Effects of probiotic supplementation on respiratory infection and immune function in athletes: systematic review and meta-analysis of randomized controlled trials.

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Effects of probiotic supplementation on respiratory infection and immune function in athletes: systematic review and meta-analysis of randomized controlled trials.

ABSTRACT

Objective: The aim of this study was to evaluate the effectiveness of probiotic supplementation on upper tract respiratory infection and inflammatory markers in elite athletes.

Data sources: We identified sources by searching the PubMed, EBSCO host, Scopus, and Web of Science databases using the following search terms: “probiotic” OR “probiotics” AND “exercise” OR “sport” OR “athletes” AND “URTI” OR “respiratory infection” OR “upper respiratory tract infections” OR “inflammation” OR “inflammatory OR “cytokines”.

Study selection: We screened the title and abstracts of 2498 articles using our inclusion criteria. A total of 14 articles were selected for further analysis.

Data Extraction: Data from the included studies were extracted by 2 independent reviewers. These data included the study design, participant characteristics, inclusion and exclusion, intervention characteristics, outcome measures, and the main results of the study.

Data Synthesis: The meta-analysis did not show any significant effect of probiotic supplementation on the number of days of illness or the mean number and duration of URTI episodes, but there was a significant effect of probiotic supplementation on total symptom severity score (-0.65, 95% CI: -1.05; -0.25, p = 0.02). Lower levels of IL-6 (-2.52 pg/ml, 95% CI: -4.12, -0.51, p = 0.001) and TNF-α (-2.31 pg/ml, 95% CI: -4.12, -0.51, p = 0.008) were also reported after supplementation.

Conclusions: This meta-analysis provides evidence that probiotic supplementation, especially among professional athletes, is an effective way to decrease the total URTI
symptom severity score. Additionally, probiotic supplementation may decrease TNF-α and
IL-6 levels. There is a need for more studies with larger groups to better estimate this effect.
It is necessary to determine the best timing, duration, composition and dose of such
supplementation.

Keywords: upper respiratory tract infection, athletes, probiotic, inflammatory
INTRODUCTION

Heavy, acute, and prolonged intense physical exercise, insufficient rest and sleep, emotional stress, and inadequate nutrition may generate serious health problems among athletes, such as inflammation or respiratory infections, which may affect their physical performance and sports achievements. It has been observed that such factors can lower the resistance of athletes by reducing the number and activity of natural killers (NK) cells, decreasing neutrophil activity, impairing proliferation of T-lymphocytes, decreasing the level of anti-inflammatory cytokines, increasing levels of pro-inflammatory cytokines, and increasing salivary IgA inducing respiratory tract infections. Indeed, many elite athletes have reported significant bouts of infections that have interfered with their ability to compete and training. Epidemiological studies also imply that extensive training is associated with an increased risk of upper respiratory tract infection (URTI). Thus, dietary strategies have been sought for many years to improve the immune function of athletes and reduce their risk of URTI.

Probiotics are live microorganisms which, when administered in adequate amounts, may confer a health benefit on the host. It has been shown in last meta–analysis among general adult populations that probiotics were better than placebo in reducing the number of participants experiencing episodes of acute URTIs, the rate ratio of episodes of acute URTI and reducing antibiotic use. This indicates that probiotics may be more beneficial than placebo for preventing acute URTIs. Wang et al. also showed that probiotic supplementation in children led to fewer days of URTI and fewer days absent from day care or school than in children who had taken a placebo.

Furthermore, many studies have demonstrated the beneficial effect of probiotic supplementation on inflammatory markers in the general population. For example, the systematic review and meta-analysis of Kazemi et al. showed that probiotics and synbiotic
decreased some of these. The intervention was most effective in reducing CRP and TNF-α in healthy or diseased general populations. It could be suggested that the use of probiotics in physically active individuals might serve as a strategy to further improve respiratory symptoms and inflammatory status, and in consequence increase the physical performance of athletes. Although a recently published review has indicated that probiotic supplementation improves the immune system and reduces the severity and incidence of upper respiratory tract infections in athletes, to the best of our knowledge there has been no systematic literature review or meta-analysis that has confirmed these results.

The aim of this study was thus to evaluate the effectiveness of probiotic supplementation on respiratory infection and inflammatory markers in elite athletes, based on the data available from randomized controlled trials.

Materials and Methods

This systematic review was designed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (#XXX).

Search strategy

The search for relevant articles published up to the end September 2020 was conducted in the Medline, Embase, SciELO, Scopus, and Lilacs databases between 01 February 2020 and 30 September 2020. The databases were searched using the following key words and their varying combinations: “probiotic” OR “probiotics” AND “exercise” OR “sport” OR “athletes” AND “URTI” OR “respiratory infection” OR “upper respiratory tract infections” OR “inflammation” OR “inflammatory OR “cytokines” without restrictions. Moreover, the references of related reviews and original articles were also reviewed. The full search strategy is described in Figure 1.
Study selection

The inclusion criteria were based on the PICOS framework and was as follows: 1) studies involving adult healthy professional athletes of both sexes (≥18 years); 2) interventions with probiotics; 3) the inclusion of a control or placebo group; 4) outcomes not previously defined (as an open question: all outcomes were reported in the studies); 5) studies focusing on inflammatory parameters and/or respiratory infections were included; 6) clinical trials, randomized clinical trials (RCT), or crossover design.

Data extraction

Two investigators (KL, JB) independently performed literature search and selection. Publications were assessed according to the titles, abstracts and full texts in subsequent stages. Each selected publication was studied critically. During the data abstraction process, no attempt was made to contact the authors for further information beyond what had been published. Discrepancies were resolved by consensus or arbitration. Data extracted from each study were as follows: authors, year, cohort age and sex, exercise, intervention and control, main outcome, and results.

Bias assessment

We made use of the Cochrane risk of bias assessment tool in order to judge the methodological of each trial, with the aim of evaluating the performance and methods of randomization, the extent of blinding (whether it affected data collectors, data analysis, outcome assessors, or participants), allocation concealment, incomplete outcome data, selective reporting, and other possible sources of bias. In line with the Cochrane handbook’s criteria for judging bias risk, each study was stated to have a high, low, or unclear risk of bias.

Statistical analysis
Statistical analysis was carried out using the Statistica 13.0 software (StatSoft, Tulsa, OK, USA). The therapeutic effect of probiotic supplementation on URTI and cytokine levels with the placebo was estimated using the standardized mean difference (SMD) with a 95% confidence interval (CI). Publication bias was examined through visual inspection of a funnel plot and further evaluated using Begg’s and Egger’s tests, with p < 0.05 indicating a significant publication bias. Heterogeneity across studies was evaluated using Cochran’s Q-statistic (with p < 0.1 implying a significant difference) and the $I^2$-statistic (with $I^2 = 0\%$ meaning no heterogeneity and $I^2 = 100\%$ meaning maximal heterogeneity). A random-effect model was selected when the value of $I^2 \geq 50\%$. All statistical tests were two-sided, and p values below 0.05 were considered statistically different.

RESULTS

Search results

A flow chart showing the study extraction is presented in Figure 1. Throughout the initial search strategy, we identified 2501 articles but finally, fourteen RCTs met the inclusion criteria and were included in the final meta-analysis.

Population and study characteristics

Most of the studies used supplements in capsules or beverages. Sachets with probiotic bacteria were used in one study. The number of study participants, the participant characteristics, the duration of the intervention, the type of supplement, and the sport discipline are presented in Table 1. A total of 1309 study participants (771 study participants from the supplemented group (PRO) and 538 from the placebo group (PLA) were included from fourteen selected studies. The mean age of participants ranged from $20.1 \pm 1.5$ to $36.5 \pm 8.6$ years. The studies were designed as randomized controlled trials (RCT)...
or crossover trials. Interventions were based on supplementation of probiotic bacteria, such as *Bifidobacterium animalis* \(^{17,27}\), *Bifidobacterium bifidum* \(^{14,17,18,24}\), *Bifidobacterium lactis* \(^{18}\), *Bifidobacterium longum* \(^{14}\), *Lactobacillus acidophilus* \(^{17,18,27}\), *Lactobacillus casei* \(^{18,20}\), *Lactobacillus casei Shirota* \(^{21,22}\), *Lactobacillus fermentum* \(^{18,19}\), *Lactobacillus gasseri* \(^{14}\), *Lactobacillus helveticus* \(^{16}\), *Lactobacillus plantarum* \(^{15,18}\), *Lactobacillus rhamnosus* \(^{18,23}\), *Lactobacillus salivarius* \(^{26}\), *Saccharomyces boulardii* \(^{18}\), *Streptococcus thermophilus* \(^{18}\) and *Bacillus subtilis* \(^{25}\). Although the date of publication of the selected papers was unrestricted, all articles included in this systematic review were published after 2007.

**Effect of probiotic supplementation on upper respiratory tract infection**

The eight studies included in our meta-analysis measured the effects of probiotic supplementation on URTI. Data from the included articles allowed us to assess the effects of probiotic supplementation on the number of days of illness, the number of URTI episodes, total symptom severity score, and mean duration of URTI symptoms. Begg’s rank correlation test (number of days of illness: \(p = 1.00\), the number of URTI episodes: \(p = 0.142\), total symptom severity score: \(p = 1.00\) and mean duration of URTI symptoms: \(p = 0.29\)) and Egger’s linear regression test (number of days of illness: \(p = 0.66\), number of URTI episodes \(p = 0.306\), total symptom severity score \(p = 0.099\), mean duration of URTI symptoms: \(p = 0.84\)) suggested that there was no significant publication bias.

The mean number of days of illness, the mean number of URTI episodes, the total symptom severity score, and the mean duration of URTI symptoms among supplemented group ranged respectively from 0.37 ± 0.72 to 5.3 ± 6.8 \(^{19,23}\), from 0.6 ± 0.9 to 4.92 ± 1.96 \(^{16,19}\), from 1.23 ± 0.36 to 110.92 ± 96 \(^{16,19}\), and from 3.5 ± 6.6 to 12.2 ± 14.8 \(^{19}\), while in the placebo group the respective ranges were from 1.08 ± 1.32 to 5.8 ± 6.6 \(^{14,19}\), from 0.5 ± 0.7 to
6.91 ± 1.22, from 1.65 ± 0.57 to 129.73 ± 40.33, from 5.1 ± 14.7 to 10.64 ± 4.67 (Table 1).

The meta-analysis did not show any significant effect of probiotic supplementation on the number of days of illness or on the mean number or duration of URTI episodes, but there was a significant effect of probiotic supplementation on total symptom severity score. After performing the analysis using only single-strain supplements, the effect remained significant (Figures 2 and 3, Table 2).

Effect of probiotic supplementation on inflammatory parameters in athletes

Eight studies in the meta-analysis measured the effect of probiotic supplementation on inflammatory parameter levels. Data from these articles allowed us to assess the effects of probiotic supplementation on IL-6, IL-10, TNF-α blood, and salivary IgA level. Egger’s linear regression test (IL-6: $p = 0.94$, IL-10: $p = 0.06$, TNF-α: $p = 0.16$, Ig A = 0.741, salivary IgA = 0.399) suggested that there was no significant publication bias.

After intervention, IL-6, IL-10, TNF-α, Ig A, and salivary IgA among supplemented group ranged from 0.71 ± 2.93 to 14.1 ± 1.3, from 0.95 ± 1.69 to 131.7 ± 14.0, from 1.15 ± 2.0 to 15.2 ± 1.9, from 123 ± 62 to 527 ± 317, and from 57 ± 29 to 260 ± 183, while in the placebo group it ranged from 1.22 ± 2.1 to 19.7 ± 2.2, from 1.16 ± 1.4 to 84.6 ± 1.8, from 1.66 ± 2.22 to 22.1 ± 2.4, from 128 ± 58 to 606 ± 170, and from 59 ± 32 to 264 ± 182 respectively (Table 1).

The meta-analysis did not show a significant effect of probiotic supplementation on IL-10 and IgA levels, but there was a significant effect of probiotic supplementation on IL-6 ($I^2 = 81.99\%, Q = 22.21, p = 0.001$) and TNF-α ($I^2 = 91.92\%, Q = 49.49, p < 0.000$) (Figures 4 and 5, Table 3).
Discussion

Modulation of the immune system to increase defenses against URTIs is one of the most extensively researched areas in professional sport at present. Our study has shown that probiotic supplementation positively affects both IL-6 and TNF-α levels. After probiotic supplementation, especially when single-strain probiotics were used, the total symptom severity score of respiratory infections was lower than in placebo cases. However, there was no effect of probiotic supplementation on the number of days of illness, the mean number of URTI episodes, the mean duration of URTI symptoms, or on IL-10 and IgA levels (whether in blood or saliva).

Besides the studies used in this meta-analysis, several other RCTs assessing the effects of probiotic supplementation on URTI and cytokine levels in elite athletes have also been published. For example, in the studies of Cox et al.28, Komano et al 29, and Strasser et al. 30, supplementation was associated with a reduction in the number of days, in the severity of respiratory illness, and in the incidence of URTI. The study of Lamprecht et al. 31 found probiotic supplementation to beneficially affected TNF-α, but not IL-6; this was in contrast to Jager et al.32, where probiotic supplementation resulted in an overall decrease in circulating IL-6. Additionally, the IL-10 data from the study of Michalickova et al.16 was excluded from analysis, because the mean value of this parameter was extremely high.

The study of Shing et al.33 found no effects of probiotic supplementation on plasma concentrations of IL-6, IL-10 and TNF-α. Unfortunately, the lack of mean and SD values in this study meant that we could not use their data in our meta-analysis.

It is known that the occurrence of respiratory infections significantly reduces the sports performance of athletes. In looking for the reasons for the development of infections, it was found that one of the basic reasons is the weakening of the effectiveness of both nonspecific and specific humoral immune mechanisms related to mucous membranes. Pyne
et al.\textsuperscript{34} reported that this manifests mainly as a decrease in secreted salivary immunoglobulin A (sIgA); when decreased, this has also been linked to a higher incidence of URTI. Fahlman et al.\textsuperscript{35}, in their one-year observational study, suggested that a season of football training can result in a significant decrease in both s-IgA and the secretion rate of s-IgA, as well as an increase in the incidence of URTI. Attention has been paid to probiotic supplementation because it has been suggested that it can stimulate T-cell immune responses \textit{in vitro}, which would be important, as many studies have shown that athletes have decreased numbers of T cells following intensive anaerobic exercise.\textsuperscript{36} The review by Wosinska et al.\textsuperscript{36} emphasized that \textit{Bacteroides acidifaciens} induces IgA production in murine models and, as a consequence, elevates the production of IgA+ B cells and B cells. These findings are important because IgA plays a pivotal role in upholding intestinal homeostasis, namely by preventing the adherence of pathogens in the intestine. These results may suggest the effectiveness of probiotic supplementation in the prevention of URTI. Unfortunately, our findings are opposite to those of these reviews, showing no significant increases in salivary or blood IgA levels after probiotic treatments, compared to placebo. Due to the limited number of publications, we were unable to perform subgroup analysis by sex, age group, design and duration of intervention, or dose and type of probiotics. These factors may also have affected our findings.

While recreational and moderate exercise may have anti-inflammatory and immunomodulatory effects, the intense exercise of elite athletes can induce inflammation through the synthesis and release of many cytokines (IL-6, IL-1beta, MIP-1alpha, IL-8, TNF-\alpha, IL-10, IL-1RA).\textsuperscript{37} A recent meta-analysis that included adults with diabetes revealed that probiotic supplementation lowers serum CRP and TNF-\alpha, and increased NO levels, but did not affect IL-6 levels.\textsuperscript{38} However Milajerdi et al.\textsuperscript{39} showed that probiotic supplementation significantly reduced serum concentrations of pro-inflammatory cytokines, including IL-6,
IL-12, IL-4 hs-CRP, and TNF-α, but did not affect IL-1B, IL-8, IFN-g, or IL-17 concentrations. Nazari et al. in their meta-analysis found that probiotic consumption resulted in a significant decrease in plasma concentrations of IL-6 and TNF-α, without significant increases in interferon-γ (SMD = 0.43; 95% CI: 0.09 to 0.76; P = .012). In the meta-analysis of Milajerdi et al., a significant increase in serum concentrations of IL-10 as an anti-inflammatory cytokine was also documented after probiotic supplementation. Similar results were found in our meta-analysis, showing that elite athletes had lower levels of TNF-α and IL-6 after probiotic supplementation; however, unlike the findings of Milajerdi et al., there were no significant changes in IL-10 concentration.

The beneficial effects of probiotic supplementation on IL-6 and TNF-α level are particularly important for athletes, as an increase in IL-6 secretions is one of the causes of inflammatory, fatigue, pain, mood changes, and concentration disorders. It has been observed that physical exercise induces muscle damage and a complex cascade of nonspecific inflammatory responses. IL-6 mRNA is detectable in skeletal muscle after prolonged, intense exercise, indicating that IL-6 is produced locally in the skeletal muscle. The increases in plasma TNF-α concentration after exercise also suggest a source in damaged muscles. The increase in the cytokine level may be associated not only with a decrease in the athlete’s sports performance, but also with changes in immune health and increased incidence of URTIs. Probiotics can help regulate inflammation in a number of ways, improving the structure and function of intestinal epithelial barriers. Probiotics and some of their secreted metabolic products can act as ligands for innate immune system receptors, directly affecting key proinflammatory pathways. As has been observed, probiotic single-strain and multispecies probiotic supplementation activates the T- and B-lymphocytes, and subsequently increase the production of important regulatory cytokines, including interleukin-10 (IL-10).
In our analysis, it can also be suggested that probiotic supplementation had a positive effect on total symptom severity score. The multistrain probiotic formulation could increase the chance of adhesion and colonization of the host by the probiotic strain. If the strains are compatible with each other, they confer synergetic effects. But in this case, the positive effect of probiotic supplementation on total symptom severity score was mainly affected by single-strain supplements. There were no significant differences in the number of days of illness, the mean number of URTI episodes, or the mean duration of URTI symptoms.

To the best of our knowledge, this is the first meta-analysis to summarize the effects of oral probiotic supplementation on respiratory tract infection and inflammatory cytokines in elite athletes. Nazari et al. \(^{40}\) made an attempt to assess the effects of probiotic consumption on inflammatory markers, but their meta-analysis also included studies with sedentary subjects and nonprofessional athletes. \(^{46,47}\) Besides, the strengths of our meta-analysis include its comprehensive literature search, the specified inclusion and exclusion criteria for the studies, the explicit methods of data extraction, the measures taken to reduce the influence of bias, and the assessment of heterogeneity. Our work also has some limitations. Firstly, as mentioned above, there were few publications that could be included in the meta-analysis. Secondly, there were many variations between the studies, including methodology, duration of intervention, sport disciplines, type of training and follow-up, probiotic formulation, doses, and duration of the intervention, formulation of probiotics, and the ability of the strains.

In some studies, supplements were given in form of probiotic capsules, while others employed beverages and probiotic sachets. Meybodi et al. \(^{48}\) suggested that supplements are able to transfer high numbers of viable probiotics into the gastrodigestive tract without much loss during storage. In contrast, the viable number of probiotics in food products, especially fermented types, can considerably decrease, but foods provide their matrix protection to
probiotics. Unfortunately, there are still too few studies to determine the best type of probiotic supplement.

Conclusion

This meta-analysis has provided evidence that probiotic supplementation, especially among professional athletes, is an effective way to decrease the total symptom severity score of URTI, especially when this supplementation was taken as single-strain probiotic. Additionally, probiotic supplementation may decrease TNF-α and IL-6 levels. There is a need for more studies with larger groups to better estimate this effect. Moreover, it is necessary to estimate the optimum timing, duration, composition, and dose of such supplementation.
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Legends to figures

Figure 1. The search process

Supplementary table 1a. Newcastle–Ottawa Quality Assessment Scale: assessment form for cohort studies

Table 1. Characteristics of studies and population (n = 1309)

Figure 2. Effects of probiotic supplementation on total symptom severity score in URTI.

Figure 3. Effects of probiotic supplementation (single-strain probiotics only) on total symptom severity score in URTI.

Table 2. Analysis of the effect of probiotic supplementation on URTI. Meta-analysis data presented as SMD.

Figure 4. The effect of probiotic supplementation on IL-6 (pg/ml).

Figure 5. The effect of probiotic supplementation on TNF-α (pg/ml).

Table 3. Analysis of the effects of probiotic supplementation on cytokine levels. Meta-analysis data presented as SMD.
Table 1. Summary of studies of probiotics for URTI and cytokines concentrations in comparison with control (n = 1309)

| Study                  | Type of study | Population (n), sex (% male), age | Intervention (dose, source, duration) | Control | Main outcome | Inter group differences | Adverse effect |
|------------------------|---------------|----------------------------------|--------------------------------------|---------|--------------|------------------------|----------------|
| Gill et al. [2016]     | crossover     | Runners (male) PRO: n = 8; PLA: n = 8; ALL: 26 (9) | beverage containing 1 × 10⁹ Lactobacillus casei 1 week | beverage without Lactobacillus casei | IgA (mg/L) | 527(317), 505(170) | 260(183), 264(182) | - |
| Gleeson et al. [2011]  | parallel      | Runners, cyclists, swimmers, triathletes, racket sports and team gamers (man and females) PRO: n = 32; 32 (14); PLA: n = 26; 25 (9) | drink containing 6.5 × 10⁷ Lactobacillus casei Shirota 16 weeks | drink without Lactobacillus casei Shirota | the mean number of URTI episodes (n) total symptom-severity score mean duration of URTI symptoms (days) | 1.3(2.3), 1.4 (1.5) | 1.5 (2.3), 1.4 (1.5) | - |
| Gleeson et al. [2012]  | parallel      | Runners, cyclists, swimmers, triathletes, racket sports and team gamers (man and females) PRO: n = 27; 25 (5); PLA: n = 27; 24 (4) | capsules with 2 × 10⁹ CFU of Lactobacillus salivarius CFU, 1.78 maltodextrin and 0.01 g magnesium stearate 16 weeks | 0.78 maltodextrin and 0.01 g magnesium stearate | the mean number of URTI episodes (n) total symptom-severity score mean duration of URTI symptoms (days) | 1.2 (4.9), 4.9 (1.5) | 127(101), 143 (95) | - |
| Gleeson et al. [2016]  | parallel      | Runners, cyclists, swimmers, triathletes and team gamers (man and females) PRO: n = 126 (58%); 20.3 (0.2); PLA: n = 117 (59%); 20.6 (0.2) | PRO: drink with 6.5 × 10⁷ Lactobacillus casei Shirota 20 weeks | drink without Lactobacillus casei Shirota | the mean number of URTI episodes (n) total symptom-severity score mean duration of URTI symptoms (days) | 0.7 (01), 0.6 (01) | 62(5), 64(6) | - |
| Haywood et al. [2014]  | parallel      | Elite rugby union players PRO: n = 30 PLA: n = 30 ALL: 24.7 (3.6) | capsules with Lactobacillus gasseri: 2.6 billion colony-forming units (CFU) Bifidobacterium bifidum: 0.2 billion organisms, Bifidobacterium longum: 0.2 billion organisms 4 weeks | capsules without probiotic bacteria | Number of days of illness total symptom-severity score mean duration of URTI symptoms (days) | 3.4 (4.6), 5.8 (6.6) | 8.7(25.1), 13.33 (14.6) | - |
| Huang et al. [2019]    | parallel      | Male triathlon teams Study I PRO: n = 9; 19–24 PLA: n = 9; 19–21 | capsules with 1.5 × 10⁸ Lactobacillus plantarum PS123 CFU and 100 mg exipient of microcrystalline cellulose 12 weeks | 400 mg exipient of microcrystalline cellulose | IL – 6 (pg/ml) | 6.6(0.7), 9.3(1.2) | 9.4(1.9), 8.7(1.1) | - |
| Kekkonen et al. [2007] | parallel      | Marathon runners (male and female) PRO: n = 61; 40 (22–58) PLA: n = 58; 40 (23–69) | 3 × 10⁹ Lactobacillus rhamnosus LGG CFU/milk-based fruit drink or 5 × 10⁹ CFU/capsule 12 weeks | milk-based fruit drink or capsule without probiotic bacteria | IL – 6 (pg/ml) | 14.1 (1.9), 18.2 (2.4) | 13.1 (7.4), 84.6 (1.8) | - |
| Khani et al. [2018]    | parallel      | Male sprint athletes PRO: n = 53 PLA: n = 3 ALL: 21 (3) | 2 × 10⁷ Bifidobacterium bifidum (BIB2) CFU 12 weeks | fruit juices without probiotic bacteria | IgA (mg/L) | 5.3(6.8), 3.9(5.9) | 7.9(1.7), 6.3(4.3) | NR |
| Michalickova et al. [2016] | parallel     | Badmintonists, swimmers, cyclists, alpinists, athletes, 2 × 10⁹ CFU of Lactobacillus helveticus Lafti L10 1% magnesium stearate and 99% | | | the mean number of URTI episodes (n) total symptom-severity score | 4.92(1.96), 6.91(12.2) | 110.92(96), 127.93(40.33) | NR |
| Study                      | Parallel | Participants | Probiotics                                      | Duration | Outcomes                                                                 |
|----------------------------|----------|--------------|-------------------------------------------------|----------|--------------------------------------------------------------------------|
| Pugh et al. [2019]         | parallel | Runners (male and female) | 25 billion *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Bifidobacterium animalis subp. Lactis* CFUs | 4 weeks  | Mean duration of URTI symptoms (days): IL – 10 (pg/ml) 3.5(6.6), 7.4(10.3) 365.2(75.07), 434.8(71.51) |
| West et al. [2011]         | parallel | Cyclists and triathletes (male) | 1 × 10^10 *Lactobacillus fermentum* (VRI-003) CFU | 11 weeks | Microcrystalline cellulose number of days of illness: 3.70(0.72), 3.26(1.9) 0.60(0.9), 0.10(1.5) 1.23(0.36), 1.65(0.57) 3.5(6.7), 3.4(10.3) 0.92(2.14), 1.22(2.1) 0.95(1.09), 1.16(1.44) 1.27(2.02), 1.66(2.22) |
| Townsend et al. [2018]     | parallel | Male baseball players | 1.2 billion *Bacillus subtilis* CFU/capsule | 12 weeks | IL – 10 (pg/ml) 2.89(1.08), 3.27(1.02) 2.07(0.76), 2.78(0.95) IgA (ng/mL) 176.6(86.6), 156.1(98.3) |
| West et al. [2014]         | parallel | Cyclists and triathletes (female) | 2 × 10^8 *Bifidobacterium animalis subsp. lactis* B‡04 CFU | 11 weeks | Sucrose base without the probiotic bacteria number of days of illness: 4.4(1.3), 4.3(1.9), 4.4(1.6) 6.1(3.3), 6.2(4.8), 5.7(3.6) |

**Notes:** PRO: probiotic group; PLA: placebo group; Mean (SD): mean (standard deviation); URTI: upper respiratory tract infections; CFU: colony-forming unit; NR: not reported;
Table 2. Analysis of the effect of probiotic supplementation on URTI.

| Subgroup                          | A meta-analysis (95% CI) | I² (%) | Q (%) | P-value | Number of studies |
|-----------------------------------|--------------------------|--------|-------|---------|------------------|
| Total symptom severity score     |                          |        |       |         |                  |
| ➔ single-strain                   | -0.65 (-1.05 -0.25)      | 27.21  | 6.87  | 0.02    | 5                |
|                                   | -0.64 (-1.04 -0.24)      | 36.18  | 6.27  | 0.02    | 4                |
| Number of days of illness        |                          |        |       |         |                  |
| ➔ single-strain                   | 0.04 (-0.49 0.58)        | 65.97  | 14.69 | 0.876   | 4                |
| ➔ multistrain                     | 0.25 (-0.17 0.59)        |        |       |         |                  |
|                                   | 0.21 (-0.18 0.60)        |        |       |         |                  |
| Number of URTI episodes (only single-strain) | -0.21 (-0.61 0.19) | 82.48  | 34.25 | 0.30    | 5                |
| Duration of URTI symptoms        |                          |        |       |         |                  |
| ➔ single-strain                   | -0.20 (-0.95 0.56)       | 65.87  | 23.44 | 0.61    | 7                |
|                                   | -0.51 (-2.04 1.02)       |        |       |         |                  |
| ➔ multistrain                     | 0.1 (-0.91 1.11)         |        |       |         |                  |

URTI: upper respiratory tract infections.
Table 3. Analysis of the effects of probiotic supplementation on cytokine levels.

| Parameters   | A meta-analysis (95% CI) | $I^2$ (%) | $Q$ (%) | P-value | Number of studies |
|--------------|--------------------------|-----------|---------|---------|-------------------|
| TNF-α (pg/ml)| -2.31 (-4.12 -0.51)     | 91.92     | 49.49   | 0.01    | 3                 |
| IL-6 (pg/ml) | -2.52 (-4.39 -0.66)     | 81.99     | 22.21   | 0.008   | 3                 |
| IL-10 (pg/ml)| 2.08 (-0.37 4.52)       | 94.57     | 92.06   | 0.096   | 4                 |
| IgA (mg/L)   | 9.34 (-14.59 33.28)     | 1.1       | 5.056   | 0.444   | 4                 |
| IgA (µg/min) | -0.6 (-13.15 11.95)     | 0         | 0.356   | 0.926   | 3                 |
Figure 1. The search process
**Figure 2.** Effects of probiotic supplementation on total symptom severity score in URTI.
**Figure 3.** Effects of probiotic supplementation (single-strain probiotics only) on total symptom severity score in URTI.
The effect of probiotic supplementation on IL-6

| Study                  | D   | (PU d.) | (PU g.) | Weight % |
|------------------------|-----|---------|---------|----------|
| Huang et al. [2019] I  | -2.70| -3.61   | -1.79   | 26.79%   |
| Huang et al. [2019] II | -5.60| -7.37   | -3.83   | 22.74%   |
| Pugh et al. [2019]     | -2.62| -11.17  | 5.93    | 4.09%    |
| West et al. [2011] women | -1.58| -3.39   | 0.37    | 21.77%   |
| West et al. [2011] man  | -0.30| -1.70   | 1.10    | 24.61%   |
| **Total (95% CI)**     | -2.52| -4.39   | -0.66   | 100.00%  |

**Figure 4.** The effect of probiotic supplementation on IL-6 (pg/ml).
The effect of probiotic supplementation on TNF-α

| Study                     | D   | (PU d.) | (PU g.) | Weight % |
|---------------------------|-----|---------|---------|----------|
| Huang et al. [2019] I     | -3.90 | (-5.19) | (-2.61) | 20.12%   |
| Huang et al. [2019] II    | -6.90 | (-9.02) | (-4.78) | 17.12%   |
| Townsend et al. [2018]    | -0.71 | (-1.39) | (-0.03) | 21.73%   |
| West et al. [2011] man    | -0.39 | (-1.45) | 0.67)   | 20.82%   |
| West et al. [2011] female | -0.56 | (-1.62) | 0.70)   | 20.21%   |
| Total (95% CI)            | -2.31 | (-4.12) | (-0.51) | 100.00%  |

**Figure 5.** The effect of probiotic supplementation on TNF-α (pg/ml).
**Supplementary table 1a.** Assessment of risk of bias in included studies

| No. | Authors.                  | Random sequence generation | Allocation concealment | Blinding of participants and personnel | Blinding of outcome assessment | Incomplete outcome data | Selective reporting | Other biases |
|-----|---------------------------|----------------------------|------------------------|----------------------------------------|-------------------------------|------------------------|---------------------|--------------|
| 1   | Gill et al. [2016]        | L                          | U                      | L                                      | L                             | L                      | U                   | U            |
| 2   | Gleeson et al. [2011]     | L                          | L                      | L                                      | L                             | L                      | U                   | U            |
| 3   | Gleeson et al. [2012]     | U                          | L                      | L                                      | L                             | L                      | U                   | L            |
| 4   | Gleeson et al. [2016]     | L                          | L                      | L                                      | L                             | L                      | L                   | L            |
| 5   | Haywood et al. [2014]     | U                          | L                      | L                                      | L                             | U                      | L                   | L            |
| 6   | Huang et al. [2019]       | L                          | L                      | L                                      | L                             | L                      | U                   | L            |
| 7   | Kekkonen et al. [2007]    | U                          | U                      | U                                      | U                             | L                      | L                   | L            |
| 8   | Khani et al. [2019]       | U                          | U                      | U                                      | U                             | U                      | U                   | U            |
| 9   | Michalickova et al. [2016]| L                          | L                      | L                                      | L                             | L                      | U                   | U            |
| 10  | Pugh et al. [2019]        | L                          | L                      | L                                      | U                             | U                      | U                   | U            |
| 11  | Pumpa et al. [2019]       | L                          | L                      | L                                      | L                             | L                      | L                   | U            |
| 12  | Townsend et al. [2018]    | L                          | L                      | L                                      | U                             | U                      | L                   | L            |
| 13  | West et al. [2011]        | U                          | L                      | L                                      | L                             | L                      | U                   | L            |
| 14  | West et al. [2014]        | L                          | U                      | U                                      | U                             | U                      | U                   | U            |

L, low risk; U, unclear risk; H, high risk based on the Cochrane Collaboration's tool for assessing risk of bias qualitatively.