Ayar M, et al., J Food Sci Nutr 2021, 7: 115
DOI: 10.24966/FSN-1076/100115

HSOA Journal of Food Science and Nutrition

Research Article

The Effect of Probiotic Supplements on C-Reactive Protein, Lipid Profile and Body Composition in Elite Greco-Roman Wrestlers

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Abstract

Probiotics are used clinically for the treatment of allergic, metabolic, inflammatory, gastrointestinal, and respiratory diseases. There has been growing interest in their effects on athletes' health and performance in recent years. This study investigated the effect of probiotic supplements on the immune system, lipid profile, and body composition in the Greco-Roman national wrestling team's athletes. A total of 14 athletes participated in the study. They were divided into two groups, with one receiving probiotics and the other as a placebo group. The first group of athletes took probiotic tablets for 15 days, while the second took placebo tablets for the same period. The athletes were followed through the same exercises and individual-specific nutrition programs. Blood tests and anthropometric measurements were taken at the beginning and end of the study. The study identified a significant decrease in CRP, monocyte, and triglyceride levels for athletes on probiotics ($p < 0.05$). When body composition was assessed for both groups, results showed a body fat decrease and significantly higher weight loss for the probiotic group ($p < 0.05$). The study found that athletes taking probiotics showed improvement in their immune system parameters, triglyceride values, body weight, and fat percentages.

Keywords: Body composition; Immune system; Lipid profile; Probiotics; Sports nutrition; Wrestling

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Citation: Ayar M, Öğünç YT, Üstün-Aytekin Ö (2021) The Effect of Probiotic Supplements on C-Reactive Protein, Lipid Profile and Body Composition in Elite Greco-Roman Wrestlers. J Food Sci Nutr 7: 115.

Received: September 06, 2021; **Accepted:** September 13, 2021; **Published:** September 23, 2021

Introduction

Wrestling is an individual and performance sport found around the world [1] and divided by weight categories that United World Wrestling determines [2]. These weight categories allow athletes to compete on equal terms [3]. Some immune system parameters can be altered negatively because of high-intensity training in wrestlers. High-intensity physical exercise can have adverse effects, such as increasing upper respiratory tract infections, weakening the immune system [4] and increasing oxidative stress [5]. Studies have emphasized the importance of probiotic administration to modulate the immune response based on exercise [6]. According to the World Health Organization (WHO), probiotics are microorganisms that provide health benefits to the host when used in sufficient quantities [7]. The International Olympic Committee (IOC) has been supported by WHO and declared probiotics are live microorganisms that increase the number of beneficial bacteria in the gut when taken orally for several weeks, and that there are many potential benefits for modulating intestinal health and immune function [8]. Over exercising can cause gastrointestinal disorders such as nausea, vomiting, diarrhea, and reflux, depending on intestinal microbiota [9]. High-intensity exercise has also been known to damage muscles, increase tissue inflammation and may negatively affect the performance of athletes. Chen et al., reported that the use of some probiotic strains may have anti-inflammatory effects and that probiotics could affect athlete performance not only indirectly but also by increasing energy availability [6].

Athlete performance can be affected by body composition and physical properties. Body composition in wrestlers was evaluated using reference values. For male wrestlers aged 16 and under, the minimum body fat is considered to be 7%, while for wrestlers aged 16 minimum body fat is 5% [10]. Jager et al., reported that probiotic supplements provided improvements in body composition and body fat percentages in athletes [11].

In this study, we evaluated the effects of orally taken probiotic supplements on body fat percentage, body weight, blood lipid profile, and some immune system parameters in elite wrestlers.

Materials and Methods

Research was conducted using 14 athletes attending the Greco-Roman Wrestling a National team camp, who were given oral and written explanations about the purpose and possible risks of this study. Written permission forms were obtained from athletes indicating their willingness to participate in the study.

Research was designed as a probiotic and placebo group by using pretest-posttest model. Volunteer approval forms and personal information forms, which were prepared according to the research model, were distributed to athletes, and blood tests as well as anthropometric measurements were taken at the beginning and end of the study. The probiotic group were given probiotic supplements orally for 15 days, while the placebo group were given tablets containing starch instead.

The probiotic and placebo groups were randomly formed from the 14 athletes invited from the Greco-Roman Wrestling National team

camp. We ensured that athletes participating in the study followed diets prepared by the national team dietitian and complied with training programs of the national team coaches.

Study design

The universe of the study was Greco-Roman Wrestling a National Team Athletes. In the study, it was planned to reach the entire universe. Anthropometric measurements were taken and blood tests analyzed of the athletes who participated in the study at its beginning and end. All tests were conducted at the medical center by the National Greco-Roman Wrestling Team's doctor.

Nutritional supplements

Probiotics are measured in colony forming units (cfu), which show the number of viable cells. The probiotic supplement's content consists of $2x10^9$ cfu/tablet *Lactobacillus acidophilus*, $2x10^9$ cfu/tablet *Bifidobacterium longum*, $1x10^9$ cfu/tablet *Bifidobacterium bifidum*, $1x10^9$ cfu/tablet *Lactobacillus plantarum*, $1x10^9$ cfu/tablet *Lactobacillus rhamnosus,* $5x10^8$ cfu/tablet *Lactobacillus bulgaricus*, $5x10^8$ cfu/tablet *Lactobacillus paracasei*, $5x10^8$ cfu/tablet *Lactobacillus breve*, $5x10^8$ cfu/tablet *Lactobacillus reuteri*, $5x10^8$ cfu/tablet *Streptococcus thermophilus*, $5x10^8$ cfu/tablet *Bifidobacterium infantis* and prebiotics (1200mg of inulin). Tablets containing starch were given to the placebo group athletes for the same duration in similar boxes purchased from the manufacturer.

Nutrition and training program

All athletes ate their meals at the Turkey Wrestling Federation dining hall and followed the same training program prepared by the national team's coaches. Their diets were supervised by a dietitian.

Biochemical analysis

Blood samples were collected (20ml) in two separate tubes at the medical center. High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), total cholesterol, C-Reactive Protein (CRP), and hemogram (complete blood count) parameters were examined in the blood samples.

Anthropometric measurements

Lohman-Brozek's formula was used to predict body fat percentages in wrestlers [12]. The height of athletes was measured using a stadiometer (SECA, Germany) with a sensitivity of 0.01m. The body weight of athletes was measured using an electronic scale (CAS-PB, China) with a precision of 0.01kg.

Athletes' body fat percentage was determined using the skinfold thickness measurement method by use of a skinfold caliper (Holtain, UK), which applied 10 g of pressure to 1 mm^2 with an error of ±2mm. Each measurement was performed at the beginning and end of the day and repeated three times. Subscapular, triceps, and abdominal measurements were performed on three different parts of the body to determine their fat percentage using Lohman-Brozek's formula.

Statistical analysis

Data was evaluated using Kolmogrov Smirnov and Shapiro Wilk's test. Results showed data was not suitable for normal distribution. Nonparametric tests were used to compare the groups. These included Mann Whitney's U test and Wilcoxon Signed Rank's test. The Mann Whitney test was used to compare two groups of variables outside normal distribution, and Wilcoxon Signed Rank's test was used for intragroup evaluation of variables outside normal distribution. Relations between quantitative variables were examined through correlation analysis. Analysis was carried out using SPSS 20.0 software with a 95% accuracy level.

Limitations

The study was designed as a single-blind randomized controlled trial. To determine what athletes would form probiotic and placebo groups, the 14 athletes were randomly divided into two probiotic groups (n=10) and placebo groups (n=10). The study was limited to a 16-day Greco-Roman wrestling national team period.

Results

The study was conducted on 14 athletes (placebo group [n=7] and probiotic group [n=7]). Ages, anthropometric measurements and biochemical findings for all groups were given in table 1. According to Table 1, there were no significant differences between the ages, duration of wrestling, anthropometric measurements, and biochemical findings of athletes in the probiotic and placebo groups at the beginning of the study ($p>0.05$). After 15 days, the same measurements were taken from all athletes and results were compared with previous measurements. Body weight and BMI values were found to be significantly lower in the probiotic group while no statistically significant differences for the same measurements could be observed in the placebo group (Table 2).

Athletes' skinfold thickness measurements were also taken and no significant differences were found in the subscapular skinfold thickness values of the probiotic group and placebo group. However, triceps and abdominal skinfold thickness values decreased significantly of probiotic group compared to the placebo group.

Subscapular, triceps, and abdominal skinfold thickness measurements did not change significantly in the placebo group. Significant decreases in total body fat percentages were observed in both groups between the beginning and end of the study. The decrease in skinfold thickness measurements also followed the decrease in body fat percentages. Low levels of body fat in wrestlers are desired because of their positive effect on performance.

Cholesterol, LDL and HDL levels did not differ significantly between the groups, but a significant decrease in triglyceride levels was observed in the probiotic group. No significant changes in triglyceride levels were observed in the control group. The difference in triglyceride levels was statistically higher in the probiotic group compared to the control group.

While CRP, one of the acute immune phase proteins, decreased significantly in the probiotic group by the end of the study, this change was not reflected in the placebo group. In the comparison of changes between groups, the study determined that the decrease of CRP in the probiotic group was significantly higher than that in the placebo group.

There were no differences in white blood cell and lymphocyte levels in either group. Monocyte (MONO) levels decreased significantly at the end of the study in the probiotic group, whereas no significant difference could be seen in the placebo group. The differences in MONO levels at the beginning and end of the study for the probiotic group were statistically higher than for the control group. No side effects were observed during the study for either the placebo or probiotic group.

Groups	Probiotic Group (n=7)			Placebo Group (n=7)			
	±SS	Min-Max	Median	±SS	Min-Max	Median	p*
Age (years)	25.00±3.21	21-31	25.0	23.14±3.08	21-30	22	0.180
Wrestling time (years)	13.86±3.53	10-20	15.0	11.29±3.99	8-20	10	0.119
Body Weight (kg)	87.80±16.77	64.5-104.5	88.70	76.33±2.13	57.3-121.0	70.0	0.277
BMI (kg/m2)	28.49±3.73	22.6-32.3	28,8	25.33±4.22	21.6-34.6	24.2	0.142
Subscapular (mm)	14.99±0.23	8.2-22.6	12.9	13.07±7.20	9-29	10.2	0.277
Triceps (mm)	10.94±2.09	8.40-14.40	11.2	8.93±4.45	5-17.2	7.8	0.159
Abdominal (mm)	17.60±7.11	7.5-25.2	19.0	14.3±1.19	7.2-36	7.6	0.277
Body Fat (%)	15.53±3.87	9.68-19.86	15.5	13.31±6.83	8.8-26.9	10.1	0.110
Cholesterol (mg/dL)	191.14±28.7	143-221	204.0	176.1±38.9	106-227	182	0.443
Triglycerides (mg/dL)	95.43±15.96	72-115	100.0	102.1±62.8	51-238	82	0.371
LDL-C (mg/dL)	117.48±25.6	77.6-143.0	133.8	102±29.9	55.8-133.5	109.6	0.482
HDL-C (mg/dL)	54.57±1.01	42-74	53.0	53.42±8.9	40-66	57	0.940
CRP (mg/dL)	0.21±0.15	0.1-0.5	0.15	0.13±0.14	0.03-0.44	0.08	0.141
WBC (K/µL)	6.82±1.31	4.5-8.8	6.85	6.56±0.95	5.22-7.85	6.34	0.482
LYM (K/µL)	2.50±0.47	1.8-3.0	2,56	2.73±0.65	1.92-3.89	2.73	0.609
MONO (K/µL)	0.63±0.20	0.25-0.85	0.62	0.56±0.22	0.35-1.01	0.48	0.371

Table 1: Anthropometric measurements and biochemical findings of the athletes at the beginning of the study.

Note: *p value was calculated with Mann-Whitney U test, p<0.05 was considered statistically significant.

Groups	Probiotic Group (n=7)			Placebo Group (n=7)			Intra Group
	Pre-test ±SS	Last-test ±SS	p*	Pre-test ±SS	Last-test ±SS	p*	p**
Body Weight (kg)	87.80±16.77	86.79±16.12	0.028	76.33±21.32	76.64±21.35	0.285	0.338
BMI (kg/m2)	28.49±3.73	28.17±3.56	0.028	25.33±4.22	25.43±4.19	0.285	0.012
Subscapular (mm)	14.99±5.23	14.51±4.82	0.074	13.07±7.19	12.60±6.91	0.062	0.898
Triceps (mm)	10.94±2.09	9.54±1.28	0.018	8.93±4.45	8.67±3.84	0.171	0.046
Abdominal (mm)	17.60±7.11	15.47±6.36	0.028	14.30±11.88	12.57±10.28	0.075	0.338
Body Fat (%)	15.53±3.87	14.33±3.36	0.018	13.31±6.83	12.58±6.18	0.058	0.110
Cholesterol (mg/dL)	191.14±28.7	181.2±37.36	0.499	176.1±38.9	197.0±31.1	0.028	0.701
Triglycerides (mg/dL)	95.43±15.96	64.86±14.31	0.018	102.1±62.8	91.6±63.5	0.116	0.011
LDL-C (mg/dL)	117.48±25.6	115.74±40.7	0.735	102±29.9	123.0±26.1	0.043	0.749
HDL-C (mg/dL)	54.57±10.11	52.57±7.00	0.445	53.42±8.9	55.86±9.8	0.173	0.948
CRP (mg/dL)	0.21±0.15	0.05±0.02	0.018	0.13±0.14	0.18±0.22	0.344	0.002
WBC (K/µL)	6.82±1.31	5.82±1.47	0.116	6.56±0.95	6.58±0.96	0.735	0.085
LYM (K/µL)	2.50±0.47	2.38±0.45	0.463	2.73±0.65	2.59±0.87	0.398	0.949
MONO (K/µL)	0.63±0.20	0.45±0.12	0.027	0.56±0.22	0.52±0.15	0.462	0.040

Table 2: Anthropometric measurements and biochemical findings of the athletes at the end of the study.

Note: *p value was calculated with Mann-Whitney U test, p<0.05 was considered statistically significant. *p value was calculated with Wilcoxon signed rank test, p<0.05 was considered statistically significant. **p value at the end of the study, the intra-group pre-test and post-test show the difference between the groups.

Discussion

High-intensity exercise is associated with increased incidence of upper respiratory infection and gastrointestinal tract disorders, causing immunosuppression and oxidative stress. Therefore, interventions aimed at reducing and preventing effects of intense exercise that may indirectly cause decrease in physical performance are very important [13]. A number of positive health benefits of probiotics have been identified for areas such as gastrointestinal system function and diseases, immune system function, hyperlipidemia, hypertension and allergic conditions [14]. It is a fact that hypercholesterolemia and low HDL-C levels are more pronounced in athletes practicing anaerobic sports such as tennis, sprinting, jumping, gymnastics, ice skating, as well as strength sports such as weightlifting, boxing, wrestling and judo [15] and some studies show that high-intensity resistance exercises can negatively affect the immune system [5,16].

Probiotics can help regulate immune system disorders that occur as a result of high-intensity resistance exercises by positively affecting immune system parameters, especially CRP, against damage caused by this type of exercise [17,18]. According to our study's results, there was a significant decrease in CRP levels in the probiotic

group compared to the placebo group. Salehzadeh et al., conducted a study of 30 athletes with similar BMIs randomly split into two groups where one group was given yogurt with probiotic content and the other group was given normal yogurt for ten weeks. Blood samples taken from the group consuming the yogurt with probiotics 24 hours earlier and after the exercise program showed a significant decrease in hs-CRP levels compared to the other group [19]. Results of their study supports the present study.

O'brien et al., also reported that after an intense endurance exercise, a group consuming kefir showed a significant decrease in CRP levels compared to a non-consuming group [20]. In their study, athletes' CRP levels in the probiotic group decreased significantly between its beginning and end, while no significant changes in CRP levels were observed in the placebo group [19].

Aghaee et al., reported that oral administration of probiotic supplements for 16 male athletes between the ages of 19 and 25 significantly decreased monocyte values during their 30 days of training. However, they observed an increase in lymphocyte, monocyte and granulocyte counts in the control group [21]. This result also supports this study.

It is known that probiotics improve some lipid profile parameters in athletes performing high-intensity anaerobic exercises [14,22]. Studies have also shown that probiotic use has positive effects on athletes' lipid profiles. Abbasi et al.'s study randomly divided 14 male bodybuilders into two groups – an experimental group and a control group-and applied a 30-day training program. The experimental group took tablets containing probiotics (*Lactobacillus casei* ($5.1x10^9$ cfu/g), *Lactobacillus acidophilus* ($2x10^9$ cfu/g), *Lactobacillus bulgaricus* ($2x10^8$ cfu/g), *Bifidobacterium breve* ($2x10^{10}$ cfu/g), *Bifidobacterium longum* ($7x10^9$ cfu/g) and *Streptococcus thermophilus* ($5.1x10^9$ cfu/g), while the control group athletes took tablets containing starch. Blood samples were collected from both groups at the beginning and end of the study. The blood tests showed that total cholesterol and triglyceride levels were lower in the probiotics group than in the placebo group [23]. Stancu et al., showed that the triglyceride levels obtained were similar to those from a 6-week animal modeling study [24]. Another study conducted by Mahboobi et al., found that 500mg probiotic supplements ($7x10^9$ cfu/day *Lactobacillus casei*, $2×10^9$ cfu *Lactobacillus acidophilus,* $1.5×10^9$ cfu *Lactobacillus rhamnosus*, $2×10^8$ cfu *Lactobacillus bulgaricus*, $2×10^{10}$ cfu *Bifidobacterium breve*, $7×10^9$ cfu *Bifidobacterium longum*, $1.5×10^{10}$ cfu *Streptococcus thermophilus* and Fructooligosaccharide) did not change lipid profiles in prediabetic individuals in an 8-week intervention [25]. The differences in results between studies can be attributed to the kinds of exercises carried out, probiotic strains used and their quantities.

Probiotic supplements are effective for reducing body fat percentages and body weight in athletes performing resistance exercises [26]. When body composition was evaluated, one could see that body weight loss was higher in the group using probiotics than the placebo group. However, there was no significant change in the percentage of body fat. In some studies that examined probiotic supplementation with resistance or endurance training, body weight and percentage of fat decreased in overweight and obese individuals [26-28].

Conclusion

In conclusion, this study determined that the use of probiotic-containing supplements for 15 days reduced CRP, monocyte, and triglyceride levels and contributed to a decrease in body weight. It showed its positive effect on the immune system for elite athletes performing high-intensity exercise. When probiotic's effects on body weight are taken into account, it may be beneficial for wrestlers to use them to accompany their diet programs. The positive effects of probiotic supplements on inflammatory markers such as CRP, their effect on lipid profile, and weight loss are all promising. While many studies support these results, some studies also show probiotics to have no effect on lipid profile and CRP levels, perhaps as a result of the differences in exercises, the type of probiotics being used or the amounts administered. Longer-term and in-depth studies are needed on the subject.

Acknowledgment

This research was financially supported by authors and received no specific grant from any funding agency. The authors declare no conflict of interest regarding this article.

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