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Research Article

Effects of Probiotic Supplementation on Immune Response in Soldiers: A Randomized, Double-Blinded, Placebo-Controlled Trial

Mahtab Noorifard[1], Elahe Ebrahimi[2], Arasb Dabbagh Moghaddam[3], Zatollah Asemi[4], Ramin Hamidi Farahani[5] and Seyed Milad Mousavi Jazayeri[2,*]

[1]Department of Infectious Disease and Infectious Disease Research Center, AJA University Medical Sciences, Tehran, Iran
[2]Department of Microbiology and Infectious Disease Research Center, AJA University Medical Sciences, Tehran, Iran
[3]Department of Public Health and Community Medicine, Infectious Disease Research Center, Faculty of Medicine, AJA University Medical Sciences, Tehran, Iran
[4]Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
[5]Department of Infectious Disease, Faculty of Medicine, AJA University Medical Sciences, Tehran, Iran

[*]*Corresponding author*: Department of Microbiology and Infectious Disease Research Center, AJA University Medical Sciences, Tehran, Iran. Email: mousavijazayeri.sm@gmail.com

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Abstract

Background: Athletic soldiers undergo strenuous and high-endurance training to prepare for competition. Stress and deprivation of proper nutrition can weaken the immune system in athletic soldiers. Therefore, enhancing the immune system function in addition to enhancing the performance of athletic soldiers can lead to a reduction in health costs for the military.
Objectives: In this study, the effects of probiotic supplementation on the immune response of soldiers were assessed.
Methods: In a randomized, double-blinded, placebo-controlled trial, 42 athletic soldiers were randomly divided into two groups of 21 members. Participants in the probiotic group daily received one glass of fruit juice containing *Bacillus coagulans* (2×10^9 CFU/g). The control group consumed the placebo at the same time. At the beginning and end of the study, as well as at specific times, IgA, CD4, lactate, and urea were measured in blood samples of all participants.
Results: Probiotic supplementation increased the concentration of IgA (+115.7 $\pm$ 28.3 vs. -108.3 $\pm$ 25.7 mg/dL, $P < 0.001$) and CD4 levels (+129.1 $\pm$ 42.6 vs. -54.5 $\pm$ 114.6 cells/μL, $P < 0.001$) after three months in the test group. In addition, a significant decrease was seen in serum lactate (-5.7 $\pm$ 10.1 vs. +10.1 $\pm$ 7.4. mg/L, $P < 0.001$) and urea (-9.9 $\pm$ 6.7 vs. +2.3 $\pm$ 5.6 mg/dL, $P < 0.001$) concentrations following probiotic supplementation compared to the placebo.
Conclusions: Probiotic consumption for 12 weeks has a positive effect on the immune response of soldiers.

Keywords: Immune Response, Nutrition, Probiotic Supplementation, Soldiers, Sports

1. Background

Strong and repeated physical activity and being under the stress of exercise pressure have negative effects on professional athlete's health. They also experience severe mental and physical stress due to their presence in deprived areas and war conditions. Proper stress and food deprivation can cause the immune system to weaken, leading to a variety of physical illnesses in soldiers. Therefore, strengthening the performance of the immune system by using appropriate dietary supplements, in addition to increasing the performance of soldiers, can reduce the cost of health for the military. Increasing the risk of disease in athletes during prolonged exercises can be associated with intermittent disorders in cellular and humoral immune function, altering the rate of immunoglobulin secretion. Probiotics are similar bacteria to human gastrointestinal microflora used in food supplements, which by improving the microbial balance of the intestine, play an important role in human health and nutrition (1, 2).

Health promotion protects the body against various infections. Probiotics enrich the digestive system with good bacteria, restore the balance between them, and diminish the damage caused by the imbalanced normal flora. These microorganisms can modulate the immune system (3), limit the establishment of pathogens (4), and control inflammatory bowel disorders. Hence, probiotics increase the hosts' health level. The beneficial effects of probiotics included reducing both diarrhea and constipation, protecting from yeast infections, preventing the growth of pathogens, increasing the growth of useful bacteria, reducing toxin levels, raising immunity and resistance to infection, producing vitamins and nutrients, inducing organic acids to be more produced, diminishing cholesterol levels and allergic reactions, and exerting antioxidant effects (5).

Probiotics can improve the function of the digestive system by reducing the risk of disease and, therefore, can directly improve athletic performance (6). Respiration increases during intense physical activity, which, in turn, leads to increased oxygen transmission to the body. In the absence of sufficient oxygen in the aerobic system to produce energy, the body produces energy from anaerobic metabolism (glycogen). Probiotics also can lead to the immune system's function improvement. By decreasing the rate of respiratory infection (7), they improve respiratory and circulatory function and cause delayed fatigue by increasing blood flow (8). The positive effects of using probiotic supplements in immune responses have been proven in many studies. Previous studies have shown that the use of several species of *Lactobacillus* increases the amount of CD4+, CD8+, and cytotoxic T cells (9).

The results of some other studies have shown that probiotics stimulate interferon secretion and lead to a rise in hosted phagocytic capacity (10). Kotani et al. (11) reported that taking *Lactobacillus* increases IgA secretion in saliva. Studies have shown that the decrease in the number of natural killer (NK) lymphocytes and cells and the defective function of T cells in the human due to stress may increase the risk of autoimmune diseases and upper respiratory tract infections (9). The immunogenicity of probiotics has several aspects, including the induction of phagocytosis, secretion of immunoglobulin A (IgA), modification of T-cell responses, an increase of Th1 responses, and a decrease of Th2 responses (1, 2, 12). Furthermore, probiotic strains have a potential for in-vitro immunization by increasing the levels of anti-inflammatory cytokines, IL-10s, and lowering the levels of anti-inflammatory cytokines (7). Researchers also reported a relationship between *Bacillus coagulans* and the immune system (13, 14). The use of *Bacillus coagulans* in various forms (powder, pills, and capsules) has been reported in the treatment of various human diseases, such as gastrointestinal diseases and urinary tract infections (15). Immune system disorders may be caused by exercise stress and loss of energy balance. Hard exercises may reduce the number of lymphocytes, disrupt the activity of NK cells through oxidative inflammation, and decrease the concentration of immunoglobulin and levels of antimicrobial proteins. The response of antigen detection cells and dendritic cells to probiotics plays an important role in the regulation of mucosal homeostasis and induces mucosal immunity (16).

2. Objectives

Considering the importance of improving the health and performance of athletes, it is essential to achieve a diet effective on their physical and mental abilities. In this study, the effect of probiotic juice containing *Bacillus coagulans* was studied on improving the immune function of soldiers.

3. Methods

3.1. Participants

This randomized double-blinded study enrolled 42 athletes (age 18 to 25 years, weight 60.6 $\pm$ 7.5 kg, height 169 $\pm$ 5.6 cm). After receiving consent from the individuals, they were randomly divided into two groups. The exclusion criteria included smokers and immune-compromised patients. All participants were asked to not use any probiotic supplement or food during the study.

3.2. Experimental Design

The height, weight, and body mass of all participants were measured at the beginning and the end of the study (Table 1). All participants received fruit juice for 12 weeks based on the above pattern: Group 1 consumed probiotic fruit juice, and group 2 received only fruit juice with no supplement (placebo). Individuals had regular exercise three times a week. Two participants were excluded from the study due to non-compliance with the criteria.

Table 1. Solutions and Measurement Steps with Kits

	Blank	Standard	Specimen
R1 (euffer), mL	1.0	1.0	1.0
R2 (enzyme), μL	50	50	50
Standard sample[a], μL	-	10	-
R3 (Alk.Reagent)[b], μL	200	200	200

[a]Mixing and incubation for 5 min at 37°C.
[b]Mixing and incubation for 5 min at 37°C; measuring absorbance of the specimen and standard against blank reagent.

3.3. Experimental Protocol

3.3.1. Nutritional Interventions

Fruit probiotic juice containing 2×10^9 CFU/g *Bacillus coagulans* (provided by TakgeneZist Company) was given to group 1, while group 2 had only fruit juice without any supplementation (placebo).

3.3.2. Blood Collection

At the beginning of the study and every 15 days within three months after the training (3800 m swimming), 10 ml of peripheral blood was taken from each person in a sitting position. Samples were collected in tubes containing EDTA. The specimens were then centrifuged (Hettich D-78532, Tuttlingen, Germany), and the serum was collected.

3.3.3. Measurement of IgA

The immunoglobulin A (IgA) level in blood was assessed via the ELISA method. Blood samples were collected in vials, and serum samples were isolated. The serum antibody titer was measured based on the protocol of Pars Azmon Kit.

3.3.4. Measurement of CD4 Cell Count

The CD4 cell count was measured by the flow cytometry technique. Blood samples were collected in EDTA tubes and centrifuged. Washing with phosphate-buffered saline (PBS) was performed to isolate the cell layer; then, 10 ml of anti-CD4 monoclonal antibody was added, and the samples were incubated for 20 min in darkness. Then, they were rinsed twice with PBS to produce fluorescent antibodies (containing fluorescein isothiocyanate fluorochrome). Cells containing the monoclonal antibody and stained cells were plotted in a BD FACSCalibur flow cytometry system. The results were reported based on the percentage of PBMC in total suspension. All reagents, hardware, and software were purchased from Becton Dickinson (UK) and used as per the manufacturer's instructions.

3.3.5. Measurement of Lactate

Lactate was measured by the colorimetric method according to the instructions of the kit (Zelbio, Padgin Teb), as follows: 6 cc of metaphosphoric acid was added to the blood sample and then centrifuged at 3000 rpm for 15 min. Three separate cuvettes for blood, calibrator, and blank were selected, and 2 cc of Tris hydrazine buffer was added to each of them; then, 0.1 cc of the supernatant, lactate calibrator, and metaphosphoric acid (3 g/L) were added to each cuvette. Next, 30 μL of lactate dehydrogenase and 200 μL of NAD (27 mol/L) were added. The samples were then stored at room temperature for 15 min until the absorbance was read at 540 nm. The lactate content of blood samples was calculated using the following formula:

$$Lactate\ concentration = \frac{Sample\ absorption}{Standard\ absorption} \quad (1)$$

3.3.6. Measurements of Urea

Blood urea measurements were performed according to the above protocol (Table 1). Sample absorption and control absorption were measured at 580 nm, and the urea concentration in the sample was calculated using the following formula:

$$Urea\ concentration = \frac{Sample\ absorption}{Standard\ absorption} \times 50 \quad (2)$$

3.4. Statistical Methods

Data obtained from the tests were analyzed using GraphPad Prism (version 6.01). An Analysis of Variance (ANOVA) was used to determine the effects of probiotic supplementation on the immune response in the two groups. The paired-samples *t*-test was used to identify the intragroup (height, weight, and BMI) differences at the beginning and the end of the experiment. The independent-samples *t*-test was used to diagnose the differences in general characteristics between the two groups. The P values of less than 0.05 were considered significant.

4. Results

In this research, two participants were excluded from the study due to the non-observance of the study criteria. After 12 weeks, there was no significant difference between the probiotic and control groups in mean height, weight, and BMI (Table 2).

Table 2. General Characteristics of Study Participants[a]

	Placebo (N = 20)	Probiotic (N = 20)	P[b]
Age, y	18.1 ± 1.2	18.0 ± 1.3	0.90
Height, cm	168.8 ± 2.0	168.6 ± 2.4	0.77
Weight at study baseline, kg	61.1 ± 3.8	59.6 ± 2.9	0.15
Weight at end-of-trial, kg	61.3 ± 3.3	60.0 ± 2.9	0.19
Weight change, kg	0.2 ± 2.5	0.4 ± 1.7	0.68
BMI at study baseline, kg/m^2	21.5 ± 1.4	21.0 ± 1.1	0.22
BMI at end-of-trial, kg/m^2	21.5 ± 1.4	21.1 ± 1.1	0.32
BMI change, kg/m^2	0.06 ± 0.9	0.1 ± 0.6	0.69

[a]Values are expressed as means ± SD.
[b]Obtained from independent *t*-test.

In the first month, the use of a probiotic supplement did not significantly affect the variables studied, but over time, the effect of probiotic consumption increased. The IgA levels (+115.7 ± 28.3 vs. -108.3 ± 25.7 mg/dL, P < 0.001) and the number of CD4 cells increased significantly (+129.1 ± 42.6 vs. -54.5 ± 114.6 cells/μL, P < 0.001) (Table 3). Also, a decreasing acceptable in lactate concentrations (-5.7 ± 10.1 vs. +10.1 ± 7.4. mg/L, P < 0.001) and urea (-9.9 ± 6.7 vs. +2.3 ± 5.6 mg/dL, P < 0.001) was observed between the test group and the control group (Tables 3 and 4).

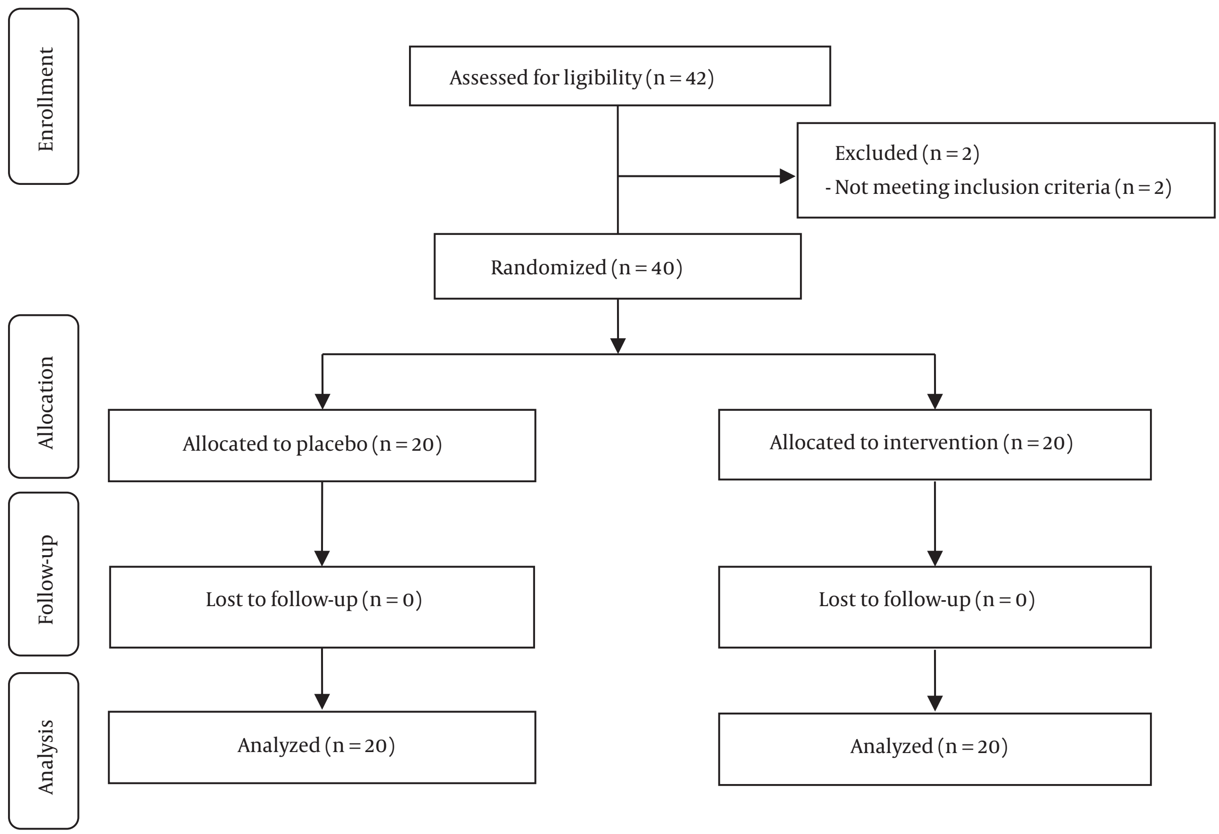

Figure 1. A summary of patient flow diagram

5. Discussion

This was the first study to investigate the effects of probiotic fruit juice containing *Bacillus coagulans* on athletic soldiers. We found that the consumption of probiotic supplementation in 12 weeks has a positive effect on the athletic soldiers' immune response. In professional athletes with intense and prolonged training, anomalies in IgA secretion may increase temperature, and mucosal immune deficiency can reduce their performance. Gut bacteria play an important role in many aspects of biology, including metabolism, endocrine gland secretion, neuronal function, and immune function. The reaction of probiotics with intestinal epithelial cells can induce the antimicrobial compounds secretion and cytokines, resulting in the activation of B and T cells in the digestive tract lymphoid tissue (17). Our findings also indicated a significant increase in IgA levels among soldiers under study. Also, increased IgA may accelerate the activity of macrophage and phagocytosis, which can increase the strength of the immune system and lead to the treatment of infectious diseases (18).

Recent research has shown that probiotics are associated with increased phagocytosis and IgA production, affecting the acquired immune system. Acquired immunity is specific, and its development requires time (19). In the first month of our study, the consumption of probiotics was not very effective on the levels of IgA and CD4, but with time, the effect of probiotic consumption was more pronounced. In 2007, Tiollier et al. (20) studied the effect of probiotic use on immune function and respiratory infections in soldiers. The results showed that probiotic use is effective in increasing the level of health and improving the function of the immune system and reducing the incidence of respiratory infections. In 2014, Pyne et al. examined the effect of probiotic supplements (*Lactobacillus acidophilus* and *Bifidobacterium*) on professional athletes, and found that probiotic supplements were effective in improving immune system function, reducing gastrointestinal disease, and improving athletic performance. Providing the nutrients needed by athletes will have a great impact on their health and increase productivity (8). In 1989, Kia Salar et al. studied the effect of probiotic yogurt on fe-

Table 3. Baseline and End-of-trial Values of Immune Responses in Study Participants[a]

	Placebo (N = 20)	Probiotic (N = 20)	P[b]
IgA, mg/dL			
Baseline	176.7 ± 21.6	231.1 ± 27.8	< 0.001
Wk4	118.5 ± 14.3	280.5 ± 26.9	< 0.001
Wk12	68.3 ± 7.6	346.8 ± 25.2	< 0.001
Change	-108.3 ± 25.7	115.7 ± 28.3	< 0.001
CD, cells/μL			
Baseline	510.9 ± 109.7	582.1 ± 28.4	0.008
Wk4	528.9 ± 39.4	644.6 ± 39.9	< 0.001
Wk12	452.3 ± 31.3	711.2 ± 38.8	< 0.001
Change	-54.5 ± 114.6	129.1 ± 42.6	< 0.001
Lactate, mg/L			
Baseline	53.3 ± 8.2	34.3 ± 8.2	< 0.001
Wk4	51.8 ± 9.8	34.4 ± 5.5	< 0.001
Wk12	63.4 ± 5.1	28.6 ± 7.0	< 0.001
Change	10.1 ± 7.4	-5.7 ± 10.1	< 0.001
Urea, mg/dL			
Baseline	39.7 ± 4.8	30.6 ± 4.3	< 0.001
Wk4	38.9 ± 5.1	33.1 ± 4.9	0.001
Wk12	42.0 ± 3.3	20.7 ± 3.8	< 0.001
Change	2.3 ± 5.6	-9.9 ± 5.6	< 0.001

[a]Values are expressed as means ± SD.
[b]Obtained from independent *t*-test.

Table 4. Adjusted Changes in Metabolic Variables in Soldiers Receiving Either Placebo or Probiotic Supplements[a, b]

	Placebo (N = 20)	Probiotic (N = 20)	P[c]
IgA, mg/dL	-131.1 ± 5.3	138.6 ± 5.3	< 0.001
CD, cells/μL	-89.3 ± 9.1	163.9 ± 9.1	< 0.001
Lactate, mg/L	19.1 ± 2	-14.7 ± 2.0	< 0.001
Urea, mg/dL	7.2 ± 1.0	-14.7 ± 1.0	< 0.001

[a]Values are expressed as means ± SD.
[b]Values are adjusted for baseline values, age, and baseline BMI.
[c]Obtained from ANOVA.

male endurance swimmers. The study found that taking probiotics increased the function of the immune system, reduced the rate of respiratory infections, and increased swimmers' physical strength and setting records.

The results of this study were confirmed by the results of Ghadami et al. (10) and West et al. (16), that reported probiotic consumption had a positive effect on the mucosal immune system CD4 cells and dendritic cells. The results of these studies are in good agreement with ours. Another study showed that *Lactobacillus fermentum* decreased the risk of respiratory infections by improving mucosal immune function (21). Probiotics are effective in the restoration and maintenance of the compounds and the normal flora of the human intestine in health (22). The results of Sullivan et al. (23) study showed that probiotic supplement containing lactic acid bacteria increased the physical strength of athletes, delayed fatigue, and was effective in treating chronic fatigue syndrome. Johnson reported that the consumption of *Bacillus coagulans* significantly increased CD4 cells, improved the immune system's performance, and had anti-inflammatory effects (14). Furthermore, Klatt et al. (24) showed that the use of probiotics increased CD4 cell counts and improved immune function. Baron showed that the consumption of *Bacillus coagulans* improved immune system defense ability against viruses such as the influenza virus and enteroviruses (13). According to the results of this study and other ones, there are positive effects for probiotics on human health and the prevention of immunity reduction caused by intense activity in athletic soldiers.

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Footnotes

Authors' Contribution: Elahe Ebrahimi, Seyed Milad Mousavi Jazayeri, and Arasb Dabbagh Moghaddam designed the study, extracted the data, and drafted the original article. Zatollah Asemi, Mahtab Noorifard, and Ramin Hamidi Farahani performed statistical analysis of the data.

Conflict of Interests: There were no conflicts of interest associated with this study.

Ethical Approval: The study was corresponded to the Helsinki Declaration and under the supervision of the Medical Ethics Committee of the AJA University of Medical Sciences. The attendance of all participants in this study was with personal satisfaction. The process was fully explained to all the participants. This study was approved by the Aja University of Medical Sciences Ethics Committee (code: IR. AJAUMS.1394.01).

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