Recent advances in clinical probiotic research for sport

Ralf Jäger\textsuperscript{a}, Alex E. Mohr\textsuperscript{b}, and Jamie N. Pugh\textsuperscript{c}

Purpose of review
This is a review of the most up-to-date research on the effectiveness of probiotic supplementation for outcomes related to athletes and physical activity. The focus is on clinical research incorporating exercise and/or physically active participants on the nutritional effectiveness of single and multistrain preparations.

Recent findings
Findings of the included clinical studies support the notion that certain probiotics could play important roles in maintaining normal physiology and energy production during exercise which may lead to performance-improvement and antifatigue effects, improve exercise-induced gastrointestinal symptoms and permeability, stimulate/modulate of the immune system, and improve the ability to digest, absorb, and metabolize macro and micronutrients important to exercise performance and recovery/health status of those physically active.

Summary
The current body of literature highlights the specificity of probiotic strain/dose and potential mechanisms of action for application in sport. These novel findings open new areas research, potential use for human health, and reinforce the potential role for probiotic’s in exercise performance. While encouraging, more well designed studies of probiotic supplementation in various sport applications are warranted.

Keywords
exercise, gut microbiome, physical activity, probiotic, sport

INTRODUCTION
In humans, the effects of probiotics in relation to exercise has been less described in comparison with clinical conditions and sedentary populations, and even less so when considering athletic populations. However, the body of probiotic research in physically active individuals and competitive athletes is expanding, including investigations in gastrointestinal health, exercise performance, recovery, physical fatigue, immunity, and body composition [1\textsuperscript{**}]. Probiotic preparations comprise live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [2]. The beneficial effect of probiotic supplementation profoundly relies on strain, dose, duration, form, and host physiology as well as the target population and the outcome of interest [3]. As such, recommendations for probiotics should consider all of these factors and benefits from specific studies should not lead to general conclusions for all probiotic products.

Probiotics are available commercially in capsule or tablet forms, as powder sachets, in the form of liquids, and in specific foods such as yogurt and nutrition bars. Commonly used probiotic strains for the application of exercise include \textit{Lactobacillus}, \textit{Bifidobacterium}, and \textit{Bacillus} genera, however, new microbiome research and technological advances are identifying potential next-generation probiotic candidates [4]. In the context of exercise, and especially athletes, the present body of literature suggests their microbiota has several key differences in comparison with other populations, likely driven, in part, by exercise and diet [5\textsuperscript{*}]. These characteristics may influence the effects of probiotics on the
Despite the existence of shared, core mechanisms for probiotic function, many health benefits of probiotics are strain-dependent and dose-dependent.

The main function of the gut is to digest food and absorb nutrients. In athletic populations, certain probiotics strains can increase absorption of key nutrients such as amino acids from protein.

Certain probiotic strains have been linked to maintain immune and gastrointestinal health compromised due to strenuous exercise.

Recent studies indicate potential benefits of certain probiotic strains on athletic performance and recovery from exercise through different mechanism-of-action, including the management of exercise-induced inflammation.

Dose ranging studies to determine minimum effective and optimal dose, studies evaluating the minimum duration of supplementation to see a benefit, and studies comparing sedentary, recreationally active and professional athletes are currently lacking.

The current article reviews the most up-to-date clinical research on probiotic supplementation in relation to exercise, published in 2019–2020 (Table 1). Key concepts explored include the effectiveness of probiotic supplementation on performance and exercise adaptation, gastrointestinal and immune health associated with exercise, and nutrient utilization (Fig. 1). Understanding whether probiotic supplementation plays a role in exercise performance and recovery is of interest to those who work to improve their training, competition performance, and health. Moreover, this knowledge may be of general benefit to human health and is important for clinicians, consumers, industry, and regulating bodies alike.

**Effectiveness of probiotic supplementation on performance and exercise adaptation**

Although considered for their role in gastrointestinal health, probiotics have more recently been studied for their potential ergogenic effects on exercise performance. In a 6-week double-blind placebo-controlled clinical study, young healthy amateur runners supplemented with either a low [3 × 10^{10} colony forming units (CFU)/day] or high [9 × 10^{10}CFU/day] daily dose *Lactobacillus plantarum* TWK10 and underwent an exhaustive treadmill exercise test [85% maximal volume of oxygen utilization (VO2MAX)] with performance measurements and related biochemical indexes [6]. Both probiotic groups had significant improvements in endurance performance, compared placebo, with greater effects in the high-dose group. There was also an increase in serum glucose concentrations during the maximal treadmill running test in the high-dose group, indicating that *L. plantarum* TWK10 supplementation may be beneficial for energy harvest during exercise.

While research examining exercise performance has been addressed in previous works, little has been done in terms of physiological stress and adaptation of exercise. To explore these features, Huang et al. [7**] investigated the effects of *L. plantarum* PS128 supplementation (3 × 10^{10}CFU/day), in triathletes. *L. plantarum* PS128 supplementation, combined with training, significantly alleviated circulating markers of oxidative stress (including thioredoxin and myeloperoxidase indices) and positively modulated the inflammatory response (6–13% decrease: TNF-α, IL-6, and IL-8; 55% increase in IL-10). In addition, *L. plantarum* PS128 substantially increased plasma branched-chain amino acids (BCAA; 24–69%; $P \leq 0.05$) and elevated exercise performance (i.e., 30 s Wingate test and VO2MAX endurance test; $P \leq 0.05$), as compared with placebo. BCAA’s have been reported to play a role in fatigue reduction in endurance exercise and are important for homeostasis of muscle energy metabolism and energy index for adaptation to exercise training [8]. Together, these studies suggest a role in which certain probiotic strains may enhance energy harvesting, and have health-promotion, performance-improvement, and antifatigue effects. Such benefits may be mediated by metabolic products from the microbiota as a result of supplementation with certain probiotic strains, although further research is warranted.

**Effectiveness of probiotic supplementation on gastrointestinal health associated with exercise**

The terms ‘gastrointestinal health’ or ‘gut health’ have been used increasingly in both the scientific literature and food industry. However, given the varied and far reaching functions of the gastrointestinal tract, it is difficult to discuss or study gastrointestinal health completely. Rather, probiotic strains have been used to assess their efficacy in a variety of gastrointestinal health-related outcomes such as symptoms of gastrointestinal distress or acute illness, microbiome changes, and indirect markers of barrier function. One such issue that is often faced by those who participate in endurance training is

resident microbiome and host physiology, as well as consideration for probiotic application.
| Reference             | Subject characteristics a | Probiotic/delivery form | Duration | Exercise                                                                 | Design                      | Outcome                                                                 |
|-----------------------|----------------------------|-------------------------|----------|---------------------------------------------------------------------------|----------------------------|-------------------------------------------------------------------------|
| Axelrod et al. [10]   | Endurance trained athletes (VO2MAX: ≥50 ml/kg/min); 31.0 ± 2.3; n = 7 USA | *Lactobacillus salivarius* UCC118: 2 × 10^9 CFU daily Capsule | 4 Weeks  | Strenuous treadmill running performed before and after each supplementation period to induce GI hyperpermeability | Double-blind RCT, crossover | Attenuated exercise-induced intestinal hyperpermeability, via intestinal permeability of sucrose |
| Huang et al. [7]      | Healthy adult male triathletes; Study 1: 18, 20.2 ± 0.7 years Study 2: 16, 22.3 ± 1.2 years; n = 34 Taiwan | *Lactobacillus plantarum* PS128 3 × 10^10 CFU daily Capsule | Study 1: 4 weeks Study 2: 3 weeks | Sprint triathlon (swimming 750 m, biking 20 km, running 5 km) | Double-blind RCT, parallel-group | Attenuated posttriathlon performance declines Reduced postrace inflammatory cytokines, reduced oxidative stress, increased plasma BCAA levels |
| Huang et al. [6]      | Healthy adults; 20–30 years; n = 54 Taiwan | *L. plantarum* TWK10 Placebo, low (3 × 10^10 CFU), and high-dose (9 × 10^10 CFU) TWK10 administration groups (n = 18 per group, with equal sexes), daily | 6 Weeks | Exhaustive treadmill exercise [85% VO2MAX] | Double-blind RCT, parallel-group, dose–response trial | TWK10 significantly elevated the exercise performance in a dose-dependent manner and improved the fatigue-associated features correlated with better physiological adaptation. TWK10 administration improved body composition |
| Pugh et al. [9]       | Healthy adult male and female marathon runners (ran marathon race quicker than 5 h within the previous 2 years); 34.8 ± 6.9 years; n = 24 UK | *Lactobacillus acidophilus* (CUL60 and CUL21), *Bifidobacterium bifidum* (CUL20), *Bifidobacterium animalis* ssp. lactis (CUL34) > 25 × 10^6 CFU daily in total, no information on individual strains, daily Capsule | 4 Weeks (prerace) | Marathon race | Double-blind RCT, parallel-group | Lower incidence and severity of GI symptoms No difference in race times |
| Pumpa et al. [12]     | Elite male rugby union athletes; 27.0 ± 3.2 years; n = 19 Australia | *Lactobacillus rhamnosus,* *Lactobacillus casei,* *L. acidophilus,* *L. plantarum,* *L. fermentum,* B. lactis, B. bifidum, S. thermophilus 120 × 10^6 CFU, no information on individual strains 500 mg Saccharomyces boulardii (added during stage 3), daily Capsule | 17 Weeks | 27-Weeks, divided into three stages: first, control period (10 weeks); second, domestic competition (7 weeks); third, international competition (10 weeks) | Double-blind RCT, parallel-group | No effect on salivary IgA. Salivary cortisol increased. Increase in salivary alpha-amylase levels during stage 3 |
| Reference                       | Subject characteristics | Probiotic/delivery form | Duration | Exercise                    | Design                  | Outcome                                                                 |
|--------------------------------|-------------------------|-------------------------|----------|-----------------------------|-------------------------|--------------------------------------------------------------------------|
| Vaisberg et al. [16*]           | Amateur marathon runners with previous history of postrace URTI; 39.5 ± 9.4 years; n = 42 | L. casei Shirata, 4 × 10^10 CFU, daily Fermented milk beverage | 30 Days (prerace) | Marathon race               | Double-blind RCT, parallel-group | Improved airway and systemic immune and inflammatory responses postmarathon. No significant effect on URTI |
| Axling et al. [22*]              | Female iron-deficient athletes; Control: 21.6 ± 6.0; Treatment: 22.3 ± 3.5; n = 53 | L. plantarum 299v 1 × 10^10 and 20 mg of iron (ferrous-fumarate) compared with 20 mg of iron alone, daily | 12 Weeks | Ergometer cycling test      | Double-blind RCT, parallel-group | Profile of Mood States questionnaire showed increased vigor with Lp299v vs. iron alone after 12 weeks (3.5 vs. 0.1, P = 0.015) Lp299v, together with 20 mg of iron, could result in a more substantial and rapid improvement in iron status and improved vigor compared with 20 mg of iron alone |
| Jäger et al. [19]                | Physically active males; 24.2 ± 5.0 years; n = 15 | 5 × 10^9 CFU Lactobacillus paracasei LPDG & 5 × 10^5 CFU L. paracasei LPCS01, coingested with 20 g pea protein, daily | 2 Weeks | Recreationally active       | Double-blind RCT, crossover | Increased methionine, histidine, valine, leucine, isoleucine, tyrosine, total BCAA, and total EAA maximum concentrations and area under the curve |
| Pugh et al. [18]                | Male cyclists; 23 ± 4 years; n = 7 | Multistrain probiotic: L. acidophilus CUI60, L. acidophilus CUI21, B. bifidum CUL20, and B. animalis ssp. lactis CUI34, 25 × 10^9 CFU, daily | 4 Weeks | 2-h cycling challenge [55% maximal aerobic power output] | Double-blind RCT, crossover | Small increases in absorption and oxidation of the ingested maltodextrin and small reductions in fat oxidation (P < 0.038) No effect on time-trial performance |

GI, gastrointestinal; RCT, randomized controlled trial; URTI, upper respiratory tract infection.

*aAge reported as mean ± SD. When unavailable, age range was reported.*
exercise-induced gastrointestinal distress. Although it is generally accepted that a reduction in splanchnic blood flow is one of the main factors precipitating exercise-induced gastrointestinal symptoms, there are many other elements that contribute to different symptoms (e.g., nausea, vomiting, bloating, and diarrhea). To evaluate the effects of probiotic supplementation on gastrointestinal symptoms, circulatory markers of gastrointestinal permeability, damage, and markers of immune response during a marathon race, 24 recreational runners were randomly assigned to either supplement with a multistrain probiotic consisting of *Lactobacillus acidophilus* (CUL60 and CUL21), *Bifidobacterium bifidum* CUL20, and *Bifidobacterium animalis* ssp. Lactis (totaling $2.5 \times 10^9$ CFU) or placebo for 28 days prior to a marathon race [9]. Although there were no differences in race finish times between probiotic and placebo groups, those in the probiotic supplement group were better able to maintain their running velocity in the final stages of the race, compared with placebo. Of note, for all participants, a significant correlation was found between reductions in running velocity during the final third of the race and the severity of subjective gastrointestinal symptoms. Supplementation of probiotics, though, resulted in fewer and less severe gastrointestinal symptoms, both in training and during a marathon race using standardized carbohydrate and hydration strategies. In contrast, probiotic supplementation had no effect on sCD14, IL-6, IL-8, IL-10, cortisol, or I-FABP concentrations or gastrointestinal permeability, which all increased postmarathon race. As such, the exact mechanism by which gastrointestinal symptoms were attenuated could not be identified, further highlighting the multifaceted nature of such distress. However, while gastrointestinal permeability and barrier function are not always indicated with functional exercise-associated gastrointestinal symptoms, this does

**FIGURE 1.** Supplementation with certain probiotic strains has been shown to increase gastrointestinal and immune health, improve nutrient absorption, speed up recovery, and improve athletic performance (illustration by Stephen Somers, Milwaukee, Wisconsin, USA).
not preclude the potential importance of limiting gastrointestinal barrier disruption during exercise and the therapeutic role probiotics may play.

To assess the efficacy of *Lactobacillus salivarius* UCC118 (2 x 10⁸ CFU/day) on exercise-induced gastrointestinal permeability and the gut microbiome in healthy adults, Axelrod et al. [10⁴⁴], performed a randomized, double-blind, placebo-controlled crossover study on seven healthy, endurance trained athletes with 4-week treatment periods. Athletes undertook strenuous treadmill running performed before and after each supplementation period and urine recovery of lactulose, rhamnose, and sucrose was used to assess gastrointestinal permeability. *L. salivarius* UCC118 significantly reduced sucrose (Δ = 38 ± 13% vs. 169 ± 79%; P < 0.05) recovery, with no substantial change in lactulose or rhamnose. Shotgun metagenomic sequencing of fecal samples revealed that *L. salivarius* UCC118 supplementation appeared to remodel the gut microbiome with 99 differentially regulated microorganisms, including a significant reduction in the phylum *Verrucomicrobia*. This apparent remodeling of the gut microbiome, not often observed in clinical probiotic studies [11], may be suggestive of protective effects of *L. salivarius* UCC118 by orchestrating changes in the gut’s resident microorganisms.

**Effectiveness of probiotic supplementation on immune health associated with exercise**

Of particular relevance to those that exercise (particularly athletes) is the reduction in incidence and/or severity and duration of symptoms from illnesses like upper respiratory tract infections (URTIs). Certain probiotics may offer a proactive approach for preventing these types of illnesses which can minimize training days lost, and in turn enhance exercise performance. This is especially true for those that travel, who may experience higher levels of stress, increased risk of transmission of illness, and circadian disfunction. In a 27-week, double blind randomized controlled trial, a small group of rugby athletes were assigned a high-dose multistrain probiotic (120 x 10⁹ CFU) or placebo group during control, domestic competition, and international competition periods [12⁴⁴]. During the final period, there was a significant increase in salivary alpha-amylase (marker of mucosal immune response) in those consuming the probiotic [13]. The mechanism on how the probiotic may have stimulated this increase was not explored in the current study, however, orally administered probiotics may interact with the approximately 200 m² of gastrointestinal mucosa and gut associated lymphoid tissue where more than 70% of immune cells are localized [14]. It is therefore not surprising that many of the recognized health benefits of probiotics are conferred mainly through stimulation/modulation of the immune system [15].

The mucosal lining of the gastrointestinal tract represents the first line-of-defense against invading pathogens and is an important interface with the host immune system. Exhaustive physical exercise negatively impacts immunity, reducing of the count and function of immune cells, as well as altering the inflammation-responsive, including pro/anti-inflammatory cytokines. Assessing these immune and inflammatory responses, Vaisberg and colleagues had 42 male marathon runners with previous history of postrace URTI ingest a fermented milk containing *Lactobacillus casei* Shirota (4 x 10¹⁰ CFU/ day) or placebo 30 days prior to a marathon [16⁴⁴]. Supplementation resulted in improved systemic and airways immune responses, including reduced neutrophil infiltration in the nasal mucosa and modulation of pro and anti-inflammatory cytokine response in the upper airway (i.e., decreased IL-6, IL-13, TNF-α, IL-5, & IL-1β; increased IL-10, as compared with placebo). These results demonstrated that the daily intake of the probiotic was able to induce an anti-inflammatory response that can mitigate the deleterious effects of marathon on the mucosal inflammation.

Although the current body of literature is weighted in favor of probiotics ability to reduce the incidence of URTIs and related symptoms, there is a large number of differing strains used with likely narrowly distributed mechanisms [1⁴⁴⁴⁴]. Moreover, the immune response is complex and not easily measured, exemplified by the large array of measurable immune cells, cytokines, and chemokines. The two studies reviewed above have taken important and necessary steps to investigate both URTI incidence and symptomology, as well as multiple markers of the immune response. Future work should continue this trend to better understand how different probiotic strains may affect immunity.

**Effectiveness of probiotic supplementation on nutrient absorption and utilization associated with exercise**

More recent work has aimed to assess various probiotic strains for their ability to digest, absorb, and metabolize nutrients important to exercise performance and recovery/health status of those physically active. For example, maintaining adequate carbohydrate availability for skeletal muscle and the central nervous system during longer duration exercise is well noted to improve performance and
delay fatigue [17]. However, absorption of ingested large amounts of carbohydrate during exercise is limited by transport systems within the gut. To test whether a probiotic could increase the absorption and oxidation of maltodextrin, Pugh and colleagues had a small group of trained male cyclists perform a 2-h cycling challenge before and after 4 weeks of supplementation with a multistrain probiotic (25 × 10⁹ CFU) or placebo using a double blind, randomized controlled, crossover design [18]. Probiotic supplementation led to a small but significant increase in total carbohydrate oxidation in the 60–120-min exercise periods and the oxidation of the consumed maltodextrin drink, as well as significant increases in the plasma glucose and insulin concentration. However, there were minimal increases in the absorption of the ingested maltodextrin and no effect on subsequent time-trial performance. Although this initial data highlights the potential of probiotics to increase absorption and oxidation of consumed carbohydrates and subsequent exercise metabolism, further studies are needed to replicate these findings.

Probiotics have also been linked to improved protein utilization [19], potentially through optimizing gut microbiota composition and increased proteolytic activity. Different types and quality of dietary protein can affect amino acid absorption following protein supplementation. Compared with animal protein sources, plant protein sources are generally incomplete proteins, contain less BCAAs, and differ in the absorption kinetics and the amount of amino acids absorbed by the host, factors known to affect adaption effects important to athletes such as skeletal muscle protein synthesis. Therefore, there is an interest in nutritional strategies to raise the blood amino acid concentrations after ingesting a plant protein source to overcome compositional shortcomings. To assess the amino acid concentration in the blood after the administration of a plant protein with or without coadministration of a probiotic supplement (5 × 10⁹ CFU Lactobacillus paracasei LP-DG and 5 × 10⁹ L. paracasei LPC-S01) a randomized, double-blind, crossover pilot study was performed in physically active males [20**]. Probiotic administration significantly increased methionine (+16.3%), histidine (+49.2%), valine (+24.7%), leucine (+25.2%), isoleucine (+26.1%), tyrosine (+11.6%), total BCAA (+26.8%), and total essential amino acid (+15.6%) maximum concentrations and area under the curve (Fig. 2). To corroborate these findings, in-vitro data showed increased proteolysis and a synergistic effect of the two strains compared with either strain alone.
for pea protein. The increased amino acids in this study are of key importance to athletes and exercise recovery. This research is also notable because this is some of the first evidence to show that probiotics helps to overcome the compositional shortcomings of plant proteins and could help to elevate BCAA levels in the blood to comparable levels of animal proteins. This would remove the need to increase the dose of plant proteins to achieve similar benefits of protein supplementation on muscle health.

A further area of recent interest in regards to probiotics and nutrient absorption is within the field of inorganic iron supplementation. Iron is crucial for oxygen transport, mitochondrial energy production, and cellular immune responses and has been shown to negatively affect physical performance and adaptation to training when low [21]. Increasing the absorption of iron could be a strategy for improving iron status and avoid the use of traditional high-dose iron supplements and thereby adverse side effects. In a recent double blind, 12 week randomized control trial in nonanemic female athletes with low-iron stores receiving a daily supplement of 20 mg of ferrous fumarate, L. plantarum 299v increased plasma ferritin (iron status), in those with ferritin levels above 20 μg/l at baseline compared with control [22*]. Significantly, those with ferritin levels below 20 μg/l did not differ from each other in terms of the ferritin response (increase). The results indicated that probiotic intake with 20 mg iron (as ferrous fumarate) could result in a more substantial and rapid improvement in iron status compared with iron alone. Further, the beneficial effects of the probiotic on iron utilization (e.g., serum ferritin) from an inorganic source of iron without any change in inflammatory markers are notable. In a recent meta-analysis this strain has been identified as moderately effective (effect size = 0.55, 95% confidence interval 0.22–0.88, P = 0.001) in iron absorption in humans using a variety of methods [e.g., stable iron kinetics, 59Fe whole-body retention and isotope activities in blood samples, double-isotope ([55Fe and 59Fe]) [23].

Currently, research investing the potential of probiotics to aid nutrient metabolism in relation to exercise remains limited. However, the recent positive findings noted above are promising and investigators are encouraged to continue this line of research for both macro and micronutrients. It may be that the exercise associated gut microbiota may be more receptive to improved nutrient utilization as it appears to possess a functional capacity that is primed for tissue repair and a greater ability to harness energy from the diet with increased capacity for carbohydrate, cell structure, and nucleotide biosynthesis [5*].

**CONCLUSION**

Although there has been continual research on probiotic supplementation in the application of sport and exercise, many questions remain concerning mechanisms of action and strain/dose specificity. Results from the included studies in this review are encouraging and open up potential new lines of inquiry.

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**Conflicts of interest**

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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