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Effects of a *Lactobacillus salivarius* Probiotic Intervention on Infection, Cold Symptom Duration and Severity, and Mucosal Immunity in Endurance Athletes

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The purpose of this study was to examine the effects of a probiotic supplement during 4 mo of spring training in men and women engaged in endurance-based physical activities on incidence of upper respiratory tract infections (URTI) and mucosal immune markers. Sixty-six highly active individuals were randomized to probiotic (n = 33) or placebo (n = 33) groups and, under double-blind procedures, received probiotic (PRO: *Lactobacillus salivarius*, 2×10^{10} bacterium colony-forming units) or placebo (PLA) daily for 16 wk. Resting blood and saliva samples were collected at baseline and after 8 and 16 wk. Weekly training and illness logs were kept. Fifty-four subjects completed the study (n = 27 PRO, n = 27 PLA). The proportion of subjects on PRO who experienced 1 or more wk with URTI symptoms was not different from that of those on PLA (PRO .58, PLA .59; p = .947). The number of URTI episodes was similar in the 2 groups (PRO 1.6 ± 0.3, PLA 1.4 ± 0.3; p = .710). Severity and duration of symptoms were not significantly different between treatments. Blood leukocyte, neutrophil, monocyte, and lymphocyte counts; saliva IgA; and lysozyme concentrations did not change over the course of the study and were not different on PRO compared with PLA. Regular ingestion of *L. salivarius* does not appear to be beneficial in reducing the frequency of URTI in an athletic cohort and does not affect blood leukocyte counts or levels of salivary antimicrobial proteins during a spring period of training and competition.

Keywords*:* exercise training, mucosal immunity, leukocytes, respiratory illness, gut microbiota

Probiotics are food supplements containing live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. There is now a reasonable body of evidence that regular consumption of probiotics can modify the population of the gut microbiota and influence immune function (Borchers, Selmi, Meyers, Keen, & Gershwin, 2009; Gill & Cross, 2002; Matsuzaki, 1998; Mengheri, 2008; Minocha, 2009), although it should be noted that such effects are strain specific. Some studies are suggestive of increased resistance to enteric pathogens and promotion of antitumor activity with probiotic supplementation, and there is emerging evidence that probiotics may be effective in alleviating some allergic and respiratory disorders in children (Hatakka et al., 2001; Kopp-Hoolihan, 2001). Furthermore, it has been reported that probiotic supplementation enhances host resistance to upper respiratory tract infection (URTI) in the general population (Berggren, Lazou Ahrén, Larsson & Önning, 2011; de Vrese et al., 2006), and several recent studies have indicated that some *Lactobacillus* probiotics can reduce URTI incidence in athletes (Cox, Pyne, Saunders, & Fricker, 2010; Gleeson, Bishop, Oliveira, & Tauler, 2011; West et al., 2011), who are generally considered a marginally immunocompromised population due to the depressive effects of hard physical exercise, psychological stress, sleep disturbance, and negative energy balance on the immune system (Gleeson, 2007; Walsh, Gleeson, Pyne, et al., 2011).

Reduced levels of salivary antimicrobial proteins (e.g., immunoglobulin A and lysozyme) in athletes may contribute to their increased risk of URTI (Fahlman & Engels, 2005; Gleeson et al., 2012; Gleeson et al., 1999; Neville, Gleeson & Folland, 2008; Walsh, Gleeson, Shephard, et al., 2011; West et al., 2010). The number of circulating leukocytes or specific lymphocyte subsets such as T-cytotoxic lymphocytes and natural killer (NK) cells might also influence susceptibility to viral infections such as URTI (Walsh, Gleeson, Shephard, et al., 2011).

The aims of the current study were to examine the effects of 4 months of daily oral supplementation with a probiotic containing the gram-positive bacteria *Lactobacillus salivarius* on infection incidence, salivary antimicrobial proteins, and blood leukocyte and

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lymphocyte subset counts in a cohort of university-based endurance athletes during a period of spring training and competition.

Methods

Subjects

Sixty-six healthy subjects who were engaged in regular sports training (predominantly endurance-based activities such as running, cycling, swimming, triathlon, team games, and racket sports) volunteered to participate in the study. Subjects ranged from recreationally active to Olympic triathletes, and their self-reported training loads (based on the answer to the question "On average how many hours in each week do you train/compete?") averaged 9 hr/week. Subjects were required to complete a comprehensive health-screening questionnaire before starting the study and had not taken any medication in the 4 weeks before the study. All subjects recruited to the study confirmed that they had not taken any probiotic in the previous 4 months. All subjects were fully informed about the rationale for the study and of all experimental procedures to be undertaken. They provided written consent to participate in the study, which had earlier received the approval of Loughborough University ethical advisory committee. Subjects were enrolled if they met all inclusion criteria and presented none of the exclusion criteria (determined by both questionnaire and interview).

Subjects could be included if they were currently healthy, had been involved in endurance training for at least 2 years, engaged in at least three sessions and at least 3 hr of total moderate- to high-intensity training time per week, and were 18–35 years of age. Subjects representing one or more of the following criteria were excluded from participation: smoking; use of any medication; currently taking probiotic supplements; suffered from or had a history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological, or psychiatric illness; objected to the prescription of diet (abstinence from fermented milk products and other probiotic supplements).

A total of 66 healthy individuals were recruited as subjects and randomly assigned to one of two treatments with stratification by gender only; under double-blind procedures, 33 subjects received the probiotic and 33 received the placebo preparation. Of these 66 subjects, 38 were women and 28 were men, with the mean age of the study cohort at recruitment being 23.9 ± 4.7 years ($M \pm SD$). Sample-size estimation of 32 subjects per treatment group was based on an expected rate of 2.0 ± 1.0 URTI episodes ($M \pm SD$) during the winter months (Neville et al., 2008; Gleeson et al., 2011), a target 30% reduction in number of episodes, statistical power of 80%, and a Type I error of 5%.

First Laboratory Visit

The study began in the month of February. For the first visit to the laboratory, subjects arrived in the morning between 8:30 and 10:30 a.m. after an overnight fast of approximately 12 hr. They were asked to empty their bladder before body mass and height were recorded. Information about the study was given to them, and they then signed an informed-consent form. Subjects then sat quietly for 10 min and completed a health-screen questionnaire and inclusion/exclusion-criteria questionnaire before providing an unstimulated saliva sample by passive dribble for 2 min into a preweighed sterile collection tube. Saliva samples were weighed and stored frozen at –80 °C before analysis. Subsequently, a resting venous blood sample (11 ml) was obtained by venipuncture from an antecubital forearm vein into two Vacutainer tubes (Becton Dickinson, Oxford, UK) containing either K_3EDTA or heparin. Hematological analysis was immediately carried out on the EDTA sample (including hemoglobin, hematocrit, and total and differential leukocyte counts) using an automated cell counter (A^c.T5diff hematology analyzer, Beckman Coulter, High Wycombe, UK). If subjects met the criteria for inclusion of the study, they were randomly assigned to the treatment or placebo group and asked to start taking the supplement the next day.

Study Intervention

The *L. salivarius* probiotic and placebo treatments were supplied in the form of 2 g powder in a sealed aluminum sachet, which was to be opened and the contents added to a glass of water (50–100 ml) as part of a meal at breakfast time and consumed within 15 min of preparation on a daily basis for the 4-month intervention period. The probiotic supplement contained 0.2 g of *L. salivarius* (2×10^{10} bacterium colony-forming units) with 1.78 g maltodextrin and 0.01 g magnesium stearate. The placebo contained the same amount of chemical vehicle (maltodextrin and magnesium stearate) as the probiotic but did not contain any bacteria. The probiotic and placebo supplements were stored at approximately 4–7 °C (domestic refrigerator). Subjects returned to the laboratory every 4 weeks to receive a fresh supply of supplement. A compliance log of sample collection was taken. Subjects were asked to keep a record of any days that they missed taking the supplement.

Study Protocol

During the 4-month intervention period with probiotic (PRO) or placebo (PLA), subjects were asked to continue with their normal training programs. Consumption of supplements (vitamins and minerals, etc.), additional probiotics, or any fermented dairy products (e.g., yogurt, sour cream, crème fraîche) was not permitted during this period. Subjects completed a health (URTI symptoms) questionnaire on a weekly basis. They were not required to abstain from medication when they were suffering from illness symptoms, but they were required, on a weekly basis, to report any unprescribed medications taken, visits to the doctor, and any prescribed medications.

The illness symptoms listed on the questionnaire were sore throat, catarrh (excessive mucus) in the throat, runny nose, cough, repetitive sneezing, fever, joint aches and pains, weakness, headache, and loss of sleep. The nonnumerical ratings of light, moderate, or severe (L, M, or S, respectively) of severity of symptoms were scored as 1, 2, or 3, respectively, to provide a quantitative means of data analysis (Fricker et al., 2005; Gleeson et al., 2012; Gleeson et al., 2011), and the total symptom score for every subject each week was calculated by multiplying the total number of days each symptom was experienced by the numerical ratings of L, M, or S symptoms of 1, 2, or 3, respectively. In any given week, a total symptom score ≥12 was taken to indicate that a URTI was present. This score was chosen because to achieve it a subject would have to record at least three moderate symptoms lasting for 2 days or two moderate symptoms lasting for at least 3 days in a given week. A single URTI episode was defined as a period during which the weekly total symptom score was ≥12 and separated by at least 1 week from another week with a total symptom score ≥12. Subjects were also asked to rate the impact of illness symptoms on their ability to train (normal training maintained, training reduced, or training discontinued; L, M, or S, respectively).

Subjects were also asked to fill in a standard short form International Physical Activity Questionnaire (IPAQ; http://www.ipaq.ki.se/downloads.htm) at weekly intervals, thus providing quantitative information on training loads in metabolic equivalent (MET)-hr/week (Craig et al., 2003).

After 8 and 16 weeks, subjects came to the laboratory again after an overnight fast. They were required to abstain from any strenuous physical activity for 24 hr before coming to the laboratory to avoid acute effects of exercise on the measured blood and saliva variables. During these visits body mass was recorded and an unstimulated saliva sample and venous blood samples were collected as described for the first visit to the laboratory.

Blood Cell Counts and Lymphocyte Subsets

Blood samples in the K_3EDTA Vacutainers (4 ml) were used for hematological analysis with an automated cell counter (A^c.T5diff hematology analyzer, Beckman Coulter, High Wycombe, UK). The intra-assay coefficient of variation for all measured variables was less than 3.0%.

Lymphocyte subsets (CD3, CD4, CD8, CD19, CD56) to enumerate total T cells, T-helper cells, T-cytotoxic cells, B cells, and NK cells were determined by three-color flow cytometry (Becton Dickinson FACS-Calibur) with CellQuest analysis software (Becton Dickinson Biosciences, Oxford, UK) as described previously (Lancaster et al., 2004). Forward-scatter versus side-scatter plots were used to gate on the lymphocyte population by morphology, and 10,000 lymphocyte events were acquired per analysis. Estimations of the absolute CD3+, CD3+CD4+, CD3+CD8+, NK cell (CD3-CD56+), and B cell (CD3-CD19+) numbers were derived from the total lymphocyte count.

Saliva IgA and Lysozyme

Saliva was analyzed for immunoglobulin A (IgA) using an ELISA kit (Salimetrics, USA) and for lysozyme using an ELISA kit (Biomedical Technologies, Stoughton, MA). For both assays, saliva samples were assayed in duplicate, standards (also in duplicate) were included on every plate, and samples for each subject were analyzed on the same plate. The intra-assay coefficients of variation for IgA and lysozyme were 10.1% and 5.0%, respectively. Saliva flow rates (ml/min) were determined from the weight of saliva collected, assuming saliva density to be 1.0 g/ml (Cole & Eastoe, 1988), divided by the collection time. Secretion rates for IgA and lysozyme were calculated by multiplying their respective concentrations in saliva by the saliva flow rate.

Statistical Analysis

The difference in proportion of subjects who presented with symptoms of infection during the trial between the PRO and PLA groups was assessed by a chi-square test. Comparisons of the proportion of days or weeks with infection symptoms were also made with a chi-square test. For subjects with infection symptoms, the total symptom-severity score and the mean duration of infection symptoms, comparisons between the treatments for single measurements was carried out using independent (unpaired) *t* tests. Changes in saliva and blood variables during the study were analyzed using a two-factor (Treatment × Time) between–within-subjects ANOVA with repeated measures (time: 0, 8, and 16 weeks of intervention). Corresponding assumptions of homogeneity of variances and intercorrelation were checked, as was sphericity in the data. Any significant *F* ratios subsequently shown were assessed using Student's paired *t* tests with Holm–Bonferroni correction for multiple comparisons applied to the unadjusted *p* value. Statistical significance was accepted at $p < .05$. Data are expressed as $M \pm SD$.

Results

Adherence to the Study

Of the 66 subjects, 56 successfully completed 8 weeks of the study, and 54 subjects completed the full 16 weeks of the study. Several subjects withdrew due to injury, persistent nonrespiratory illness (preventing them from performing training), or undisclosed reasons. Adherence to the intervention was good; subjects who completed the study reported that they had missed taking the supplement on average only on 4 days (range 0–13 days). Saliva and blood samples were obtained on all three visits from 53 and 48 subjects, respectively. Subjects were asked at the

end of the study which treatment they thought they had taken. Sixty percent said they did not know, 23% thought they were on PRO, and 17% thought they were on PLA. Of the subjects who expressed an opinion, 43% were correct in their selection of treatment and 57% were incorrect; hence, the study blinding was effective.

On PRO the baseline characteristics of the subjects (n = 27) who completed the study were as follows: age 25 ± 5 years, body mass 74.3 ± 14.9 kg, height 175 ± 10 cm, body-mass index 24.2 ± 3.4 kg/m^2, and self-reported weekly training load 9.1 ± 4.2 hr/week. On PLA the baseline characteristics of the subjects (n = 27) were as follows: age 24 ± 4 years, body mass 69.4 ± 11.6 kg, height 173 ± 9 cm, body-mass index 23.2 ± 2.7 kg/m^2, and self-reported weekly training load 8.7 ± 3.8 hr/week. There were no statistically significant differences between the two treatment groups.

Training Loads

Analysis of the IPAQ questionnaires indicated that the weekly training loads were relatively stable between and within experimental groups over the 16 weeks of the study (Figure 1) and that the means were not significantly different for the PRO and PLA groups: 67.8 ± 23.2 and 60.8 ± 20.1 MET-hr/week, respectively (p = .285). This is equivalent to about 10 hr of moderate to vigorous activity per week.

Infection Symptom Incidence

Analysis of the URTI symptom questionnaires indicated that 10% ± 4% of the cohort experienced a URTI episode each week. Twenty-four subjects did not experience a single URTI episode during the study period, and 29 experienced at least one. The proportion of subjects on PRO who experienced 1 or more weeks with URTI symptoms was not significantly different from that on PLA (PRO .58, PLA 0.59; p = .947). The number of URTI episodes was similar in the two groups (PRO 1.5 ± 2.3, PLA 1.4 ± 1.5; p = .710). During weeks when a URTI episode was present, the proportion of subjects who took medication was similar in the PRO and PLA groups (.55 and .53, respectively), and the proportion who went to see their doctor was small and not significantly different in the PRO and PLA groups (.11 and .24, respectively; chi-square test statistic = .949, p = .330).

Saliva Antimicrobial Proteins

There were no significant time, treatment, or interaction effects for saliva IgA concentration or secretion rate. There was a significant main effect of time for saliva lysozyme concentration (and secretion rate), with values at 16 weeks lower than at baseline, but there was no effect of treatment on this (Table 1).

Blood Leukocyte Counts and Lymphocyte Subsets

There were no significant time, treatment, or interaction effects for blood counts of total leukocytes, neutrophils, and monocytes. For blood lymphocyte counts, there was significant main effect of time, with values higher after 16 weeks than at baseline, and a significant effect of treatment, with counts higher on PRO than on PLA (Table 2). However, the magnitude of the difference in mean lymphocyte counts between the PRO and PLA groups was not different at baseline, 8 weeks, or 16 weeks.

There were no significant time, treatment, or interaction effects for percentages of T cells (CD3+), T-cytotoxic cells (CD3+CD8+), or NK cells (CD3-CD56+), although the interaction effect for NK cells was close to signifi-

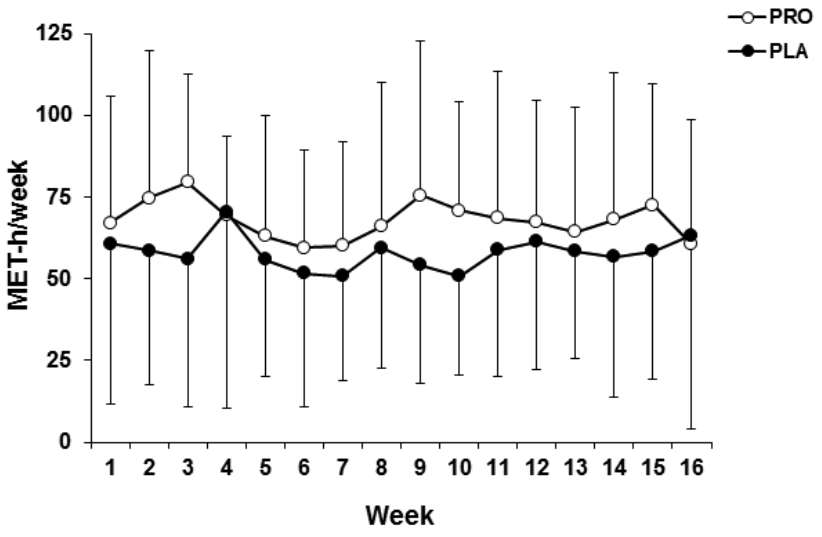

Figure 1 — Training loads in MET-hr/week over the 16-week study period for subjects who completed the study, $M \pm SD$. No statistically significant difference between treatments.

Table 1 Saliva IgA and Lysozyme Before and After 8 and 16 Weeks of the Intervention, *M* (*SD*)

	Before	8 weeks	16 weeks	*p* (interaction, time, treatment)
IgA, mg/L				
placebo, *n* = 27	138 (81)	143 (95)	128 (58)	.797, .381, .625
probiotic, *n* = 27	135 (90)	127 (101)	123 (62)	
IgA secretion rate, µg/min				
placebo	65 (55)	63 (41)	59 (32)	.763, .552, .568
probiotic	58 (57)	63 (36)	57 (29)	
Lysozyme, mg/L				
placebo	4.7 (3.6)	3.7 (2.3)	3.6 (2.5)	.700, .027[a], .560
probiotic	4.4 (3.8)	3.7 (2.4)	2.9 (2.6)	
Lysozyme secretion rate, µg/min				
placebo	2.3 (2.4)	1.6 (1.1)	1.7 (1.3)	.533, .029[b], .604
probiotic	2.1 (2.5)	1.7 (1.1)	1.3 (1.3)	

[a]Before > 16 weeks, $p = .021$. [b]Before > 16 weeks, $p = .022$.

Table 2 Total and Differential Leukocyte Counts (Cells × 10^9/L) Before and After 8 and 16 Weeks of the Intervention, *M* (*SD*)

	Before	8 weeks	16 weeks	*p* (interaction, time, treatment)
Leukocytes				
placebo, *n* = 25	5.50 (1.35)	5.52 (1.47)	5.60 (1.21)	.970, .460, .802
probiotic, *n* = 22	5.56 (1.24)	5.57 (1.28)	5.77 (1.04)	
Neutrophils				
placebo	2.96 (1.11)	2.97 (1.14)	2.87 (0.80)	.883, .997, .119
probiotic	2.59 (0.76)	2.57 (0.77)	2.65 (0.83)	
Monocytes				
placebo	0.48 (0.11)	0.47 (0.15)	0.49 (0.15)	.797, .748, .651
probiotic	0.48 (0.13)	0.50 (0.19)	0.50 (0.13)	
Lymphocytes				
placebo	1.84 (0.42)	1.87 (0.52)	1.99 (0.46)	.957, .013[a], .014[a,b]
probiotic	2.18 (0.54)	2.22 (0.66)	2.36 (0.61)	

[a]Before > 16 weeks, $p = .010$. [b]Probiotic > placebo.

cance ($p = .068$), with decreases over time in the PLA group and increases over time in the PRO group (Table 3). There was a significant effect of time for T-helper cells (CD3+CD4+), with values higher after 8 weeks than at baseline. There was a significant main effect of treatment for B cells (CD3-CD19+), with values higher on PRO than PLA, but it seems that this is likely to be a chance difference that was present at baseline.

The circulating numbers of the different lymphocyte subsets were obtained by multiplying the lymphocyte count (measured using the Coulter counter) by the percentage of the specific subset (measured using the flow cytometer). As there were significant time and treatment effects for the blood lymphocyte count, it is not surprising that likewise there were some significant main effects for the various subset counts. These are shown in Table 4. There were significant main effects of treatment (all higher on PRO than PLA) for T cells, T-cytotoxic cells, and B cells and increases with time for T-helper cells and the T-helper:T-cytotoxic (CD4+/CD8+) ratio. However, there were no significant interactions for any of the lymphocyte subset counts.

Discussion

The current study investigated the effects of probiotic supplementation on self-reported URTI symptoms in a group of highly active individuals engaged in their normal level of training and competition during the months of February to May. The main findings were that the proportion of subjects who experienced one or more URTI episodes was not significantly different in the PRO group than with PLA. During weeks when a URTI episode was present, the mean total symptom-severity score and duration of symptoms were not different on PRO than on PLA. The proportion of subjects who took medication when suffering URTI symptoms was similar in the PRO and PLA groups, and the difference in the proportion who went to see their doctor was small and not significantly different between the groups. Saliva

Table 3 Percentages of Total Lymphocytes Before and After 8 and 16 Weeks of the Intervention, *M* (*SD*)

	Before	8 weeks	16 weeks	*p* (interaction, time, treatment)
T cells CD3+				
placebo, *n* = 25	60.2 (9.5)	61.9 (8.7)	60.4 (9.7)	.143, .596, .754
probiotic, *n* = 20	60.1 (10.0)	60.6 (6.2)	63.7 (5.9)	
T-helper cells CD3+CD4+				
placebo	34.1 (6.3)	36.4 (7.1)	35.4 (7.6)	.548, .016[a], .380
probiotic	31.7 (7.7)	34.0 (8.6)	34.8 (7.5)	
T-cytotoxic cells CD3+CD8+				
placebo	23.6 (5.9)	23.5 (5.4)	23.0 (5.3)	.204, .757, .414
probiotic	24.3 (8.0)	24.4 (7.4)	25.0 (6.7)	
B cells CD3-CD19+				
placebo	8.9 (3.0)	8.8 (3.1)	8.6 (3.2)	.942, .524, .042[b]
probiotic	11.2 (4.9)	11.0 (4.0)	10.7 (4.1)	
Natural killer cells CD3-CD56+				
placebo	11.4 (5.8)	11.0 (5.7)	10.4 (5.4)	.068, .802, .811
probiotic	10.0 (4.9)	10.2 (4.8)	11.6 (4.8)	

[a]Before > 8 weeks, $p = .012$. [b]Probiotic > placebo, $p = .093$.

Table 4 Lymphocyte Counts (Cells $\times$ 10^9/L) Before and After 8 and 16 Weeks of the Intervention, *M* (*SD*)

	Before	8 weeks	16 weeks	*p* (interaction, time, treatment)
T cells CD3+				
placebo, *n* = 20	1.09 (0.32)	1.14 (0.36)	1.17 (0.29)	.446, .120, .018[a]
probiotic, *n* = 25	1.35 (0.46)	1.31 (0.41)	1.45 (0.39)	
T-helper cells CD3+CD4+				
placebo	0.61 (0.18)	0.66 (0.22)	0.68 (0.19)	.714, .045[b], .153
probiotic	0.70 (0.25)	0.71 (0.21)	0.78 (0.20)	
T-cytotoxic CD3+CD8+				
placebo	0.43 (0.15)	0.43 (0.16)	0.45 (0.14)	.453, .170, .037[c]
probiotic	0.57 (0.31)	0.54 (0.27)	0.60 (0.28)	
B cells CD3-CD19+[d]				
placebo	0.16 (0.07)	0.16 (0.07)	0.17 (0.07)	.562, .508, .010
probiotic	0.26 (0.16)	0.24 (0.12)	0.25 (0.17)	
Natural killer cells CD3-CD56+				
placebo	0.21 (0.12)	0.20 (0.12)	0.20 (0.11)	.620, .134, .415
probiotic	0.21 (0.10)	0.22 (0.11)	0.26 (0.12)	
CD4+:CD8+ ratio				
placebo	1.53 (0.46)	1.63 (0.50)	1.62 (0.49)	.462, .003[e], .636
probiotic	1.46 (0.62)	1.58 (0.81)	1.49 (0.64)	

[a]Probiotic > placebo, $p = .124$. [b]Before > 16 weeks, $p = .037$. [c]Probiotic > placebo, $p = .098$. [d]Probiotic > placebo, $p = .143$. [e]Before > 8 weeks, $p = .003$; before > 16 weeks, $p = .038$.

lysozyme concentration fell over time, but the magnitude of change was not different on PRO compared with PLA. It is not clear why the saliva lysozyme concentration was lower at the end of the study; it could be a seasonal effect or due to an accumulation of training stress.

Other than a main effect of treatment for blood lymphocyte counts (higher on PRO than PLA) and some lymphocyte subsets (T cells, T-cytotoxic cells, and B cells), there were no differences in any of the immune variables measured for PRO compared with PLA at Week 0 (baseline) or after 8 or 16 weeks of intervention. The lack of positive clinical consequences does not provide evidence for any beneficial effect of daily *L. salivarius* ingestion in a cohort of highly physically active people. This is in contrast to several recent studies that have reported beneficial effects of other *Lactobacillus* probi-

otics on URTI incidence and some aspects of immune function in endurance athletes. For example, Cox et al. (2010) observed fewer days of respiratory illness and lower severity of respiratory illness in subjects taking a daily *Lactobacillus* probiotic, which was attributed to higher spontaneous IFN-γ production (Cox et al., 2010). Others have reported lower URTI incidence (Gleeson et al., 2011; West et al., 2011) and improved maintenance of salivary IgA levels (Gleeson et al., 2011a; Tiollier et al., 2007) with *Lactobacillus* probiotics during periods of physical stress. The dose of probiotic used in the current study was similar to that of the other studies cited here, although it was administered as a soluble powder rather than as part of a fermented dairy product; however, this should not have influenced its efficacy because the manufacturer carried out tests to confirm the bacterial viability of the supplied probiotic product.

Several factors may have confounded the lack of impact of the probiotic supplement on infection outcomes:

- The mean weekly physical activity levels estimated from the IPAQ questionnaires indicated that training loads were on average about 10% higher for subjects in the PRO group than in the PLA group, although this difference was not statistically significant.
- The blood lymphocyte, T cell, T-cytotoxic cell, and B cell counts were significantly higher at baseline in the PRO group. The subjects were randomly allocated to the two treatments, with stratification only for gender, so these differences in cell counts arose purely by chance.
- Most subjects were female, and West et al. (2011) have reported that *L. fermentum* probiotic supplementation in a cohort of 64 male and 35 female elite endurance athletes was associated with a lower symptom load for chest infections and less use of cold and flu medications in males taking the probiotic compared with placebo but that differences in clinical outcomes for females between the two groups were not evident.
- URTI incidence was not as high as expected; the study began in February and therefore missed the winter months when URTI incidence is generally highest in the UK.
- The number of subjects may not have been sufficient, and the study was underpowered to detect any small differences in URTI incidence.
- As effects of probiotics are known to be strain specific, it may be that the strain used in this study was just not effective.

Minocha (2009) cautions that results from meta-analyses and systematic reviews that combine results of studies from different types of probiotics to examine the effects in any disease state should be interpreted with caution. Specific strains are effective in specific disease states, and different probiotic strains vary in their ability to modulate the immune system (Gill & Prasad, 2008). No two probiotics are exactly alike, so we should not expect reproducible results from studies that employ different species or strains, variable formulations, and diverse dosing schedules. Therefore, the efficacy of each strain needs to be carefully demonstrated through rigorously designed (randomized, double-blind, placebo-controlled) studies.

In the general population, several recent large-scale human studies have demonstrated that regular, long-term intake of various probiotic-prebiotic combinations improved health by reducing both the incidence and the severity of respiratory diseases during the winter season (Pregliasco et al., 2008; Winkler, de Vrese, Laue, & Schrezenmeir, 2005). Other studies have reported that probiotics are associated with shortening of common cold symptoms and reduced severity of symptoms without an effect on infection incidence in adults during spring periods (de Vrese et al., 2006; Guillemard, Tondu, Lacoin, & Schrezenmeir, 2010).

We conclude that regular ingestion of *L. salivarius* does not appear to be beneficial in reducing the frequency of URTI in a predominantly female athletic cohort and does not affect levels of salivary antimicrobial proteins or blood leukocyte and lymphocyte subset counts during a spring period of training and competition.

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