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Chapter

The *Bifidobacterim bifidum* (BIB2) Probiotic Increased Immune System Factors in Men Sprint Athletes

Ali Hossein Khani, Seyed Milad Mousavi Jazayeri, Elahe Ebrahimi and Ayoub Farhadi

Abstract

Foods supplemented with probiotics enhance athletes' immune system functions, improve body health and consequently decreases athlete's health maintenance costs. Probiotics improve immune system function against pathogens via affecting on innate immune system, humeral immunity and cytokines. The effects of consumption of Iranian probiotic *Bifidobactrium bifidum* (BIB2) on athletes' immune system functions were evaluated. The results showed studied immune system factors were significantly different between test and control groups, so that IgA, IgM, lymphocyte and monocytes percentage and CD4 measurements of test group were higher than control. The *Bifidobacterim bifidum* (BIB2) probiotic consumption can affect some immune system factors; therefore its ability to improved general health should be studied more.

Keywords: probiotics, CD4, IgA, IgM, monocyte, lymphocyte, sprint athletes, *Bifidobactrium bifidum* (BIB2)

1. Introduction

There is a general belief among elite athletes and their coaches that overtraining causes resistance to infection. Epidemiological studies report that symptoms of respiratory tract infection increases in 1–2 weeks after strenuous endurance competitions. The highest percentages of patients were athletes who exceeded their training threshold level that is associated with the training load [1, 2]. The biological balance of body organs improves the health of the host, improving performance and increasing power of the immune system [3]. Probiotics are a group of living microorganisms that improve health by improving biological balance when added to foods or consumed as supplements. These organisms increase immune system function and enhance host defense against harmful microorganisms. The benefits of probiotics such as reducing toxins, increase immunity and resistance to infection, produce vitamins and nutrients, organic acids, reduce allergic reactions, respiratory infections, reduce the symptoms of irritable bowel syndrome, arthritis, rheumatoid and modulating immune responses have been shown in many studies [4, 5]. Overwhelming exercise undertaken by athletes or military personnel diminishes

immune system function and increase gastrointestinal complaints, as well as increasing the risk of disease and infections [6–8]. In many studies, reduced immunity after chronic fatigue has been seen in over trained athletes [9–11]. Nieman et al. (2000) showed that regular and continuous exercise enhances the strength of athletes' immune system; while undertaking heavy and alternate physical activities had the reverse impact. Also, some research shows that excessive exercise also damages the immune system is impaired [12, 13].

The humoral immunity in athletes is often studied by mucosal immunoglobulin measurements, especially the changes in the secretion of IgA and IgM from tissue was reported in sporting activities [13]. Due to reduction of immunoglobulins in sport activities (and increased risk of infection in the upper respiratory tract) investigating the IgA and IgM is important [14]. Because the antibodies secretion, the lymphocytes have a very important role in the immune system [15]. Monocytes are the largest cells in the bloodstream and involved in phagocytosis in the early stages of the immune response. Also, monocytes produce the cytokines that activate lymphocytes and consequently stimulate inflammation [16]. Immune system malfunctions might be caused by stress, sleep disorder, exercise, and negative energy balance. Hard exercises raise neutrophil count; however, they decrease lymphocyte count, natural killer cell activity (NK cell) via disturbance with oxidative burst, neutrophil function, immunoglobulin's level and antimicrobial proteins level in saliva.

The effects of probiotics bacteria in improving immune function and preventing disease have been shown in numerous studies [6, 17–19]. The *Lactobacillus* and *Bifidobacterium* produce bactericidal and bacteriostatic agents such as lactic acid that can prevent pathogenic bacteria growth. Therefore probiotics reduce tissue inflammation directly or with antagonistic effects on pathogens [20, 21]. It has been shown that probiotics stimulate interferon-A secretion and this leads to increasing host phagocytic capacity [22, 23]. Kotani et al. reported that consuming *Lactobacillus* rised salivary IgA secretion [24]. Therefore, in this study the effects of juice supplemented by *Bifidobacterium bifidum* probiotic (2×10^9 cfu/ml) on immune factors including IgA, IgM, lymphocytes, monocytes and CD4 cells count were in the men's sprint athletes was investigated. The results could play an important role for athletes to overcome many diseases and infections, especially respiratory infections.

Effects of fruit juices supplemented by *Bifidobacterium bifidum* (BIB2) probiotic ($2*10^9$ cfu/ml) was assessed on immune system function of sprint athletes. Hence, 56 male athletes aged 21 ± 3 years and average weights of 78 ± 5 kg which divided into 2 groups: the first group received a glass (200 cc) juices containing probiotic daily for 12 weeks and control group received only simple juice (placebo) without probiotic. All volunteers had general health and did not smoke. Also they were asked not to use any probiotics products and antibiotics during the survey. The athletes had three times exercise per week. Before experiment, the probiotic characteristics and testing process were explained for all participants. All athletes voluntary participated in the experiment.

1.1 Blood sample collection

First, prior to the tests, the blood samples were collected from athletes after fasting, then once a month 24 h after 100 meters running, 8 ml of blood were collected from both studied groups in EDTA tubes. The bloods were centrifuged at 5000 rpm for 15 min and serums were collected. The blood cell counting was done using cell counter device (BC-2000 Mindray).

1.2 The IgA and IgM measurements

The IgA and IgM were measured using ELISA methodology using human IgA and IgM ELISA Kit (ab137980 and ab137982) according to protocol suggested by manufacture (abcam Inc., USA) using ELISA reader (Biotech microplate reader ELX800).

1.3 The CD4 cells count measurements

Flow cytometry is a technique for counting microscopic particles. The CD4 cells count was measured by flow cytometry technique using BD FACSCalibur system and anti-CD4 monoclonal antibody and results were reported based on the percent of peripheral blood mononuclear cell (PBMC) in total suspension.

1.4 Statistical analysis

The obtained data were analyzed using Graphpad Prism (version 6.01) software with 0.05 significant levels.

2. Results and discussion

The results of present study showed that consuming probiotic supplemented fruit juice increased serum IgA level and can be boosted immune system activities (**Table 1**). According to results shown in chart 1, CD4 cells count increased after consuming probiotics (**Figure 2**, **Table 1**) and it probably affects immune system ability to defense against antigens.

Probiotics or their products could have antimicrobial activities or they can prevent colonization of pathogens [4]. They probably have adjuvant effect so they may be stimulate phagocytosis done by leucocytes or may be increase IgA and defensins secretion. They could be attached to gut immune receptors, thus they inhibit competitively pathogenic virus's or bacteria's attachment. In addition, competition occurs for earning foods and nutrition so with suitable colonization of probiotics, we can conquer pathogenic bacteria.

Indigenous bacteria are accepted to add to the immunological protection of the host by making a hindrance against colonization by pathogenic microbes. This hindrance can be upset by sickness and by utilization of antibiotics, in this way, permitting less demanding access of the host gut by pathogens. It is presently trusted

Time	**IgA (cell/μl)**	
	Test group	**Control group**
	Means ± SD*	**Means ± SD**
Before test	364.954 ± 25.98	356.234 ± 42.36
First month	398.065 ± 40.24	350.568 ± 36.78
Second month	431.365 ± 37.96	358.653 ± 42.63
P-value		<0.05

**Standard division.*

Table 1.
The CD4 means ± standard deviation in test and control group.

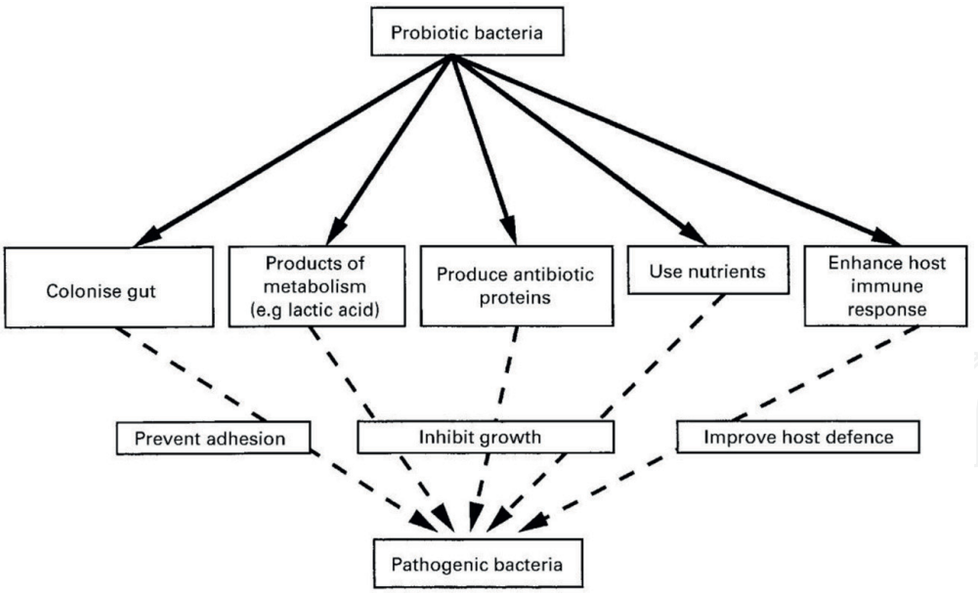

Figure 1.
Potential roles of probiotic bacteria in the human intestinal tract. Probiotic bacteria may act in a variety of ways to prevent the growth and colonization of pathogenic bacteria.

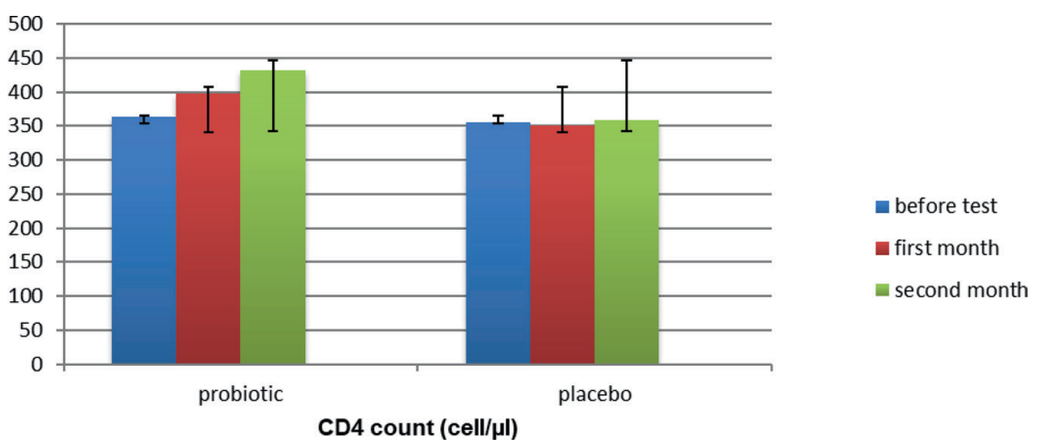

Figure 2.
The CD4 cells count (cell/µl) differences between test and control group in sprint athletes.

that this hindrance can be kept up by giving enhancements containing live 'alluring' microbes: such supplements are called probiotics [24].

n expansion to making a barrier impact, a few of the metabolic products of probiotic bacteria (e.g. lactic acid and a class of anti-microbial proteins named bacteriocins, created by a few bacteria) may hinder growth of pathogenic organisms. Moreover, the alluring bacteria may compete for nutrients with the pathogens. At last, there's a few prove that probiotic microbes may improve the intestine immune reaction against pathogenic microscopic organisms (**Figure 1**).

Studies in rats and mice uncover that lactic acid bacteria managed orally increment the numbers of T lymphocytes, CD4þ cells and antibody-secreting cells, counting those within the intestinal mucosa, and improve lymphocyte expansion, normal killer cell activity, IL-1, TNF and IFN-g generation, antibody production (counting secretory IgA), phagocytic activity and the respiratory burst of macrophages and the DTH reaction [21].

Hard and continues physical activities CAN decrease immune system function and it can lead to infectious diseases [25]. Probiotics have an important role to improve and boost an individual's health via pathogen growth prevention, amino

acids and vitamins production, detoxification, cholesterol reduction and allergic reaction inhibition [26]. In the present study, the effects of *Bifidobactrium bifidum* (BIB2) probioticwe on immune system factors of IgA and CD4 were evaluated. Results revealed significant differences between the control and test group, so that swimmers received probiotic juice had higher IgA level and CD4 cells ($P < 0.05$) count than control group (**Tables 1** and **2**, **Figures 2** and **3**). These increment might accelerate microphage activities and phagocytosis, in turn, boost immune system against respiratory infections and diseases [27]. Lee et al. reported that intake of *Lactobacillus casei* and *Bifidobacterium* improved immune system functions and decreased respiratory infections among athletes [28].

West et al. reported that probiotic consumption lead to mucosal immune system improvement and also increased CD4 and dendritic cells [29]. In other research conducted by Ohashi et al. in the same field showed that increasing IgA level and cytokine secretion occurred during probiotic consumption [30]. Furthermore, it has been showed that probiotic consumption caused potent increasing in lymphocyte and NK cells count in peripheral blood samples and they improved immune system and general health while NK cells count reduction was determined during hard exercises [31, 32]. These results mentioned above were completely in accordance with our findings. Other studies have been done in the field of relationship between CD4 cells and probiotic consumption [33–35]. For example, Jensen et al. reported that consumption of *Bifidobactrium bifidum* probiotic increased CD4 cell, improved immune system functions and had anti-inflammatory effects [34]. Besides, findings obtained by Selbovitz et al. about the effects of probiotic on individuals suffered from acquired immune deficiency syndrome (AIDS) showed that probiotics could boost immune defense against viruses and could prevent virus transmission [35].

Lactobacillus and *Bifidobacterium* are normal inhabitants of the human adult gastrointestinal tract. Complex interactions occur between probiotic bacteria and the different constituents of the intestinal ecosystem (resident microflora and epithelial and immune cells). Mucosal epithelial surfaces, such as the gastrointestinal tract or respiratory tract that host a wide variety of different microorganisms from the external environment, are suitable sites for the onset of infection with pathogens. These levels are not protected. Different mechanisms of defense are involved in permanent and effective monitoring. Secretive secretion system plays an important role in this regard. IgA secretion (sIgA) is the dominant enzyme of the antibody in the secretion of the mammalian intestine. Most IgA is produced from suprapathic plasma cells that produce IgA polymer with the J chain (pIgA).

Time	**IgA (μg/dl)**		**IgM (μg/dl)**	
	Test group	**Control group**	**Test group**	**Control group**
	Means ± SD*	**Means ± SD**	**Means ± SD**	**Means ± SD**
Before test	230 ± 47	210 ± 56	143 ± 28	129 ± 73
First month	237 ± 48	185 ± 78	147 ± 75	132 ± 84
Second month	248 ± 75	193 ± 65	150 ± 62	135 ± 46
Third month	253 ± 83	194 ± 42	152 ± 43	133 ± 73
P-value				<0.05

**Standard division.*

Table 2.
The IgA and IgM means ± standard deviation in test and control group.

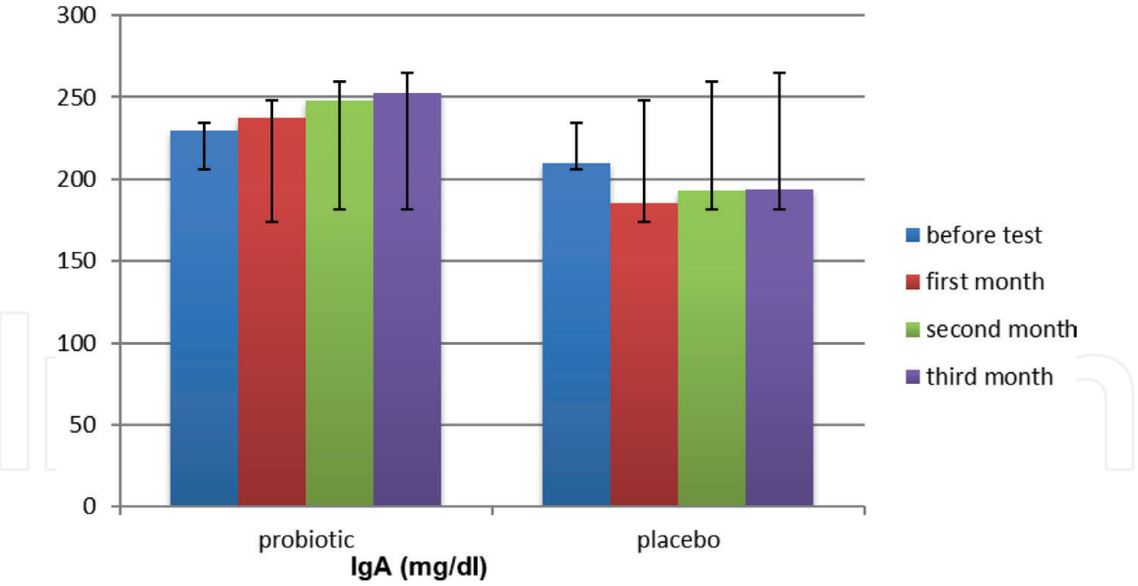

Figure 3.
The IgA concentration (mg/dl) differences between test and control group in sprint athletes.

Epithelial cells express the specific Ig receptor (pIgR). They are very important for the selective transfer of immunoglobulins to the lumen of the intestine. Immunoglobulin classes IgG and IgM are also present in the intestinal secretions, but vary in amounts and isotypes based on animal species. SIgA has many benefits. The dimer and tetrameric forms of IgA contain 4 to 8 antigen binding sites and have several "rewards" agents similar to IgM. IgA is more resistant to the activity of proteolytic enzymes that occur in gastrointestinal secretions [18]. IgA-antigen complexes do not activate the complement with inflammatory outcomes. Thanks to the content of mannan oligosaccharide side chains, sIgA could inhibit the adherence of bacteria with type I fimbriae to epithelial cells regardless of any specific antibody response. The typical response of the secretory immune system is the production of specific secretory IgA antibodies against luminal antigens to prevent other later responses on the epithelial surface. This process is called the immune exclusion and provides non-inflammatory protection in the mucous membrane. The integrity of the epithelial layer, the production of mucus, glycolipid, cytoprotein peptides and antibiotic-like agents are other host protection systems [29]. Protective microflora makes boundary impacts against risky pathogens and makes administrative specialists, such as brief and biofine fatty acids of bacteria. This impact moreover incorporates competition for receptors and metabolic foundations. It is more stamped but less caught on by the part of intestinal microflora within the modulation of homeostasis. The mucosal layer of the digestive tract is known as one of the biggest immune organs known to all sorts of immune cells [12]. It is conceivable to characterize anatomically the acceptance and effector parts of the immune reaction interior of the mucosal immune system. The most acceptance places are Peyer's patches restricted along the total little digestive tract. Lymphoid and accessory cells of Peyer's patches are secured by the follicular epithelium with M cells that serve as antigen preparing cells within the intestinal divider. Lymphocyte migration is important for the transport of immunological information between the different compartments of the intestinal immune system. The dendritic cells are the primary antigen particular cells depleting from the guts after mesenteric lymph node resection, afterward specially T-cells recycled through the intestine wall. After movement into the intestinal lamina propria, the lymphocytes may enter the space between the epithelial cells where they are present as intra-epithelial lymphocytes.

These intra-epithelial lymphocytes (IEL) may collectively constitute up to 27% of the epithelial cell populace and 40% of the peripheral T-cell populace. A tall extent of these cells is CD8+ (77% in pigs, 24% in sheep). They vary from blood T-cells. For case, they are CD90–, CD5– and carry an isoform of CD45 not found on peripheral blood T-cells. The division of the intestine wall from 5-day-old pigs come about in a 10-fold lower add up to lymphocyte surrender compared with grown-up pigs where 26.8×10^6 intra-epithelial lymphocytes and 35.2×10^6 add up to lymphocytes per g of tissue were gathered. Intestinal epithelial cells (IEC). They also contribute to the "education" of thymus independent subpopulations of intra-epithelial lymphocytes. Bacterial dependent activation of intestinal epithelial cells requires a direct contact with IEC and likely the interaction of surface molecules. For the start of the nearby resistant reaction and the actuation of particular T-cells, the entry of luminal antigens over the epithelial boundary is vital. Peyer's patches, or other lymphoid totals secured with a specialized epithelium layer with M cells are the most put for antigen section and T-cell actuation. The other sorts of immune cells such as macrophages, dendritic cells and enterocytes are moreover included within the handling of antigen at the mucosal level. T-lymphocytes from the intestinal lamina propria are ceaselessly beneath the antigen impact in vivo. They are actuated (IL-2 receptor expression, CD95) and viably respond to infection. Permanent antigen stimulation is responsible for the proliferation, maturation, and migration of T-cells to distant tissues where they act as effector cells in the immune response. T-cells produce lymphokines responsible for the aggregation of other types of immune cells (B cells, inflammatory cells) and for the modification of their microenvironment. One of the most important lymphokines is interferon γ (IFN-γ) produced by activated T-cells. It activates effector cells such as macrophages or epithelial cells (IEC) [29]. Murine IEC express MHC class II and ICAM-1 molecules and they display the antigen to T lymphocytes. This work is altered by the physiological or pathological status of the host. IEC are able to create in vitro a wide range of pro-inflammatory cytokines such as IL-8, MCP-1, TNF-α and GM-CSF in case they are affected by intrusive pathogenic bacteria. IL-8 and MCP are chemokines that pull in and actuate neutrophils and monocytes. TNF-α activate immune and inflammatory cells and GM-CSF incorporates a synergistic impact on cell actuation. The infiltration of the tissue with inflammatory effector cells to annihilate pathogens is continuously associated with a certain level of tissue harm. Subsequently the actuation is strictly controlled by external signals such as IFN-γ or TNF-α and by surface molecules like CD54 or CD95, which are able to enact or to discourage the actuation of IEC. There's a clear contrast between Gram-negative non-pathogenic microbes and lactic-acid bacteria (LAB) in their interaction with IEC. In coordinate interaction with IEC both types of bacteria actuate IFN-γ, but the stimulating impact of LAB is confined to the cellular surface molecule expression. The molecular mechanism responsible for these impacts of Gram-negative bacteria and LAB is not caught on. The capacity of Gram-negative bacteria to improve the expression of IFN-γ receptors on IEC increments the affectability of these cells to enactment with IFN-γ. On the other hand, LAB can stimulate IEC for successive actuation with this lymphokine, which is critical in local immune homeostasis. Permanent antigen stimulation of mucosal surfaces could create inflammatory lesions of the tissue. The homeostatic mechanisms should be active in the mucous layer to prohibit such undesirable effects. Apoptosis as programmed cell death shows one of the homeostatic mechanisms. Most of the T-lymphocytes in lamina propria are cells carrying a surface molecule – Fas that shifts apoptotic signals when it reacts with Fas ligand expressed on activated T-cells. Some T-cells also express Fas and Fas ligands that are potentially reactive with IEC or other T-cells [36].

Consumption of *Bifidobactrium bifidum* probiotic improves immune system defense ability against viruses such as influenza and enteroviruses [37, 38]. Also, these results were confirmed by Hu et al. [39]. Our findings indicated that consumption of *Bifidobactrium bifidum* (BIB2) probiotic increases CD4 cells count and they possibly improve the immune system response. According to results of present study and researches have been mentioned before, daily consumption of probiotics products could improve general health of individuals without any side effects. The results of this study show that probiotic juice containing *Bifidobactrium bifidum* probiotic supplementation affecting athletes sprint immune factors and showed significant differences with the control group. According to the survey was taken before the tests the participants feel a better perception of juice Probiotics than the Probiotics capsules.

In the present study athletes who consume probiotic showed the monocyte and lymphocyte cells significantly higher than control group (**Figures 4** and **5**, **Table 3**). Kekkonen et al. showed that in runners who take probiotics lower respiratory infections and gastrointestinal symptoms were reported, but the study did not find any effect of changes in monocyte cells that are inconsistent with our results [6].

West et al. reported the protective effect of probiotics on respiratory infections. In professional athletes who practice a lot in the long and intensive period, body temperature increased, the secretion of IgA confusion is created and therefore interferes in mucosal immunity [29]. Cox investigated the effect of probiotic *Lactobacillus fermentum* for 4 months on the Champ elite endurance. Unlike the results of this study did not observe any change in serum IgA, which can be because of the species and strains of bacteria [7]. Ashraf et al. showed that probiotics strengthen the immune system without causing an inflammatory response and immune modulators and directly affects the mucosal immune system. They suggested that probiotics as a natural and healthy food can increase stress resistance and immunity [40]. Probiotics can increase IgA antibody secretion, increase in phagocytic activity of macrophages and enhance the specific immune effects [41]. In study by Sakai et al. the effect of *Lactobacillus gasseri* on stimulating the production of IgA was shown [42].

Zhao and colleagues showed that *Lactobacillus plantarum* and *Bacillus subtilis* increase activity of lysozyme, superoxide (SOD) and the concentration of IgM

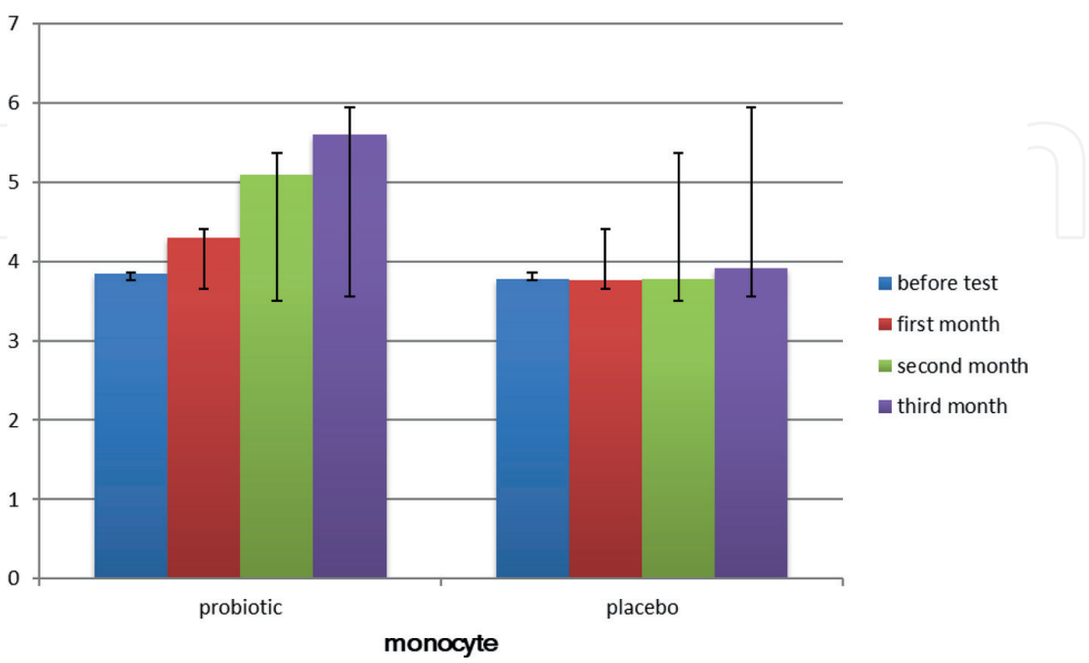

Figure 4.
The monocytes percentage differences between test and control group in sprint athletes.

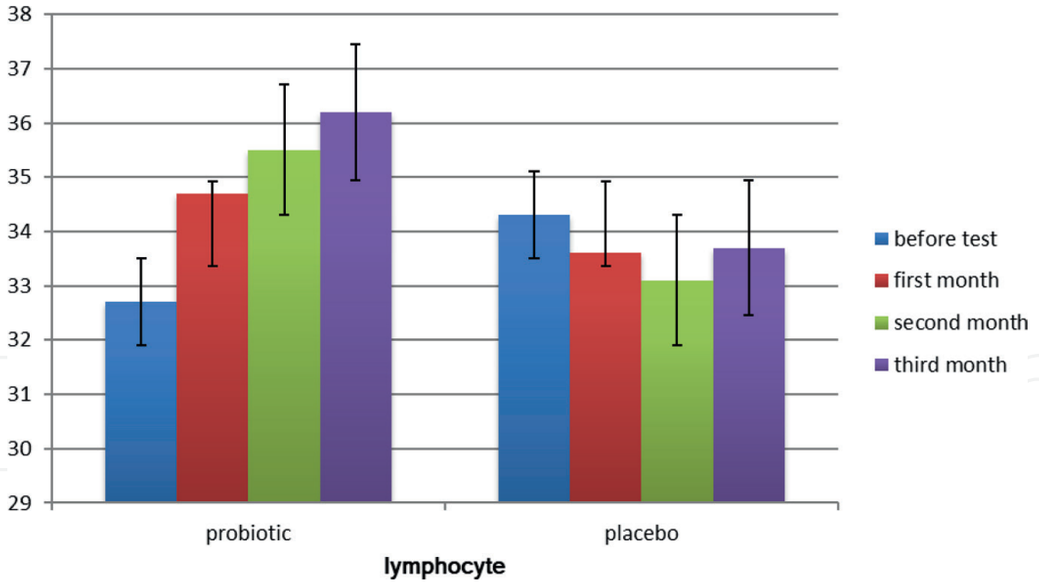

Figure 5.
The lymphocyte percentage differences between test and control group in sprint.

Time	Monocyte (%)		Lymphocyte (%)	
	Test group	Control group	Test group	Control group
	Means ± SD*	Means ± SD	Means ± SD	Means ± SD
Before test	3.85± 0.36	3.78 ± 0.42	32.7 ± 5.6	34.3 ± 4.8
First month	4.30± 0.65	3.77 ± 0.25	34.7 ± 6.4	33.6 ± 7.2
Second month	5.10± 0.42	3.78 ± 0.24	35.5 ± 5.7	33.1 ± 6.4
Third month	5.60± 1.2	3.91 ± 0.90	36.2 ± 7.3	33.7 ± 6.8
P-value				<0.05

**Standard division.*

Table 3.
The monocyte and lymphocytes means ± standard deviation in test and control group.

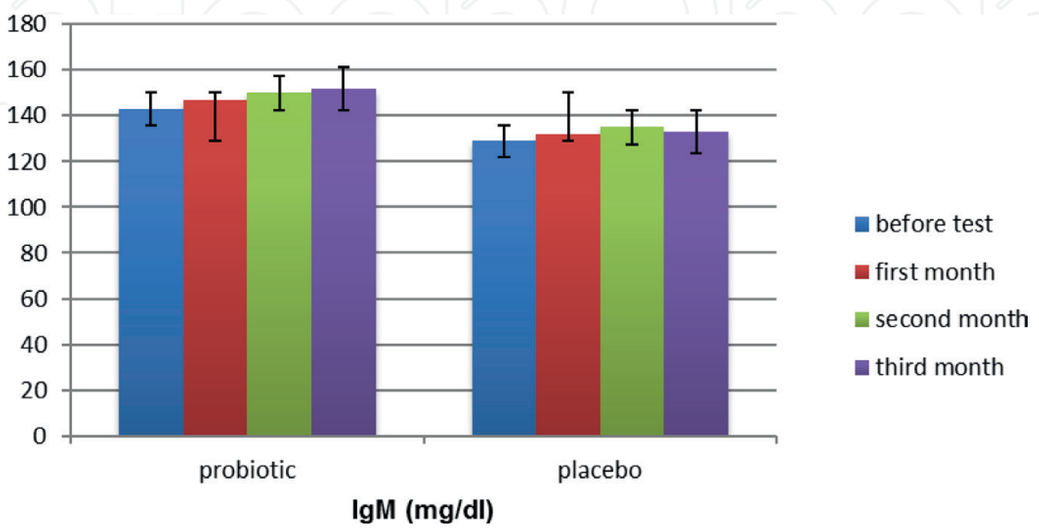

Figure 6.
The IgM concentration (mg/dl) count differences between test and control group in sprint athletes.

Time	Monocyte (%)	
	Test group	Control group
	Means ± SD*	Means ± SD
Before test	3.85 ± 0.36**	3.78 ± 0.42*
First month	4.30 ± 0.65*	3.77 ± .025*
Second month	5.01 ± 0.42*	3.78 ± 0.24*
Third month	5.60 ± 1.2	3.91 ± 0.90*
P-value		<0.05

*Standard division.
**Means with same superscript letters in each row are significantly (P < 0.05) different.

Table 4.
Comparsion the means of monocyte percentage between test and control group (n = 3) in sprint athletes.

Time	Lymphocyte (%)	
	Test group	Control group
	Means ± SD*	Means ± SD
Before test	32.7 ± 5.6a,**	34.3 ± 4.8a
First month	37.4 ± 6.4a	33.6 ± 7.2b
Second month	35.5 ± 5.7a	33.1 ± 6.4b
Third month	36.2 ± 7.3a	33.7 ± 6.8b
P-value		<0.05

*Standard division.
**Means with same superscript letters in each row are significantly (P < 0.05) different.

Table 5.
Comparsion the means of lymphocyte percentage between test and control group (n = 3) in sprint athletes.

that was consistent with our results [43]. In the present study in the athletes who drank juice containing the probiotic, the IgM was significantly higher than control group (**Figure 6** and **Table 2**). Probiotic bacteria in reaction with macrophage cells in tight junctions of epithelial cells, immune cells and dendritic cells led to the development of immune function, then macrophages and dendritic cells also generate immune responses through it. The dendritic cells spread their teeth between intestinal epithelial cells in the intestinal wall and use the probiotics to regulate immune function. The reaction of probiotic with intestinal epithelial cells induces the secretion of antimicrobial factors and cytokines and leading to activation of B and T lymphocytes in the lymphoid tissue of the gastrointestinal tract [6, 44, 32] (**Tables 4** and **5**).

3. Conclusion

The advantageous impacts of probiotics have been illustrated in numerous diseases. One of the major mechanisms of probiotic activity is through the control of resistant reaction. A few of the prevalently utilized probiotic microorganisms are *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, bifidobacteria and certain strains of *Lactobacillus casei, Lactobacillus acidophilus*-group, *Bacillus coagulans*, *Escherichia coli* strain Nissle 1917, certain enterococci, particularly *Enterococcus faeciumSF68*,

and the yeast *Saccharomyces boulardii*. Bacterial spore formers, generally of the class Bacillus dominate the scene. These probiotics are included to foods, especially fermented drain items, either separately or in combinations.

As shown below a number of mechanisms are thought to be associated with probiotic beneficial effects:

1. Production of inhibitory substances such as H_2O_2, bacteriocins, organic acids, and so on.

2. Blocking of adhesion sites for pathogenic bacteria.

3. Compete with and inhibit growth of potential pathogens,

4. Degradation of toxins as well as the blocking of toxin receptors,

5. Modulate inflammatory immune responses [45].

The results of this study show that the probiotic *Bifidobactrium bifidum* BIB2) juice supplementation can significantly affect some immune system factors including IgA, IgM, lymphocytes, monocytes and CD4 cell count in sprint athletes compared to control group.

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