Lactobacillus helveticus Lafti L10 Supplementation Modulates Mucosal and Humoral Immunity in Elite Athletes: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Michalickova, DM, Kostic-Vucicevic, MM, Vukasinovic-Vesic, MD, Stojmenovic, TB, Dikic, NV, Andjelkovic, MS, Djordjevic, BI, Tanaskovic, BP, and Minic, RD. *Lactobacillus helveticus* Lafti L10 supplementation modulates mucosal and humoral immunity in elite athletes: a randomized, double-blind, placebo-controlled trial. *J Strength Cond Res* 31(1): 62–70, 2017–To test the influence of probiotic supplementation on humoral immune response, a double-blind, placebo-controlled trial was conducted. Thirty athletes (24 males and 6 females, females: $\dot{V}O_2$max 38.2 ± 4.9 ml·kg^{-1}·min^{-1}, age 23.2 ± 1.4 years; males: $\dot{V}O_2$max 57.5 ± 9.2 ml·kg^{-1}·min^{-1}, age 24.0 ± 2.4 years, mean ± *SD*) were randomized either to the probiotic group (*Lactobacillus helveticus* Lafti L10, 2×10^{10} colony-forming units) or to the placebo group. Serum and saliva samples were collected at the baseline and after 14 weeks. Total and specific antibacterial antibody levels of IgM, IgG, and IgA classes were determined for different bacteria in the serum, and in saliva, total and specific antibacterial IgA levels were examined. Total IgM was elevated in both probiotic (18%, 15–20%; mean, 90% confidence interval; $p = 0.02$) and placebo group (35%, 22–47%; $p = 0.02$), without observed differences in changes between the groups. No significant changes in IgM levels specific for tested bacteria were found. Total IgG level was constant in both groups. A significant (16%, −2.8 to 35%, $p = 0.04$) reduction of anti–*Enterococcus faecalis* IgG was noted in the placebo group, in comparison with the probiotic group. There was a substantial decrease in total IgA level in the placebo group, when measured either in serum (15%, 12–18%, $p = 0.04$) or in saliva (35%, −1.4 to 53%, $p = 0.03$). Significantly reduced levels of serum anti–lactic acid bacteria IgA antibodies in the placebo group compared with the probiotic group were detected for *Lactobacillus rhamnosus* LA68 (24%, 5.8–42%, $p = 0.02$) and for *L. rhamnosus* LB64 (15%, 2.7–27%, $p = 0.02$). Probiotic administration could have beneficial effects on systemic humoral and mucosal immune responses.

Key Words probiotics, salivary IgA, immunoglobulins, immune system

Introduction

Because of the competitive nature of professional sports, elite athletes are constantly in need to push boundaries, which is a difficult task, especially in times of rapidly increasing global population. Strenuous exercise leads to physical stress, which has an impact on the individuals' immune system. Although moderate exercise has a beneficial effect on the immune system, compared with a sedentary lifestyle, excessive amounts of prolonged high-intensity exercise can impair immune function, leading to higher risk of upper respiratory tract infections (URTI) (37). Upper respiratory tract infection occurs in the period of strenuous exercise, particularly during winter months (18), thus negatively influencing athletes' training and consequently impairing performance during competitions.

Mucosal immunity impairment has been suggested to be a key risk factor for higher URTI incidence in elite athletes (37). Secretory IgA is reported to play a multifunctional role in mucosal immunity, including host protection by neutralizing bacterial, viral, and fungal antigens and modulation of epithelial cells (9,21). It is generally considered that salivary IgA level decreases in response to high-intensity exercise, especially if it lasts over longer periods of time (>6 months)

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31(1)/62–70

Journal of Strength and Conditioning Research

(14). Nevertheless, certain discrete dietary changes could compensate for the detrimental effects of strenuous exercise on mucosal immunity (14). Recent studies suggested that probiotic supplementation could help better mucosal immunity maintenance, or even induce its enhancement (15,31,36,38).

As part of immune modulation because of the consumption probiotics, systemic humoral immune responses could be induced as well. Several studies confirmed that immunoglobulins, main mediators of humoral immunity, were influenced by oral probiotic administration (21,28,29,32). In addition, enhancement of specific humoral response would be of special interest for professional athletes in terms of prevention of bacterial infections and minimization of their detrimental impact on training and performance.

The probiotic strain *Lactobacillus helveticus* Lafti L10 was previously reported to have inherent immunity-enhancing properties and nonpathogenic nature in animal studies (30). These data were corroborated with human trials: an enhancement of antigen-stimulated interferon-γ production after a month of daily intake of *L. helveticus* Lafti L10 at a dose of 2×10^{10} colony-forming units (CFUs) was reported in a cohort of fatigued athletes experiencing recurrent viral infections (8). Moreover, supplementation with *L. helveticus* Lafti L10 reduced the duration of URTI episodes and increased $CD4^+/CD8^+$ (T helper/T suppressor) cells ratio in a cohort of elite athletes (Marinkovic et al., submitted). Although there is an emerging amount of evidence that probiotics could modulate mucosal immune system, data regarding the influence of probiotics on professional athletes' humoral immunity are rather scarce. Therefore, the aim of this study was to test the effects of *L. helveticus* Lafti L10 supplementation not only on the total antibody levels but also on specific antibacterial antibody levels in serum and saliva.

Methods

Experimental Approach to the Problem

The study included a randomized, double-blind, placebo-controlled parallel-groups design. The athletes were randomly allocated to the probiotic ($n = 15$) or the placebo group ($n = 15$), taking into account maximal aerobic capacity (determined by cardiopulmonary testing). All the participants finished the study.

We have tested both serum and salivary antibody reactivity for several species/strains of *Lactobacillus*, and 3 clinical isolates–2 of gram-negative bacteria *Escherichia coli* and *Proteus mirabilis*, and a gram-positive *Enterococcus faecalis*. Moreover, total salivary IgA and total IgA, IgG, and IgM antibodies in serum were determined.

The supplementation started in the middle of January, in winter, and lasted for 14 weeks. The experimental group received the probiotic capsules of *L. helveticus* Lafti L10 (2×10^{10} CFUs) daily for 14 weeks. Each capsule contained 1×10^{10} CFUs of *L. helveticus* Lafti L10, so subjects were instructed to take 2 capsules per day. The control group received 2 placebo capsules also daily, which were identical in taste and appearance as probiotic capsules. The placebo capsules contained 1% magnesium stearate and 99% maltodextrin, and the probiotic capsules contained 72.2% of the bacterial mass, 26.7% maltodextrin, and 1% magnesium stearate. Capsules were composed of hydroxypropylmethylcellulose and covered by titanium dioxide (TiO_2). Both probiotic and placebo capsules were kept in a refrigerator (2–8° C).

The athletes were asked to return the remaining capsules when coming to the final testing after the intervention. The researchers counted the remained capsules; the compliance in the probiotic group was 95.2% and that in the placebo group was 94.8% ($p = 0.74$).

Both athletes and the study team were blinded to the intervention until the statistical analyses were finished.

Subjects

A total of 30 elite athletes were involved in the trial: 24 men ($\dot{V}O_2$max ranged from 49.5 to 82.0 $ml \cdot kg^{-1} \cdot min^{-1}$) and 6 women ($\dot{V}O_2$max ranged from 45.0 to 57.0 $ml \cdot kg^{-1} \cdot min^{-1}$), aged 18–28 years, nonsmokers, with training >11 $h \cdot wk^{-1}$. Professional athletes from several different sports (badminton, triathlon, bicycling, athletics, karate, kayaking, and judo) participated in the study. Exclusion criteria were sensitivity to the ingredients of probiotics and the use of probiotics and antibiotics a month before the beginning of the study, recent surgical intervention, and/or the presence of chronic diseases (immune, neurological, renal, pulmonary etc.).

Athletes were asked to take capsules after breakfast to ensure the compliance. Furthermore, subjects were required to refrain from supplements that are intended for promotion of

Table 1. Physical and anthropometric characteristics of the participants.*†

	Probiotic Lafti L10	Placebo	*p*
Number	15	15	
Males/females	12/3	12/3	
Age (y)	22.5 ± 2.9	23.6 ± 2.9	0.88
$\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$)	54.3 ± 10.9	55.2 ± 8.2	0.80
BMI	22.9 ± 2.5	22.9 ± 2.5	0.95
Training loads ($MET\text{-}hr \cdot wk^{-1}$)	96 ± 52	97 ± 56	0.94

*BMI, body mass index; MET, metabolic equivalent.
†Results are expressed as mean ± *SD*.

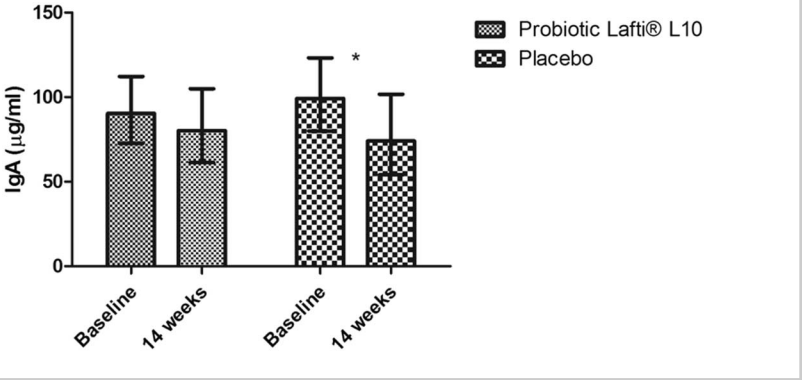

Figure 1. Levels of total salivary IgA in the probiotic and placebo groups at the baseline and after 14 weeks of supplementation. Results are expressed as mean and *SD*. *$p \leq 0.05$.

immune system (e.g.,): *Echinacea*, caffeine, *Ginseng panax*, propolis, multivitamins, and multiminerals. Moreover, the participants were asked to hold a steady training regimen and adhere to a diet not containing yogurt and fermented milk products.

All the experimental procedures in the current study followed the guidelines laid down in Declaration of Helsinki. The study was approved by the Ethics Committee of Sports Medicine Association of Serbia. Subjects were informed of the benefits and risks of the investigation before signing an informed consent approved by the committee.

Procedures

Training Loads and Maximal Aerobic Capacity Determination. Athletes were required to report their training loads weekly, filling in the standard short form of International Physical Activity Questionnaire (http://www.ipaq.ki.se/downloads.htm). Training loads in metabolic equivalents (MET-hr·wk^{-1}) were counted on the basis of completed questionnaires, according to Ainsworth (1). Maximum oxygen consumption was determined by a graded cardiopulmonary test on a treadmill (Quark b2; Cosmed, Pavona, Italy). The exercise intensity was progressively increased, and oxygen and CO_2 concentration of the inhaled and exhaled air were measured. A test was considered maximal if the participants achieved 90% or more of predicted maximal heart rate for age and gender, a plateau in oxygen consumption was reached despite increased workload, a respiratory exchange ratio was greater than 1.00, and subjects reached volitional exhaustion.

The training loads (MET-hr·wk^{-1}) did not differ between the groups (Table 1). Maximal aerobic capacity did not change during the study (data not shown).

Serum and Saliva Samples Collection. Samples were collected before the cardiopulmonary testing. Blood samples (10 ml per serum tube) were taken out of the antecubital vein. Whole, unstimulated saliva was collected in a glass tube for 2 minutes, after sitting quietly for a few minutes, leaning forward, with their heads tilted (4). All the samples were collected twice: before the study and after the study, at the same time (between 9:30 and 10:30 AM), to avoid diurnal changes. Serum and saliva were separated by centrifugation ×1500 g, 15 minutes and ×3000 g, 10 minutes, respectively, and stored frozen at −20° C until analysis.

Total Salivary IgA Determination. Saliva samples were diluted ×1000 and then analyzed for IgA concentrations using a commercial enzyme-linked immunosorbent assay (IBL, Hamburg, Germany). Samples were determined in duplicates; the intra-assay coefficient of variation was 10%.

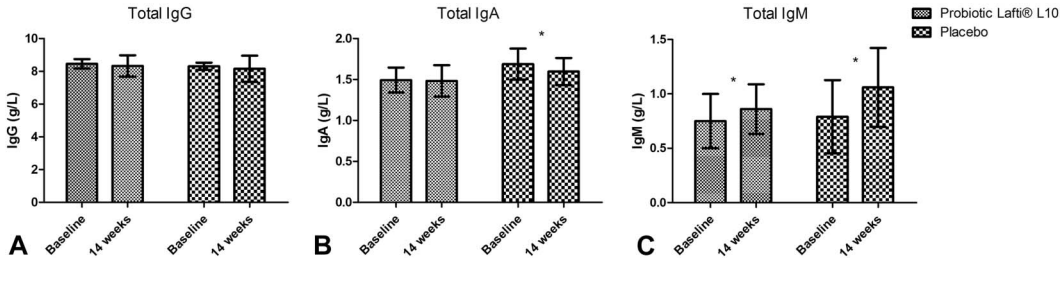

Figure 2. Total serum antibodies: (A) IgG, (B) IgA, and (C) IgM antibody levels in the probiotic and placebo groups at the baseline and after 14 weeks of supplementation. Results are expressed as mean ± *SD*. *$p \leq 0.05$.

Table 2. Specific serum IgG antibodies for lactic acid bacteria and pathogenic bacteria in serum.*

	Probiotic Lafti L10			Placebo			
	Baseline	14 wk	Change scores (90% CI)	Baseline	14 wk	Change scores (90% CI)	Mean difference (90% CI)	*p*
Lactobacillus plantarum WCSFS1	0.40 ± 0.24	0.51 ± 0.27	29 (20 to 38)	0.61 ± 0.31	0.71 ± 0.37	11 (7.4 to 14.5)	18 (−21 to 58)	0.35
Lactobacillus rhamnosus LB64	0.42 ± 0.29	0.43 ± 0.24	3.0 (0.30 to 5.8)	0.39 ± 0.29	0.49 ± 0.37	23 (19 to 27)	−20 (−42 to 2.9)	0.09
Lactobacillus rhamnosus LA68	0.24 ± 0.25	0.27 ± 0.25	7.8 (3.0 to 12)	0.27 ± 0.32	0.31 ± 0.28	22 (17 to 27)	−14 (−45 to 16)	0.35
Lactobacillus helveticus Lafti L10	0.45 ± 0.27	0.56 ± 0.20	24 (18 to 30)	0.52 ± 0.35	0.61 ± 0.36	41 (34 to 48)	−17 (58 to 24.4)	0.41
Escherichia coli	0.21 ± 0.23	0.17 ± 0.17	−14 (−18 to −11)	0.13 ± 0.09	0.14 ± 0.09	15 (8.7 to 22)	−30 (−65 to 4.5)	0.09
Proteus mirabilis	0.50 ± 0.48	0.51 ± 0.51	6.4 (2.3 to 10)	0.41 ± 0.34	0.38 ± 0.32	−6.6 (−11 to 8.6)	13 (−7.6 to 34)	0.21
Enterococcus faecalis	0.23 ± 0.12	0.21 ± 0.13	−2.0 (−4.4 to 10)	0.32 ± 0.23	0.29 ± 0.20	−10 (−25 to −8.5)	16 (−2.8 to 35)	0.04

*Results are expressed as mean ± *SD*. Change scores in the groups and mean difference in the change scores between the groups are expressed in percent. CI, confidence interval.

Salivary flow ($ml \cdot min^{-1}$) was determined by conversion of the amount of saliva in grams to milliliters, assuming that saliva density is 1 $mg \cdot ml^{-1}$, and division by time of collection (2 minutes). Salivary IgA antibody (sIgA) secretion rate ($\mu g \cdot min^{-1}$) was obtained by multiplying the absolute sIgA concentration ($\mu g \cdot ml^{-1}$) with saliva flow rate ($ml \cdot min^{-1}$) (4). Salivary protein concentration was determined by the Lowry method (24).

Total Serum Antibody Assessment. Frozen serum samples were sent to a certified human diagnostics laboratory (Laboratorija Beograd, Belgrade, Serbia) and immunoturbidimetric method (Roshe Hitachi, Rotkreuz, Switzerland) was used for the quantification of total IgA, IgG, and IgM in serum samples.

Bacterial Strains and Growth Conditions. In this study, several *Lactobacillus* species were used, such as *L. helveticus* Lafti L10 (Lallemand Health Solutions, Montreal, Canada), *Lactobacillus plantarum* WCFS1 (Wageningen Centre for Food Sciences, Wageningen, The Netherlands), *Lactobacillus rhamnosus* LA68, *L. rhamnosus* LB64 (Institute of Virology, Vaccines and Sera, Torlak), and *Lactobacillus acidophilus* ViVag (Pharma Vinci A/S, Denmark); all the strains were grown in MRS (deMan, Rogosa and Sharpe) medium (Institute of Virology, Vaccines and Sera, "Torlak," Belgrade, Serbia), without shaking, at 37° C. Overnight cultures were centrifuged at ×3000 g for 10 minutes at room temperature (RT), washed twice with phosphate-buffered saline (PBS) and counted using a hemocytometer. After the optical density of 1×10^8 of *L. helveticus* Lafti L10 was determined, all other bacteria were diluted to the same optical density. Clinical isolates of *E. coli*, *P. mirabilis*, and *Enterococcus faecalis* were grown in Nutrient broth (Institute of Virology, Vaccines and Sera, "Torlak"), and overnight cultures were also diluted to the same optical density. Before usage, all bacterial species were frozen once at −20° C.

Antibacterial enzyme-linked immunosorbent assay. Antibacterial enzyme-linked immunosorbent assay (ELISA) was essentially done as previously described (34) with minor modifications. MaxiSorp plates (Nunc A/S, Roskilde, Denmark) were filled with 50 μL/well of bacterial suspension. The plates were centrifuged at ×1500g for 10 minutes, and the supernatant liquid was decanted. The plates were left for 2 hours at 50° C to dry. The plates were blocked with 200 μL/well in 2% bovine serum albumin/PBS at 37° C for 1 h and washed 3 times with PBS, and sera was added at an appropriate dilution. For the analysis of bacteria-specific IgG and IgM, the sera was diluted ×400, and for specific IgA, sera was diluted ×50. Salivary IgA specific for bacteria was determined at a ×4 dilution. Sera or saliva was incubated for 2 hours at 37° C and washed with PBS, and the following secondary antibodies were used: monoclonal antihuman IgG (Fc specific) biotin conjugate, 2000 times diluted,

Table 3. Specific serum IgM antibodies for lactic acid bacteria and pathogenic bacteria.*

	Probiotic Lafti L10			Placebo				
	Baseline	14 wk	Change score (90% CI)	Baseline	14 wk	Change score (90% CI)	Mean difference (90% CI)	*p*
Lactobacillus plantarum WCSFS1	0.54 ± 0.22	0.49 ± 0.20	−11 (−7.3 to −2.7)	0.58 ± 0.26	0.56 ± 0.26	−17 (−19 to −16)	−6.8 (−24 to 9.0)	0.41
Lactobacillus rhamnosus LB64	0.29 ± 0.12	0.28 ± 0.13	−2.9 (−6.6 to 0.80)	0.34 ± 0.20	0.33 ± 0.16	7.8 (3.7 to 12)	−10 (−37 to 16)	0.42
Lactobacillus rhamnosus LA68	0.29 ± 0.12	0.26 ± 0.14	−13 (−16 to −10)	0.33 ± 0.21	0.30 ± 0.16	−3.9 (−7.8 to 0.10)	−9.4 (−32 to 13)	0.40
Lactobacillus helveticus Lafti L10	0.43 ± 0.13	0.39 ± 0.17	−12 (−15 to −9.4)	0.50 ± 0.29	0.48 ± 0.27	1.9 (−2.3 to 6.0)	−13 (−37 to 10)	0.26
Escherichia coli	0.29 ± 0.14	0.25 ± 0.12	−6.9 (−11 to −2.5)	0.31 ± 0.18	0.31 ± 0.16	3.1 (−1.3 to 7.5)	−9.7 (−37 to 17)	0.46
Proteus mirabilis	0.46 ± 0.16	0.46 ± 0.12	−1.6 (−5.8 to 2.6)	0.55 ± 0.27	0.54 ± 0.28	−1.9 (−5.5 to 1.6)	0.4 (−24 to 25)	0.97
Enterococcus faecalis	0.49 ± 0.16	0.44 ± 0.12	−6.5 (−10 to −2.6)	0.56 ± 0.30	0.54 ± 0.29	−5.3 (−8.1 to −2.5)	−1.2 (−22 to 20)	0.91

*Results are expressed as mean ± *SD*. Change scores in the groups and mean difference in the change scores between the groups are expressed in percent.CI, confidence interval.

Table 4. Specific serum IgA antibodies for lactic acid bacteria and pathogenic bacteria.*

	Probiotic Lafti L10			Placebo				
	Baseline	14 wk	Change score (90% CI)	Baseline	14 wk	Change score (90% CI)	Mean difference (90% CI)	*p*
Lactobacillus plantarum WCSFS1	0.16 ± 0.09	0.15 ± 0.09	−3.6 (−6.6 to 0.62)	0.17 ± 0.07	0.15 ± 0.06	−9.8 (−12 to −7.2)	6.2 (−11 to 8.5)	0.47
Lactobacillus rhamnosus LB64	0.12 ± 0.05	0.11 ± 0.05	−2.8 (−5.3 to −0.33)	0.17 ± 0.11	0.14 ± 0.09	−17 (−19 to −16)	15 (2.7 to 27)	0.02
Lactobacillus rhamnosus LA68	0.09 ± 0.06	0.08 ± 0.05	−4.3 (−7.6 to −1.1)	0.11 ± 0.07	0.08 ± 0.07	−28 (−31 to −26)	24 (5.8 to 42)	0.02
Lactobacillus helveticus Lafti L10	0.21 ± 0.08	0.18 ± 0.08	−11 (−14 to −7.4)	0.21 ± 0.06	0.18 ± 0.06	−10 (−13 to −8.4)	−0.26 (−18 to 18)	0.98
Escherichia coli	0.12 ± 0.11	0.09 ± 0.09	−11 (−15 to −7.6)	0.08 ± 0.04	0.07 ± 0.04	1.8 (−2.8 to 6.6)	−13 (−40 to 14)	0.33
Proteus mirabilis	0.14 ± 0.08	0.14 ± 0.08	−4.6 (−6.4 to −2.5)	0.14 ± 0.05	0.13 ± 0.05	−5.1 (−7.9 to −2.3)	0.5 (16.8 to 17.8)	0.95
Enterococcus faecalis	0.16 ± 0.07	0.15 ± 0.08	−5.8 (−8.1 to −3.5)	0.19 ± 0.10	0.17 ± 0.08	−3.2 (−5.6 to −0.82)	−2.6 (−18 to 13)	0.73

*Results are expressed as mean ± *SD*. Change scores in the groups and mean difference in the change scores between the groups are expressed in percents (%). CI, confidence interval.

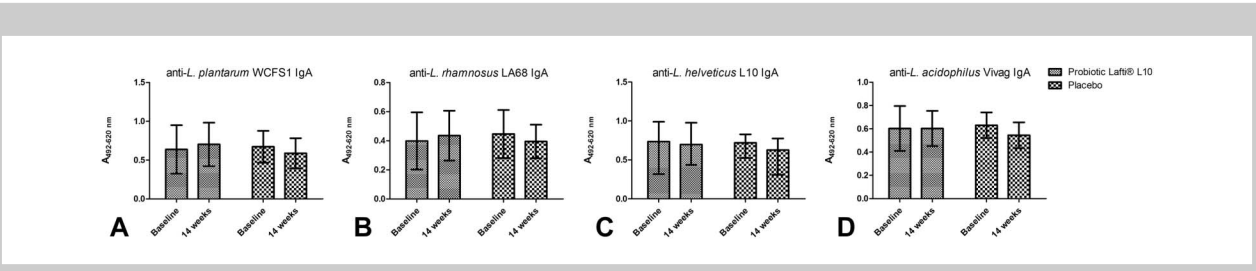

Figure 3. Specific salivary IgA antibodies: (A) anti–*Lactobacillus plantarum* WCFS1 (B) anti–*Lactobacillus rhamnosus* LA68, (C) anti–*Lactobacillus helveticus* L10, and (D) anti–*L. acidophilus* Vivag antibody levels in the probiotic and placebo groups at the baseline and after 14 weeks of supplementation. Results are expressed as mean ± *SD*.

antihuman IgM (μ-chain specific) biotin conjugate, 2500 times diluted, antihuman IgA (α-chain specific), 2000 times diluted; all were obtained from Sigma Aldrich (St. Louis, MO, USA). All secondary antibodies were incubated for 1 hour at RT. After washing with PBS, streptavidin–horse radish peroxidase (Biolegend, San Diego, CA, USA) diluted 2000 times was added and incubated for 1 hour at RT. Colored substrate used was SigmaFast OPD (Sigma Aldrich), and the reaction was developed for 10 minutes. The absorbance was read at 492 nm and at 620 nm and the latter was subtracted from the former. Each sample was done in duplicate, and intra-assay coefficients of variation were below or equal to 10% for all the bacterial ELISAs performed.

Statistical Analyses

All statistical analyses were performed with GraphPad Prism software. The normality of the data was checked by Wilk-Shapiro test. The differences in the change scores between the groups were assessed by an unpaired *T*-test. Spearman correlation was used for checking the correlation between different antibody subclasses. The results are expressed as mean value and *SD*. $P \leq 0.05$ was considered significant.

Results

Total Salivary IgA Level

There was a significant (35%, −1.4 to 53%; mean, 90% confidence interval; $p = 0.03$) reduction in total salivary IgA concentration in the placebo group (−28%, −38 to −20%, $p = 0.02$) in comparison with the probiotic group (−8.7%, −15 to 1.7%, $p = 0.34$), Figure 1. No significant changes were observed for protein concentration (1.5%, −4.7 to 10%, $p = 0.85$), salivary flow (−9.2%, −99 to 76%, $p = 0.80$), and salivary IgA secretion rate (3.5%, −25 to 34%, $p = 0.65$) between the groups.

Analysis of Total IgG, IgA, and IgM Antibody Levels in Serum

Mean difference between change scores in the groups was not significant for total IgG (0.09%, −10 to 5.0%, $p = 0.99$) and IgM level (−27%, −68 to −15%, $p = 0.14$), as shown in Figures 2A and 2C. However, the level of total IgM increased in both the probiotic (18% [15–20%], $p = 0.02$) and placebo groups (35%, 22–47%, $p = 0.02$). On the other hand, there was a significant (15%, 12–18%, $p = 0.04$) decrease in total IgA level in the placebo group (−8.0%, −10 to −2.2%, $p = 0.03$), when compared with the probiotic group (0.48%, −0.45 to 1.4%, $p = 0.77$) (Figure 2B).

Analysis of Specific Antibacterial Serum IgG Levels

The levels of serum IgG specific for different lactic acid bacteria (LAB) species and for selected pathogenic bacteria are shown in Table 2. Statistical analysis showed no difference in the groups for any of the LAB species used. A significant 16% (−2.8 to 35%, $p = 0.04$) reduction over the course of study was noted for anti–*E. faecalis* IgG; it decreased insignificantly by 2.0% (−4.4 to 10%, $p = 0.88$) in the probiotic group, but decreased significantly in the placebo group by 10% (−25 to −8.5%, $p = 0.02$).

Analysis of Specific Antibacterial Serum IgM Levels

No significant differences in reactivity for LAB species were detected between the groups. This was also the case with clinical isolates of pathogenic bacteria (Table 3).

Analysis of Specific Antibacterial Serum IgA Levels

No significant differences were detected between the individual groups of sera, but statistically significant changes with time were detected in the placebo group (Table 4). The reduced levels of anti-LAB antibodies in the placebo group in comparison with the probiotic group were detected for *L. rhamnosus* LA68 at 24% (5.8–42%, $p = 0.02$) and for *L. rhamnosus* LB64 at 15% (2.7–27%, $p = 0.02$). In the case of clinical isolates of pathogenic bacteria, no differences in antibody levels were detected in different time points.

Lactobacillus-Specific Salivary IgA

No statistically significant differences were observed in the specific IgA level antibodies for any of the tested LAB species between the groups (Figure 3).

Discussion

The most important finding of this study was preservation of total salivary IgA level in the group supplemented with Lafti L10. Given the fact that mucosal surface is the first line of defense against different pathogens, this finding might have a practical application in terms of prevention of URTIs during strenuous exercise in elite athletes. Our result is in

accordance with literature data concerning highly active individuals (15,36) and other immunologically susceptible populations, such as the elderly and children (31,38). However, there are some studies showing a lack of positive impact on mucosal immunity (10,16). This fact is of no surprise because the effects of probiotics are strongly dose and strain dependent.

In line with salivary IgA level maintenance, a trend of systemic IgA level preservation occurred. However, we cannot be sure that there is a direct connection between these 2 findings because the production of secretory and serum IgA are regulated differently and located in different compartments. Consequently, the mechanisms by which mucosal and humoral immunological responses could be influenced by probiotic consumption are probably different (11,23). Namely, the majority of adult human plasma cells produce IgA antibodies (22,33), which exists as 2 subclasses, IgA1 and IgA2. The former, predominant in the human serum, is generated by B cells in the bone marrow and peripheral lymphoid organs (11,23). On the other hand, IgA2 is predominant in mucosal secretions (26) and is produced partly by B1 peritoneal cells (25%) and partly by B2 cells from mucosal associated lymphoid tissues (25).

Even though all individuals who participated in the study are young adults and elite athletes, individual differences, as in any other human population are vast, and this is also the case for levels of specific antibacterial antibodies. Apart from the diversity of each individual's genetic background, there are also differences in the histories of antigen encounter, which ultimately shape antibody repertoire. An interesting finding was that although serum IgA levels specific for *L. rhamnosus* LA68 and *L. rhamnosus* LB64 were reduced in the control group, salivary IgA was not lowered in any of the LAB species tested. Yet, the analysis of IgA levels specific for LAB either in saliva or serum showed no difference in the probiotic group. Moreover, the reduction in specific anti-LAB IgA levels might be explained by a certain level of cross-reactivity between LAB species because all the participants were told to restrain from fermented milk products and other probiotic supplements. Therefore, the consumption of *Lactobacilli* results in the maintenance of certain anti-*Lactobacillus* IgA antibody levels, which is a relatively specific effect, as we did not observe the change in the antibody levels specific for other bacteria tested. Finally, it might be concluded that specific salivary IgA is just a poor indicator of specific intestinal IgA response, as reported by previous studies (13,29).

Nevertheless, the mechanism by which probiotics could induce salivary IgA remains elusive, because the sIgA-mediated immunity is very complex. Some new findings suggest that the generation of mucosal IgA+ B-cells is both T-cell dependent and T-cell independent, but their relative contributions are still unclear (6). Mucosal IgA+ cells migrate out of the gut mucosa to the circulation, arriving at the local mucosal immune tissues, such as the salivary glands. There they produce IgA, secreted into the salivary gland duct as sIgA (7,21,35).

IgM antibody class is the first immunoglobulin in serum to elevate in concentration, generally within 1–2 weeks (28). It is the most cross-reactive antibody class, as it represents the first line of defense against pathogens. No changes in the levels of antibacterial specific IgM levels were found, which was to be expected because of the low IgM specificity.

Interestingly, total IgM antibody levels were significantly increased in both probiotic and placebo groups. Previous studies about the effects of exercise reported that the greatest effect of acute exercise on humoral response was an increase in serum IgM levels (27), although other authors reported no change (17) or even a decrease (19). Different mechanisms were proposed to explain this increase in IgM, including the nonspecific interaction between sympathetic neural system and immunity and antigen stimulation by larger amounts of microorganisms entering the body during the intensive training (27). In addition, the observed reduction of IgA and an increase of IgM, which was observed in the placebo group, is also found in IgA-deficient individuals, where the lack of IgA is compensated by the increase of IgM (5).

On the other hand, the IgG antibody class is considered to be a true and main indicator of antigen encounter. It is the most specific antibody class and provides immune memory (28). A significant 16% reduction during the study was noted for anti–*E. faecalis* IgG. It seems that supplementation with Lafti L10 helped in maintaining adequate antigen-specific response against a gram-positive uropathogenic strain of *E. faecalis*, but not against gram-negative *Proteus mirabilis* or *E. coli*. These findings indicate that supplementation with Lafti can enhance specific but not generalized immune activation. Therefore, a future study should include testing of specific responses to a greater number of antigens, especially those of common infective agents causing URTI, such as influenza.

Several trials conducted in athletes showed the ability of some strains to reduce the incidence of URTIs (10), the severity of symptoms (39), and shorten the duration of an URTI episode (40). Lafti showed the potential to reduce the duration of an URTI episode and decrease the number of respiratory symptoms (Marinkovic et al., submitted).

Similar enhancement of specific humoral response were observed against *Haemophilus influenza* (32), *Cholera* (29), and enterotoxigenic *E. coli* (28). However, to our knowledge, this is the first study to examine specific humoral responses upon probiotic supplementation in professional athletes.

Apparently, circulating sera IgG is critical for defense against URTI (12,20), but there are some contrasting findings concerning its response to prolonged exercise (37). It is reported that serum levels of both total IgG and IgG subclasses are significantly lower in swimmers in comparison with sedentary controls (27). However, total IgG did not change during the period of supplementation in both probiotic and placebo groups. Similar results were reported by Gleeson (15). In fact, this could be expected, because total levels of

immunoglobulins are less likely to respond to dietary changes, except in some extremes (HIV infections, severe malnutrition) (3). Conversely, there are studies showing that probiotics might affect circulating antibody counts: probiotic supplementation of critically ill patients resulted in a substantial increase of systemic IgA or IgG concentrations (2,38).

Correlation found for IgG, IgM, and IgA levels in different individuals was trivial, which is also in connection with the specificity of these different antibody classes.

In conclusion, we suggest that *L. helveticus* Lafti L10 supplementation could be an appropriate dietary aid in humoral and mucosal immunity maintenance, which is critical for URTI prevention in elite athletes. Further investigations should elucidate the mechanisms of the interactions between *L. helveticus* and immunity.

Practical Applications

The current study indicates that probiotic supplementation restores mucosal and humoral immunity impairment caused by intense training during winter months. Apparently, respiratory illness occurs typically in the period of heavy exercise, particularly during winter months (18). In that manner, every training disruption during preparations for forthcoming sport competitions may result in performance impairment. In addition, humoral and especially mucosal immunity plays a crucial role in the defense against pathogen translocation. Hence, our findings might have a practical implication, in the sense of prevention, or the reduction of length and severity of URTI episodes. Additionally, athletes and their coaches might take *L. helveticus* Lafti L10 into consideration as an appropriate nutritional supplement, to avoid performance impairment because of illness.

Acknowledgments

The authors acknowledge material and scientific support of Lallemand Health Solutions, Montreal, Canada. This work is supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. III 46009 and OI 172049). The authors also thank Aleksandra Todorovic, MD, from the Institute of Virology, Vaccines and Sera, Torlak, for providing clinical isolates of uropathogenic bacteria. **Laboratories where the research was conducted:** 1. Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11152 Belgrade; 2. Institute of Virology, Vaccines and Sera, Torlak, Vojvode Stepe 458, 11152 Belgrade, Serbia; 3. Vita maxima, medical office for sport medicine, Marsala Tolbuhina 8, 11000 Belgrade. The present study does not constitute endorsement of the product by the authors or any conflict of interest.

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