±16.16	7.92±169.50	7.45±68.45
Control	0.71±16.00	8.89±168.83	8.11±68.50

Table 2. The mean salivary IgA levels of groups at the pre-test and post-test

Experimental groups	Pretest IgA	Posttest IgA
Exercise with Vitamin C	13.25±69.49	11.10±97.95
Exercise	13.18±69.64	11.10±91.97
Vitamin C	13.10±68.90	11.58±78.52
Control	14.03±69.87	14.03±74.08

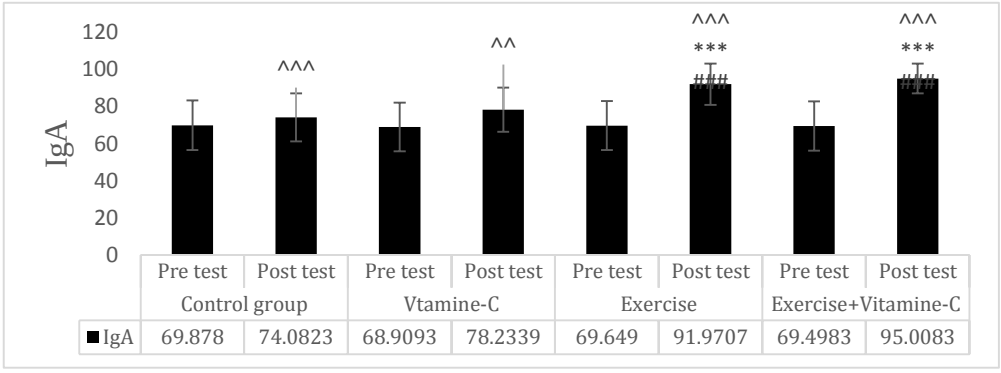

Figure 1. IgA Levels in Study Groups (^^P=0.01 and ^^^P=0.0001 show significant increase compared to pretest; ***P=0.001 shows significant increase compared to control group; ###P=0.001 shows significant increase compared to vitaminC group)

The results of one-way ANOVA indicated significant differences between the groups at the posttest. Moreover, the results of Tukey's post-hoc test showed that the salivary IgA levels of the exercise with vitamin C group (P=0.001) and exercise group (P=0.001) were significantly higher compared to the control group. However, no significant difference was observed in the IgA level between the control and vitamin C groups (P=0.49). The IgA levels in the exercise with vitamin C (P=0.001) and exercise groups (P=0.001) were significantly higher compared to the vitamin C group. Nevertheless, no significant difference was observed between the exercise

group and the vitamin C with exercise group in this regard (P=0.76).

The results of paired t-test regarding the changes at the posttest compared to the pretest of each study group indicated that the posttest IgA levels of the exercise with vitamin C supplementation group (P=0.001; t=-21.56), exercise group (P=0.001; t=-21.58), vitamin C supplementation group (P=0.001; t=2.47), and control group (P=0.001; t=8.35) were significantly higher compared to the pretest levels.

Discussion

According to the results of the present study, salivary IgA levels increased more significantly in the male high school students of the regular exercise with vitamin C supplementation group and regular exercise group compared to the vitamin C supplementation and control groups at the posttest. However, the difference between the vitamin C supplementation and control groups at the posttest was not considered significant in this regard. Notably, salivary IgA levels increased at the posttest in all the study groups compared to the pretest.

In the present study, regular exercise increased the level of salivary IgA at the posttest compared to the pretest. Therefore, it could be inferred that regular exercise could enhance the immune function through mechanisms such as the differentiation of Th1 to Th2 defense cells, increasing the level of anti-inflammatory factors (e.g., IL-10), increasing the secretion of immunoglobulins by lymphocytes, and decreasing cytokines. Furthermore, pro-inflammatory outcomes may be possible in the form of releasing interferon gamma, IL-1 receptors, and tumor necrosis factor receptor alpha, which improve the antigen expression of macrophages (Mϕ or Mp) followed by the development of phagocytic and cytotoxic in the mucosa (16). Previous findings in this regard have demonstrated that low- and moderate-intensity exercise could enhance the immune function (e.g., IgA) (19). Furthermore, another study on children with spastic cerebral palsy (age: 9.5±1.8 years) indicated that 12 weeks of resistance and endurance exercise training had significant effects on salivary immunoglobulin A, salivary alpha-amylase, and total salivary protein (20).

According to a research conducted on 216 subjects with depressive symptoms and IgA nephropathy, regular physical activity was reported to improve the cardiopulmonary function, depressive symptoms, and health-related quality of life in the patients (21). On the other hand, several studies have confirmed that intense exercise, poor nutrition, and insufficient sleep may modulate immunoglobulins and suppress the immune system (1). Furthermore, high-intensity exercise has been shown to decrease the immune function and some immune factors, such as immunoglobulins (16).

In an eight-week study aiming to investigate the effects of continuous aerobic training with a low-carb diet on the serum immunoglobulins in overweight adult men aged 36-50 years, the levels of IgA, IgG, and IgM were reported to decrease (17). Moreover, the results of a six-week study regarding the effects of aerobic exercise (three 60-minute sessions per week) on the body composition, cortisol, and salivary IgA levels of 19-year-old subjects indicated no significant impact on the salivary IgA (22). Evidence suggests that depending on intensity and duration, exercise could improve general health and the immune function (23).

According to the current research, regular exercise with vitamin C supplementation could increase the salivary IgA levels at the posttest compared to the pretest. In another research, five days of vitamin C supplementation (500 mg/day) followed by an exhaustive aerobic exercise session increased serum IgA and IgG levels on the sixth day in 32-year-old women (24). Another study showed the significant effects of vitamin C intake (500 mg per day for five days) on serum IgA and IgG in 24 male Wushu athletes following exhaustive aerobic training (3). Meanwhile, some findings have indicated that high-intensity exercise and vitamin C have no significant effects on immunoglobulin levels (19, 17). For instance, a seven-day study of young women with the mean age of 22 years showed that the effects of exercise (two sessions of exhaustive exercise) and vitamin C (1,000 mg per day) on salivary IgA, cortisol, and total salivary proteins were not significant (25).

Some studies have also denoted that factors such as vitamin dosage, type of exercise, age, and geographical location could affect the cellular and humoral immune function (2). By

participating in the structure of antioxidants, vitamin C could increase antioxidant and anti-inflammatory activity and improve the molecular and humoral immune function (26). In addition to vitamin C, the presence of other structural vitamins (e.g., vitamins E and D) in the production of antioxidants may be effective in the improvement of the cellular and humoral immune function and protection against pulmonary diseases (26, 27).

The results of the present study indicated that vitamin C consumption could increase the level of salivary IgA (immune factor) at the posttest compared to the pretest. According to the literature, consuming the minimum vitamin C dosage of 10 milligrams per day could enhance the immune function, while two grams of this vitamin per day is recommended to adults by the United States Food and Drug Administration (FDA) (27). In addition, consuming 1,000 milligrams of vitamin C per day may be beneficial and uncomplicated for pneumonia patients, while 2-4 grams of vitamin C could decrease the risk of catching a cold more significantly (27). In line with these data, vitamins C, E, and D have been shown to improve the immune function and several immune factors (26, 27).

Diarrhea is the only side-effect of high-dose vitamin C consumption, and no other complications have been attributed to this supplement (8). Notably, the consumption of 10-15 milligrams and 0.3 gram of vitamin C per day has been reported to be insufficient for the improvement of the immune function and the prevention of acute pulmonary diseases in men (28). Therefore, it could be inferred that various doses, combinations, and duration of vitamin C intake could exert different effects on the immune function and immune factors.

According to the results of the present study, the salivary IgA level of the control group increased slightly at the posttest compared to the pretest, which could be due to the fact that our study was performed during winter, in which the probability of antigens entering the body is higher, which in turn increases the immune response and secretion of more antibodies (2).

One of the limitations of current research was the lack of control over the nutrition and medication of the subjects. However, recommendations were made to avoid the unnecessary use of medications during the research. Another limitation was failure to measure other immunoglobulin levels. Therefore, it is recommended that the levels of these physiological variables be evaluated in the further investigations in this regard. Moreover, we were not able to examine various doses of vitamin C and other vitamins, as well as exercises with varied intensity, which should be addressed in similar studies.

Conclusion

According to the results, regular exercise and vitamin C supplementation alone and exercise combined with vitamin C supplementation could increase the salivary IgA levels of the male high school students after 12 weeks. Therefore, it is recommended that further investigations assess the impact of various exercises or doses of different vitamins on immunoglobulin levels.

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References

1- Abul Abas, Andrew H, Shiva P. Cellular and Molecular Immunology. 2015.

2- Rahmani Nia F, Ranjbar k. Pysical Activity and Immunology. University of Gilan. 2017.

3- Hasanloei F, Asadpour H, Karvani V. Effect of vitamin c intake following exhaustive aerobic exercise on serum immunoglobulins A and G in wushu athletes. Medbiotech J. 2017; 1(04):149-54.

4-Gunzer W, Konrad M, Pail E. Exercises-induced immune depression endurance Athletes nutritional intervention with carbohydrate, protein and fat – what is possible, what is not?. Nutrition. 2012;4(9):1187-212.

5-Roth D E, Caulfield LE, Ezzati M, Black RE. Acute lower respiratory infections in childhood: opportunities for reducing the global burden through nutritional interventions. Bull ***World Health*** Organ. 2008; 86, 356 -364.

6- Samavati Sharif MA, Heidarianpour A. The effects of endurance swimming plus vitamin c supplement on the indices of oxidative stress among male rats. ***J Ilam Uni*** Med Sci. 2017; 10;24(6):1-10.

7-Boretti A, Banik BK. Intravenous vitamin C for reduction of cytokines storm in acute respiratory distress syndrome. PharmaNutrition. 2020;12: 100190.

8- Krueger RF, Derringer J, Markon KE, Watson D, Skodol AE. Initial construction of a maladaptive

personality trait model and inventory for DSM-5. Psychol Med. 2012;42(9):1879-90.
9- Akbarpour-beni M, Kh A. Impact of aerobic training on CD25, Interleukin-2 and salivary IgA in elderly men. Feyz, ***J Kashan Univ*** Med Sci. 2012;16(1):24-30.
10-Gleeson M. Immune function in sport and exercise. ***J*** Appl Physiol. 2007;103(2):693-9.
11-Zarneshan A. The Efficacy of Aerobic and Breathing Exercise Training on Asthma Control and Physical - Psychological Health Promotion in Women with Asthma. Iran J Health Educ Health Promot. 2018; 6 (2):179-188.
12-Ghasemi G, Barati Rokati P, Salehi O. Effect of Eight Weeks of Modified Pilates Training on Pulmonary Function and Quality of Life of Veterans Exposed to Chemical Warfare. Iran J War Public Health. 2019; 11 (4):183-188.
13-Bashirian M, Zarehbinan F, Shahsavari S, Sabooni F. Comparative efficacy of two breath training methods and lower extremity aerobic exercise on amount of respiratory ventilation and quality of life of patients with chronic bronchitis. Sci J Kurd Univ Med Sci. 2009; 14 (1):23-32.
14-Farzanegi P, Azarbayjani MA, Farahmand M. The effects of single and repeated bouts of gymnastic training on salivary IgA and cortisol. J Maz Univ Med Sci. 2008; 18(67).
15- Asadbakhti A, Choobineh S, Kordi M. The effect of a soccer-specific exercise protocol on salivary IgA, IgG, IgM and cortisol in male athletes. Sport Physiology. 2012; 4(15): 83-96.
16-Martin SA, Pence BD, Woods J A. Exercise and respiratory tract viral infections. Exercise & Sport Sciences Reviews. 2009; 37(4), 157.
17-Zarghamizadeh D, Ghasemian SM. The effect of continuous aerobic training with low carbohydrate diet on the serum immunoglobulins A, G, and M in overweight adult men. Rep Health Care. 2018; 4(3):61-6.
18-German NSD. New reference values for vitamin C intake. Ann Nutr Metab. 2015; 67(1), 13.
19- Rakati B. The effect of eight weeks of modified pilates training on pulmonary function and quality of life of chemical veterans. Iran J War Public Heal. 2019;11(4):183-188.
20- Akbari fard L, Zehsaz F, Farzangi N. Concurrent exercise training effect on salivary immunoglobulin A and alpha-amylase in children with cerebral palsy. Ger ***J*** Exerc Sport Res. 2021; 51(1):79–85.
21-Yu Z-h, Ya P, Yue G, Bei YB, Heng F. Effect of physical activity on depression symptoms in patients with IgA nephropathy. J Int Med Res. 2020: 48(1) 1–9.
22-Nemati S, Shabani R. The Effect of aerobic exercise training on salivary IgA and cortisol levels response, body composition and physical fitness in children with allergic. IslamicAzad University, RashtBranch, Guilan, Iran. 2016.
23-Grande AJ, Keogh J, Silva V, Scott AM. Exercise versus no exercise for the occurrence, severity, and duration of acute respiratory infections. Cochrane Database Syst Rev. 2020;(4).CD010596.
24-Talebzadeghan M, Hashemi F, Dehghan E. Effect of a 5-day vitamin c supplements on immunological stress caused by an aerobic exercise session in young women. Jundishapur J Health Sci. 2017;16(5):535-45.
25-Fathi M, Asadi Farsani M, Adeli O. Salivary Immune Factors, vitamin C and physical activity. Journal of Sabzevar University of Medical Sciences. 2018; 25(2):2017-27.
26-Yavari M, Moosavai A, Janani L, Feyzi Z, Vafa M. Effect of supplementation of vitamin D, C and E on the lung spirometry and plethysmography in Idiopathic Pulmonary Fibrosis patients. RJMS. 2018; 25(170): 65-72.
27-Hemilä H, Louhiala P. Vitamin C may affect lung infections. J R Soc Med.2007:100 (11):495-498.
28- Gombart AF, Pierre A, Maggini S. A review of micronutrients and the immune System– Working in harmony to reduce the risk of infection. Nutrients. 2020;12(1): 236-46.