Effects of Probiotics Supplementation on the Performance and Metabolic Health of Overtraining Athletes

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Abstract Probiotics are live microorganisms that may have health benefits when consumed in adequate amounts. Many athletes suffered from fatigue, gastrointestinal distress, mood disturbances, and underperformance due to overtraining or highly intensive competition. The use of probiotics in sports has been growing in recent years due to various beneficial effects including improvement of muscle endurance and intestinal health, amelioration of symptoms of metabolic disorder, as well as reduction of oxidative stress. Moreover, approximately 70% of the immune system is located in the gut and probiotic supplementation has been shown to promote a healthy immune system and inhibit the growth of harmful gut bacteria. Recent studies showed that probiotics can do more than improve gut health. They also may indirectly help with the depression and mental stress of athletes. Here, we present a comprehensive review of the key beneficial effects of probiotics on athletes and discuss the potential mechanisms.

Keywords: Probiotics, metabolic health, athletes, overtraining, recovery, gut microbiota


1. Introduction

Regular exercise has numerous health benefits, for example, it reduces the risk of cardiovascular disease and cancer. It has also been shown that the risk of type 2 diabetes can be halved in high-risk groups through nonpharmacological lifestyle interventions involving exercise and diet. Nevertheless, uncontrolled excessive exercise may cause many adverse effects. Recent reports have indicated that extreme exercise may induce musculoskeletal injuries, cardiac arrhythmias, and biochemical and histological changes in the liver and pancreas of rats. Overtraining is a condition precipitated by an imbalance between physiological performance, training demand, and current functional capacity. When this delicate balance is disrupted, excessive training (known as overtraining) can exacerbate the drop in performance based on the association between heavy training and skeletal muscle injury. Thus, in an overtrained individual, performance does not increase after adequate rest following training fatigue. Overtraining can put the athlete at risk of developing asthma [1], infection such as upper respiratory illness [2,3], gastrointestinal complaints [4] as well as depression and anxiety [5]. Various other symptoms such as immune suppression and chronic fatigue have also frequently been reported [6]. Various studies have explored the positive relationship between athletes and probiotics, and how they can be used to ease or prevent symptoms associated with overtraining [7].

Probiotics are defined as viable microorganisms that, when ingested in an appropriate concentration, exert various beneficial effects on the host [8]. The word “probiotic” comes from Greek and means “for life.” In 1954, Ferdinand Vergin conceived the term “probiotic” in an article entitled “Anti-und Probiotika,” in which several microorganisms were studied to make a list of useful bacteria and to determine the detrimental effects of antibacterial agents and antibiotics on the intestinal microbiota [9]. As per the current state of knowledge, probiotics encompass species of lactic acid bacteria (LAB) (e.g., Lactococcus, Lactobacillus, Streptococcus, and Enterococcus), Bifidobacterium and yeast genera have a long history of safe use [10]. Probiotics have been utilized as a starter culture to produce fermented foods for centuries. They can be taken directly as a food additive and colonized in the human oral cavity. In the digestive tract, while competing with pathogenic bacteria to use limited nutrients, it can also produce antibacterial substances to inhibit the growth of pathogens, thereby achieving the effects of improving the balance of human intestinal flora and improving human health. Many traditional fermented foods or regional specialty foods, such as kimchi, yogurt, cheese, milk wine, etc., have probiotics.

Particular probiotic strains produce certain vitamins and could benefit the human host, whereas lactase-positive probiotic strains can offer relief from symptoms of lactose
intolerance. Recent studies revealed that probiotics also modulate host immunity, restrict the harmful substances that may exist in the gut, and prevent allergies and cardiovascular or urogenital disorders. They, also, reduce blood cholesterol and prevent from heart diseases. Moreover, it has been identified that probiotics decrease symptoms related to antibiotic consumption. They are reported to reduce important gastrointestinal symptoms at athletes.

Studies have shown that probiotics can improve endurance sports performance by regulating the biological activity of the musculoskeletal of the athletes, and have a positive effect on the metabolism of the musculoskeletal and the repair of injuries. Probiotics can promote the athletes’ energy metabolism, and improve gastrointestinal disorders, oxidative stress, muscle fatigue, and other symptoms, thereby indirectly improving physical performance. At the same time, probiotics can also regulate the neuroendocrine system by affecting the brain-gut axis, which can relieve fatigue, depression, anxiety, and other emotional disorders to a certain extent. This review will summarize the current status of probiotics on athletes and put a special focus on the molecular mechanisms involved. Here, we present a comprehensive review of the application status of probiotics on athletes with a special focus on the molecular mechanisms involved. Finally, future perspectives on this area will be discussed.

2. Probiotics Improve Muscle Endurance

In order to increase athletic performance, the training must be challenging enough to make adaptations to resistance training stimuli. The mechanism of exercise-induced muscle damage includes mechanical and metabolic pathways and the magnitude of damage depends on the type, intensity, and duration of the exercise, as well as the nutritional status.

A large number of human experiments have also confirmed the positive effects of probiotics on skeletal muscle metabolism and damage repair of the exercise body. *Bacillus coagulans* produce digestive enzymes that are active under gut conditions (alkaline proteases, etc.) and these proteases have been shown to digest proteins more efficiently than the endogenous human proteases alone. *Bacillus coagulans* GBI-30, 6086 enhances the health of the cells of the gut lining improving nutrient absorption including minerals, peptides and amino acids by decreasing inflammation and encouraging optimum development of the absorptive area of the villi. The study found that the muscle-damaging exercise resulted in significantly reduced perceived recovery and increased muscle soreness, increased muscle damage and reduced performance (Wingate peak power) in the protein supplemented group. The co-administration of *Bacillus coagulans* GBI-30, 6086 and protein significantly increasing recovery 24 and 72 h, and muscle soreness 72 h after exercise. The addition of the probiotic to the protein tended to reduce muscle damage and prevented the decline in peak power. Strength or vertical jump power did not significantly decline in both groups, indicating that the exercise protocol was not challenging enough to negatively influence those measures.

A study found that *L. plantarum TWK10* supplementation could enhance glucose utilization to increase endurance exercise time by increasing the number of gastrocnemius type I muscle fibers. Researchers provide evidence that *L. plantarum TWK10* affects biochemical features with long-term aerobic swimming. For future investigations, *L. plantarum TWK10* could be used in humans who focus on aerobic endurance training for protective and health purposes [11]. Combinations of probiotic cultures have proven to be more effective than the use of single cultures for improving muscle endurance caused by over-training. Twenty-one days of probiotic supplementation with probiotics containing 5 bn live cells (AFU) *B. breve* BR03 and 5 bn live cells (AFU) *S. thermophilus* FP4 in healthy, resistance-trained men attenuates performance and the range of motion decrements following a bout of muscle-damaging eccentric exercise. Further, 21 days of probiotic supplementation lowered resting IL-6 concentrations, which were sustained to 48 h post-exercise. These data suggest that the specific probiotic cocktail may assist in the recovery of performance following unaccustomed heavy eccentric exercise [12].

3. Probiotics Relieve Metabolic Disorders

It has been recognized that high to extreme amounts of exercise training have been associated with negative effects on cardiac health and metabolic balance [13]. Over-exercising can lead to metabolic changes that can cause symptoms such as bloating, abdominal cramping, diarrhea, nausea, and vomiting are very commonly seen in endurance runners. The body’s metabolism is responsible for converting the nutritional substrates from the dietary intake into fuel for muscle contractions, and into new cells used to repair tissue damaged during exercise. During rest and low-intensity exercise, energy in the form of Adenosine Triphosphate (ATP) is provided through aerobic metabolism. However, the energy for high-intensity exercise is produced by the Phosphagen and Glycolysis energy pathways, which place a significant demand on the involved muscle tissue to rapidly deliver the necessary ATP to fuel muscle activity. Typical metabolic damage caused by over-exercising includes elevated blood lactate and acidity, increased levels of human growth hormone, and increased glycogen storage in muscle tissue.

The intestinal flora has been shown to play an important role in regulating the availability of energy-producing substances for muscles during over-exercising. Nay et al. (2019) investigated the effects of gut microbiota on muscle function and found that, in gut microbiota-depleted mice, running endurance and the extensor digitorum longus muscle fatigue were decreased. After 10 days of natural reseeding, several pertinent glucose metabolism markers including glucose transporters G protein-coupled receptor 41, sodium-glucose cotransporter 1, and muscle glycogen level increased. This study implies that modulating the muscle availability of glycogen via gut microbiota represents one potential mechanism that can contribute to the gut microbiota-skeletal muscle axis [14]. Moreover, gut microbiota-derived phenolic metabolite isovanillic acid 3-O-sulfate affects muscle cells to take up and metabolize glucose by up-regulating GLUT1, GLUT4, and PI3K p85α expression and increasing phosphorylation of Akt [15]. Reduced carbohydrate absorption due to gastro-intestinal distress poses a particular problem for endurance athletes, as carbohydrates availability during
endurance exercise lasting > 60 min may be a limiting factor for performance. In a randomized trial of twenty-four recreational runners, researchers showed that supplementation with a multi-strain probiotic for 28 days prior to a Marathon race reduces the incidence and severity of gastrointestinal symptoms when compared with placebo control. Moreover, probiotic supplementation also attenuates the substantial change in the metabolome induced by running a Marathon [16].

The Gut microbiota can also improve the bioavailability of amino acids and alter the decomposition, absorption and metabolism of proteins. By using gene expression data and tissue-specific genome-scale metabolic models (GEMs), researchers found that the distribution of free amino acids in the gastrointestinal tract germ-free mice has significantly changed [17]. By using comparative genomic analysis, Li et al (2018) found that *L. plantarum* ZJ316 had 34 genes encoding intracellular peptidases, 23 genes encoding proteases with different specificities, and large amounts of genes involved in the biosynthetic pathway of most amino acids. This result suggested that probiotics have the potential to regulate amino acid biosynthesis and protein metabolism [18]. Data collected from resistance-trained males showed that probiotic supplementation probiotic BC30 (Ganedon Biotech Inc., Maryfield Heights, OH) in combination with a slow digesting protein might increase athletic performance by increasing protein absorption [19]. Ammonia is a continuously produced metabolic toxic waste product. Increased ammonia levels have been recorded following physical exertion. A pilot study with male football players showed that *L. casei* probiotic supplement could naturally generate phenylacetate contributing to ammonia removal through glutamine sequestration as phenylacetyl glutamine via the kidneys [20]. Huang et al. (2019) investigate the effects of *L. plantarum* PS128 supplementation on triathletes. Their results showed that *L. plantarum* PS128 supplementation caused a 6–13% decrease of indicated pro-inflammatory (TNF-α, IL-6, and IL-8) cytokines (p < 0.05) and a 55% increase of anti-inflammatory (IL-10) cytokines (p < 0.05) after intensive exercise stimulation. In addition, *L. plantarum* PS128 can also substantially increase 24–69% of plasma-branched amino acids and elevate exercise performance, as compared to the placebo group [21]. Moreover, Probiotics have been shown to improve the nutritional value of food through the production of enzymes and metabolites to increase free amino acids, bioactive peptides, γ-aminobutyric acid and the concentrations of other nutrients, which play a role in host energy metabolism [22].

Maintaining a high level of athletic performance and staying healthy have related common factors. The B-vitamins (thiamin, riboflavin, vitamin B-6) are necessary in the energy-producing pathways of the body. Supplementation of probiotics can improve the metabolism of B-vitamins to fortify the body and prevent some of the pitfalls of overtraining including illness and burnout. Magnúsdóttir et al. (2015) systematically assessed the genomes of 256 common human gut bacteria for the presence of biosynthesis pathways for eight B-vitamins. Their results revealed that the distribution of synthesis pathways was diverse and some genomes had all eight biosynthesis pathways [23]. A probiotic strain *L. fermentum* CECT 5716 isolated from human milk was able to produce riboflavin and folates [24]. Collectively, the body’s metabolism is accelerated during high-intensity exercise. The reduced glycogen storage and insufficient energy supply lead to muscle fatigue and restricts exercise capacity. Probiotics can improve the composition of gut microbiota and modulate the availability of amino acids and vitamins to promote the host’s energy metabolism and improve energy supply. These alterations are critical in improving muscle health, relieving body fatigue, and enhancing training performance.

### 4. Probiotics Improve Gastrointestinal Health

Gastrointestinal (GI) complaints are common among athletes with rates in the range of 30% to 70% [25]. Exercise-induced gastrointestinal syndrome (EIGS) is a common characteristic of exercise. It has been used to describe a complex response to exercise that disturbs and compromises gastrointestinal function. The onset of EIGS, and associated lower-gastrointestinal symptoms (GIS), varies across different forms of exercise: from reported GIS incidence of ≤10% in strength and power sports, team sports, and endurance exercise <4 h in duration, to ≥60% in ultraendurance running and triathlon events [26]. It is estimated that 45% of recreational endurance runners reported at least one gastrointestinal complaint and 11% severe gastrointestinal complaint during running events ranging from 10 km to marathon distance (4). In marathon running, 27% of recreational runners report moderate or more severe GI symptoms during a race [27]. Additionally, female runners and those runners who were not accustomed to feeding during running reported higher incidence of symptoms.

The mechanisms by which exercise causes gastrointestinal symptoms are not well known. It is generally accepted that the EIGS was associated with splanchnic blood flow redistribution, cytokine responses, sympathetic activation, gastrointestinal motility, and malabsorption [28,29]. Exercise perturbs gastrointestinal integrity by the redistribution of blood flow to working muscle and peripheral circulation, which aids skeletal muscle metabolism and thermoregulation, subsequently reduces total splanchnic perfusion and causes intestinal ischemia. The exercise-induced reduction in splanchnic blood flow is well characterised and results in dysregulation of the intestinal barrier [30]. Moreover, exercise may increase sympathetic hyper activation to reduce overall gastrointestinal functionality. The effects of acute exercise on gastrointestinal motility have been hypothesised to explain gastrointestinal symptoms such as heartburn (gastro-oesophageal reflux), vomiting, gastrointestinal cramps, urge to defecate, and diarrhoea. High intensity exercise may also decrease gastric motility and emptying. This cascade of physiological events can increase the potential for nutrient malabsorption further aggravated by extreme environmental conditions [31,32]. Collectively, these causes can lead to outcomes that include increased intestinal epithelial injury and gastrointestinal hyperpermeability. The gut becomes more leaky, allowing pathogenic endotoxins normally present and isolated to the intestine to pass into the bloodstream.
With the aim of improving gastrointestinal health and preventing gastrointestinal related issues, there is significant interest and belief amongst the general public for the administration of probiotic supplementation. It has been widely recognized that the intestinal barrier function is affected by microbiota’s metabolism. More and more evidence showed that appropriate probiotic supplementation could have potential to counteract exercise-associated gastrointestinal perturbation [33]. Many probiotic bacteria have demonstrated favorable effects in vitro and in vivo on epithelial integrity through direct or indirect mechanisms [34]. As shown in Figure 1, probiotics may provide protective effects to the secondary outcomes of EIGS through various ways including enhancing enterocyte stability and functional capabilities, stimulating immune cells activity (regulating the pro-/antiinflammatory pathways and immunoglobulins production), increasing short-chain fatty acids (SCFA) production, lowering intestinal pH, and stimulating mucus production [35]. The consumption of live L. rhamnosus GG protects the integrity of the gastric mucosal barrier, but had no effect at the intestinal level, highlighting the differences in physiology of the epithelial barrier in different locations of the gastrointestinal tract [36]. Several studies have reported the positive outcomes of probiotics supplement on gut permeability and endotoxemia. Specifically, a significant reduction of fecal zonulin concentrations and plasmaendotoxin concentrations. Interestingly, these studies used a multistrain probiotics supplement containing the species L. acidophilus, B. bifidum, and B. animalis ssp.lactis for at least 1 month.

There are few studies that report the positive influence of probiotics on the GI system in athletes. Brennan et al. found that highly trained endurance athletes supplemented with L. salivarius (UCC118) reported less intestinal hypermeability induced by exercise [37]. Probiotic strains including Lactobacillus and Bifidobacterium are known to improve gastrointestinal health by increasing the relative abundance of certain short chain fatty acid. A more recent study observed that oral ingestion of a commercial probiotic beverage containing L. casei (volume equivalent for 10^{11} CFU/day) for seven consecutive days, before 2 h running at 60% VO_{2\text{max}} in T_{\text{amb}} 34°C, resulted in a substantially greater endotoxemia (i.e. Δ pre-to post-exercise: 15 pg/ml and 1 h post-exercise: 46 pg/ml) in all participants, compared with matched placebo [38].

5. Probiotics Interact with Immune System

It is generally considered that exercise and training activity also compromises immune function, with most changes on balance suggesting an overall decrease in immune system function, particularly when training loads are high. A marked fall in the number of circulating leukocytes is often indicative of a chronic viral infection and low blood leukocyte counts are commonly found in athletes engaged in heavy training [39]. Some study found that intense exercise causes a significant release of proinflammatory cytokines and free radicals, and leads to muscle damage and tissue injury [40]. After high-intensity prolonged exercise, the concentration and activity NK cell, as well as serum concentration of IgG1, IgG2, and IgE are changed. Lymphocyte and neutrophil counts and B cell function are also influenced [41].

The gut microbiota interacts with the host, participates in the development and dynamic balance of the host's immune system, and protects the host from infection by pathogens. It has been proved that probiotics can combine some required characteristics to enhance the immune function of the host by modifying non-specific and humoral immunity. Probiotic strain can modulate the gut microbiota to produce several metabolites from digested food. Amongst these molecules short-chain fatty acids (SCFAs) are the most widely investigated in the regulation of inflammation and immune system. It has been demonstrated that SCFAs have anti-inflammatory effects on intestinal mucosa, thus protecting the bowel from the development of inflammatory bowel disease [42]. The signal between gut microbiota and immune system is
fundamental to regulate the homeostasis and to maintain the balance between immune tolerance to commensals bacteria and immunity to pathogens [43]. Beyond SCFAs, GM produces other metabolites from digested food that have important immunomodulatory function as indole derivatives and polyamines, these metabolites derive from dietary tryptophan and arginine respectively and have an indirect immune function. Indoles derivatives favor the integrity of the enteral mucosa and the barrier defense towards pathogens by stimulating the production of anti-microbial peptides, mucins, and proliferation of intestinal goblet cells. Polyamines as putrescine, spermidine, and spermine fulfill important roles in gene expression and proliferation; enhance the development and maintenance of the intestinal mucosa and resident immune cells [42].

Probiotics can enhance nonspecific cellular immune response characterized by activation of macrophages, NK cells, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in strain-specific and dose-dependent manner [44]. Several probiotics have been reported to augment NK cell activity. Studies have shown that *L. plantarum* 299v is capable of inducing mucin, MUC2 and MUC3, which is important in hindering pathogen colonization [45]. A randomized controlled study was held of 96 male stress-burdened office workers who were allocated to two groups and given milk as control drink or a fermented milk drink containing 100 billion *L. casei* Shirota daily for 12 weeks. The URTI-free rate was 0.78 in the probiotic group but 0.47 in the control group. NK cell activity was significantly higher at week 6 in the probiotic group than in the control group. In contrast to the change in NK cell activity, saliva cortisol-a stress marker-was significantly lower at week 6 in the probiotic group than in the control group [46]. In the study published by Vaisberg et al. in 2019, it can be observed how *L. casei* Shirota (LeS), administered for a period of time equal to 30 days, showed benefits related to the modulation of the immune response, inflammatory and mucosal upper respiratory tract, presenting protective effects [47]. Probiotic supplementation has been demonstrated to beneficially modify and support the gut microbiota composition such as *Akkkermansia* and *Prevotella*. Various studies have elucidated immune modulation induced by *Akkkermansia*. It has been reported that *A. muciniphila* is capable of up regulating genes involved in antigen presentation, as well as aiding in B and T cell maturation [48]. *A. muciniphila* and its immune modulation activity, and concluded that *Akkermansia* could activate Toll like receptor 2 (TLR) and TLR4 and induce IL8 and IL10 [49].

The immune disturbances caused by intense exercise have been associated with the development of infection especially during winter months. Overtraining-induced muscle damage is associated with an inflammatory response characterized by increased susceptibility to infections attributable to changes in the functional status of immune cells [50]. Upper respiratory tract (URT) infection and gastroenteritis are the most common infections seen in athletes but pharyngitis, infectious mononucleosis, sinusitis, otitis media and externa, acute bronchitis, pneumonia, conjunctivitis, skin infections, and blood-borne infectious diseases also occur. Infectious illnesses contribute to tissue wasting, muscle catabolism, and negative nitrogen balance. Pain, discomfort, or other symptoms of infection can be disturbing during training and competition and have a negative effect on performance. Reducing the incidence or severity of illness has positive impacts on performance during training and competition. Probiotic consumption might promote the innate and adaptive immune systems due to its modulatory effects on the immunity parameters and thus indirectly enhance physical performance. Moreover, probiotics may also provide positive effects on time to exhaustion, and muscle recovery [51].

Several studies have reported the beneficial effects of probiotics in increasing immune functions of athletes. An early research found the fatigued athletes had clinical characteristics consistent with reactivation of Epstein-Barr virus infection and significantly less secretion of interferon (IFN) γ from blood CD4 positive T cells. After one month of daily capsules containing $2 \times 10^{10}$ CFU *L. acidophilus*, secretion of IFNγ from T cells had increased significantly to levels found in healthy control athletes [52]. This study showed that probiotic therapy may reverse a T-cell defect in fatigued athletes, and enhance mucosal IFN concentrations in healthy athletes.

Athletes presenting with fatigue and impaired performance had an immune defect relevant to defective containment of Epstein-barr virus infection. Regular ingestion of probiotics reduced plasma Epstein-barr virus antibody titres, an effect that can be interpreted as a benefit to overall immune status [53]. The administration of a two-strain probiotic supplement (*Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. *lactis* Bi-07) delayed the occurrence of URT illness and significantly increased the training load during a 5-month intervention period compared with placebo [54]. Strasser et al examined the effect of a probiotic supplement on the incidence of upper respiratory tract infections (URTI) after exhaustive aerobic exercise in trained athletes during three months of winter training. Their results showed that the ratio of subjects taking the placebo who experienced one or more URTI symptoms was increased 2.2-fold compared to those on probiotics [55]. In a study involving young adult female endurance swimmers, subjects were randomly assigned into two groups, receiving either 400 mL probiotic yogurt (intervention group) or ordinary yogurt (control group) daily for 8 weeks. The results revealed that the average number of episodes of respiratory infection in the intervention group was 0.9 day, which was statistically fewer than that in the control group (1.4 days) [53]. The mechanism of antinfection effects of probiotic may attribute to the immune enhancement activity and/or antagonistic activities against pathogens. Probiotic strains can produce several types of antimicrobials including bacteriocins, hydrogen peroxide, lactic acid, acetic acid. The production of antimicrobials allows for longer survival of the producer in the intestine, as well as having the potential to outcompete pathogenic bacteria maintaining a healthy bacterial balance in the gut. Athletes tend to be prone to gastroenteritis and other infections, and the antimicrobial compounds produced by the probiotics can help in easing or preventing the symptoms.
6. Probiotics Reduce Oxidative Stress

Overtraining induces a significant rise in inflammatory and apoptotic markers. Muscle microtrauma induces reductions in strength [56] and range of motion [57] due to swelling in the injured area as well as a local inflammatory response with activated circulating monocytes and systemic inflammation and possibly immunosuppression [58]. Intense physical exercise has been reported to cause oxidative stress, which is the imbalance between oxidant and antioxidant levels in the body and results in the creation of reactive oxygen species (ROS) [56]. ROS have been linked to mechanisms related to postexercise inflammatory response and possibly with propagation of muscle damage [59]. Exercise of progressively increased and decreased training volume may induce oxidative damage to lipids and proteins as evidenced by changes in indirect blood markers of oxidative stress [60]. Moreover, the generation of ROS by over-training causes a decrease in the release of endothelial NO, whose action is the key in the control of platelet aggregation and blood hemostasis, and it also participates in the inflammatory and immune response.

Normally, ROS are generated by aerobic cells and are readily removed by endogenous free radical scavenging mechanisms including glutathione (GSH) and superoxide dismutase (SOD). However, antioxidant defense systems of the cells can be overwhelmed by large quantities of ROS. Heshmati et al. performed a comprehensive study on the literatures and database reporting the effect of probiotics supplementation on biomarkers of oxidative stress. Their results revealed that oxidative stress parameters levels, including total antioxidant capacity (TAC), GSH, SOD and nitric oxide (NO) were higher in probiotics group compared to controls. Moreover, malondialdehyde (MDA) level was lower than controls [61].

Various probiotic species have been reported to have antioxidant activities. It has been shown that survival time of \textit{L. rhamnosus} GG was significantly longer in the presence of H2O2 and hydroxyl radical as compared to non-antioxidative strains, \textit{L. paracasei} Fn032 and \textit{L. plantarum} Fn001, respectively. In addition, \textit{L. paracasei} Fn032 and \textit{L. plantarum} Fn001 were specific for free radical scavenging activities of their intracellular free extracts (ICFE) [62]. Grompone et al. developed an in vitro method for the screening of antioxidative potential of new probiotic strains and examined a total of 78 strains of lactic acid bacteria (LAB) (\textit{Lactobacillus} and \textit{Bifidobacterium}). Their results showed that a \textit{L. rhamnosus} strain (CNCM I-3690) demonstrated a high antioxidant capacity. Martarelli et al has investigated the effects of two probiotic strains, \textit{Lb. rhamnosus} IMC 501 and \textit{Lb. paracasei} IMC 502, on the oxidative stress of athletes during a four-week time frame of intense training. The results showed the probiotic course successfully lowered the oxidative stress caused by the intense exercise [63]. Studies have also demonstrated the antioxidant activity of \textit{B. animalis}, which was found to absorb hydroxyl radicals and superoxide anions in vivo, as well as, increasing the general antioxidase activity. \textit{Lb. fermentum} E3 and E18 have been found to produce glutathionine (GSH) that enables the strain with antioxidant abilities [64]. In addition, the results of Mishra et al. showed that probiotics enhance the antioxidant system by increasing the production of antioxidant peptides and repairing the intestinal microbial flora [65].

![Figure 2. Reduction of ROS by probiotic supplementation by athletes](image-url)
7. Probiotics Affect the Neuroendocrine System

There appear to be a number of hormonal abnormalities in athletes engaged in very heavy training and it has been suggested that a neuroendocrine imbalance in overtraining syndrome. For instance, finding a reduced increase in corticotrophin, growth hormone (GH) and cortisol (COR) in response to an insulin-induced hypoglycemia [66]. Researchers found an impaired pituitary hormonal response to exhaustive exercise in over trained endurance athletes. During heavy endurance training or over-reaching periods, there is evidence of reduced adrenal responsiveness to Adreno Cortico Trophic Hormone (ACTH), which is compensated by an increased pituitary ACTH release. During the early stages of overtraining syndrome, despite increased pituitary ACTH release, the decreased adrenal responsiveness is no longer compensated and the cortisol response decreases. During the advanced stage of overtraining syndrome, the pituitary ACTH release also decreases [67]. Many probiotics reduced HPA axis activity by decreasing CORT and/or ACTH levels, including most of the Lactobacillus strains tested: *L. plantarum*, *L. helveticus*, *L. fermentum*, *L. rhamnosus*, and *L. casei* [68]. Probiotics may also directly affect levels of BDNF, γ-aminobutyric acid (GABA), serotonin, and dopamine (DA).

8. Probiotics Improve Mental Health

Optimized physical conditions accompanied by a good mental conditions, are part of a continuum that enables the optimal training and performance of competitive athletes. In order to reach the goal of improved performance, the athlete has to reach his/her limits of physical capacity (or even slightly beyond). The balance between desirable and excessive training is quite tenuous and excessive training (or insufficient rest) is very common in elite athletes. Accumulating shreds of evidence have shown that excessive exercise and overtraining syndrome can be associated with impaired mental health. Among professional athletes, data shows that up to 35% of elite athletes suffer from a mental health crisis that may manifest as stress, eating disorders, burnout, and even depression [69]. The gut microbiota has emerged as one of the key regulators of mental illness. The gut microbiota and the brain communicate with each other via numerous pathways. Data indicate that gut-brain communication is bidirectional and mediated by neural and humoral mechanisms. Probiotics may direct activate the neuronal system or mediate host physiology by their metabolites.

Cognitive stress and anxiety are common emotional states experienced by athletes at high-performance levels. Stress and anxiety are characterized by a range of physiological, behavioral, and/or cognitive signs and symptoms. Numerous studies have demonstrated activation of gastrointestinal tract vagal afferents by gut hormones, cytokines, microbial signals, and mechanical stimuli, and vagal afferents have been implicated in probiotic-induced neurobehavioral changes [70]. Probiotic *L. rhamnosus* can directly increase the single-unit and multiunit firing rate of the mesenteric nerve bundle and can decrease stress-induced corticosterone and anxiety and depression [71]. Moreover, these behavioral effects of *L. rhamnosus* are abolished by vagotomy [72]. *L. gasseri* OLL2809 combined with alpha-lactalbumin decreases symptoms of depression in college athletes [73]. The combination of *Lactobacillus helveticus* and *Bifidobacterium longum* reduced anxiety behavior in Wistar rats and probiotics reduced hypothalamus-pituitary- adrenal axes (HPA) and autonomic nervous system (ANS) activity [74]. The use of *Bifidobacterium longum* R0175 for 30 days reduced the anxiety and stress levels in individuals and caused a significant improvement in anxiety and depression [75]. Thus, gut microbiota and probiotics play an important role in the central nervous system function.

Probiotics can influence host physiology with products of their own metabolism, including the neurotransactive molecules 5-hydroxytryptamine (serotonin) and gamma-aminobutyric acid (GABA). Specific examples include generation of gamma-aminobutyric acid by members of the Lactobacillaceae and Bifidobacteriaceae families, dopamine and norepinephrine by members of the Baciellaceae family, and noradrenaline and 5-hydroxytryptamine by the Enterobacteriaceae family [76]. Male germ-free mice have increased hippocampal 5-hydroxytryptamine, increased plasma tryptophan, decreased hippocampal brain-derived neurotrophic factor, and altered anxiety, suggesting that microbiota alter the development of central nervous system neurotransmitter systems [77]. GABA is an amino acid with a non-protein structure that is mainly produced by plants, animals and microorganisms. It is considered an inhibitory neurotransmitter that has aroused increasing interest over the years due to its essential role in the nervous system. The inhibitory effect of GABA occurs as a result of binding to the GABAAergic receptor system composed of three specific receptors: GABA	extsubscript{A}, GABA	extsubscript{B} and GABAC. Through these receptors, GABA can modulate mood (e.g. relaxation), sleep disorders and temporal and spatial memory [78]. Both *Bifidobacterium* and *Lactobacillus* have received considerable attention due to their large number of GABA-producing strains [79]. Strasser et al. investigated the effect of a multi-species probiotic formulation (*B. bifidum* W23, *B. lactis* W51, *E. faecium* W54, *Lb. acidophilus* W22, *Lb. brevis* W63, *L. lactis* W58) and its possible effect on tryptophan levels, often implicated in the etiology of depression [55].

Short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate, are produced by microbial metabolism of otherwise indigestible dietary fibers and also significantly impact host physiology. SCFAs directly stimulate the sympathetic and autonomic nervous system via activation of G protein-coupled receptors. While SCFAs can permeate the blood-brain barrier, the degree to which endogenous SCFAs cross into the brain to affect neurological function remains unclear, although they have been shown to maintain the integrity of the blood-brain barrier. *Lactobacilli* can produce end products, such as pyruvate, by the fermentation of carbohydrates during the glycolytic metabolic pathway, while *Bifidobacteria* can produce mainly acetate and formate by using the fermentation pathway [80]. It has been reported that *Bifidobacterium longum* R0175 taken for 30 days mitigated...
anxiety-like behaviors and stress levels measured by urinary free cortisol concentration, and significant improvements in anxiety and depression were reported by the patients [75]. Moreover, the study also demonstrated that the chronic use of a Lactobacillus helveticus and B. longum R0175 formulation could also contribute to the mental well-being of subjects with low levels of stress [81]. Collectively, it can be concluded that probiotic supplements can be considered an appropriate medication without side effects for improving the mental status of athletes. Probiotic studies that focus on the mental health of athletes represent an emerging area in the field of sports nutrition and exercise performance. However, the number of probiotic studies remains very low, with studies often including a low number of subjects, and a wide variety of questionnaires have been used to assess cognitive outcomes. Despite limited evidence, cognitive health remains an intriguing area of sports nutrition research [82].

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<th>Benefits/Impacts</th>
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<td>L. fermentum VRI-003</td>
<td>↓ Severity and incidence of URT; ↑ Whole blood culture IFNγ</td>
<td>elite distance runners; n=20</td>
<td>(83)</td>
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<tr>
<td>L. fermentum (PCC®)</td>
<td>↓ Severity of gastrointestinal symptoms; ↓ severity/duration of lower respiratory illness; ↓ cytokine imbalance</td>
<td>Competitive cyclists; n=99</td>
<td>(84)</td>
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<tr>
<td>B. longum 35,624</td>
<td>Differences in cognitive outcomes were detected showing more favorable sport recovery related scores in the probiotic supplementation group</td>
<td>Highly trained competitive swimmers; n = 17</td>
<td>(85)</td>
</tr>
<tr>
<td>L. casei Shirota</td>
<td>PRO maintained salivary immune protection and increased anti-inflammatory response on the upper airways, immediately after the marathon. Serum TNF-α level was significantly lower immediately post-marathon in the PRO group compared to that in the PLA group</td>
<td>Marathon runners; n = 42,</td>
<td>(47)</td>
</tr>
<tr>
<td>L. casei Shirota</td>
<td>↓ Incidence of upper respiratory tract infections; ↑ Saliva IgA level</td>
<td>Endurance athletes; n = 84</td>
<td>(86)</td>
</tr>
<tr>
<td>L. plantarum PS128</td>
<td>↓ Oxidative stress; ↓ Pro-inflammation; ↓ Anti-inflammation; ↑ Plasma-branched amino acids</td>
<td>Triathletes; n = 18</td>
<td>(21)</td>
</tr>
<tr>
<td>L. helveticus LaftiL10</td>
<td>Improved the immunity of athletes</td>
<td>Elite athletes; n = 30</td>
<td>(87)</td>
</tr>
<tr>
<td>Lactococcus lactis JCM 5805</td>
<td>↑ pDC maturation; ↓ Incidence of URTI; ↓ Fatigue</td>
<td>Athletes; n = 51</td>
<td>(88)</td>
</tr>
<tr>
<td>B. breve BR03 and S. thermophilus FP4</td>
<td>Positive effect on the reduced performance and range of motion followed by intense muscle damaging exercise</td>
<td>resistance-trained men; n=15</td>
<td>(12)</td>
</tr>
<tr>
<td>L. rhamnosus, L. casei, L. acidophilus, L. plantarum, L. fermentum, Bifidobacterium lactis, B. bifidum, Streptococcus thermophilus, Saccharomyces boulardii</td>
<td>↓ Incidence of gastrointestinal and upper respiratory tract infections; ↑Salivary α-amylase</td>
<td>Elite rugby union athletes; n = 19</td>
<td>(89)</td>
</tr>
<tr>
<td>L. acidophilus, L. delbrueckii subsp. bulgaricus, B. bifidum, and Streptococcus salivarius subsp. thermophilus</td>
<td>↓ Number of episodes of upper respiratory tract infections; ↓Dyspnea and ear pain</td>
<td>Female endurance swimmers; n = 46</td>
<td>(90)</td>
</tr>
<tr>
<td>L. gasseri, B. bifidum, B. longum</td>
<td>↓ Incidence of upper respiratory tract infections; ↓gastrointestinal episodes</td>
<td>Rugby players; n = 30 males</td>
<td>(54)</td>
</tr>
<tr>
<td>** L. acidophilus CUL-60, L. acidophilus CUL-21, B. bifidum CUL-20, B. animalis subsp. lactis CUL-34</td>
<td>↓ Endotoxin units; ↓gastrointestinal symptoms; Maintenance of intestinal permeability</td>
<td>Long-distance triathletes; n = 30</td>
<td>(33)</td>
</tr>
<tr>
<td>L. rhamnosus IMC 501©, and L. paracasei IMC 502© (1:1 ratio)</td>
<td>Neutralize the ROS; ↑Antioxidant potential</td>
<td>Amateur athletes; n= 24 males;</td>
<td>(63)</td>
</tr>
<tr>
<td>*L. rhamnosus IMC 501© and L. paracasei IMC 502©</td>
<td>Improve gut health, oxidative status, and mucosal immunity</td>
<td>Athletes; n = 10</td>
<td>(91)</td>
</tr>
<tr>
<td>B. bifidum W23, B. lactis W51, Enterococcus faecium W54, L. acidophilus W22, B. brevis W63, and L. lactis W58</td>
<td>Improves intestinal permeability; ↓Exercise-induced protein oxidation; ↓TNF-α level</td>
<td>Trained athletes (triathletes, runners, cyclists); n = 23</td>
<td>(92)</td>
</tr>
<tr>
<td>B. bifidum W23, B. lactis W51, E. faecium W54, L. acidophilus W22, B. brevis W63, L. lactis W58</td>
<td>↓ Incidence of upper respiratory tract infections; ↓Rate of exercise-induced tryptophan degradation</td>
<td>Trained athletes; n=29</td>
<td>(55)</td>
</tr>
<tr>
<td>L. acidophilus LAFTI®</td>
<td>↑ Fatigue</td>
<td>trained amateur athletes; n=27</td>
<td>(52)</td>
</tr>
<tr>
<td>L. gasseri OLL2809 combined with alpha-lactalbumin</td>
<td>Prevents the exercise induced drops in NK cells; positive effect on minor fatigue; ↑mood from a depressive state</td>
<td>university student athletes; n=44</td>
<td>(73)</td>
</tr>
</tbody>
</table>

Table 1. Probiotic species and the reported benefits on athletes
9. Conclusions

Literature data indicate that supplementing a human diet with proper probiotic bacteria enables a digestive tract to sustain its homeostasis, increasing the tolerance of an organism to unfavorable external stimuli [93]. Most studies have shown that intake of probiotics positively affects the gut microbiota population in terms of influencing immune function as well as intestinal epithelial cell proliferation. Recent investigations have shed light on the potential roles of probiotics in sports nutrition [94]. However, research investigating in the potential of probiotics to aid nutrient metabolism in relation to exercise remains limited. The number of publications on the use of probiotics in endurance athletes is small and has limitations or possible biases.

High physiological demands endured by elite athletes who undergo intensive and prolonged training and competitions lead to numerous health-related side effects, including gastrointestinal (GI) discomfort and symptoms [95]. For these symptoms, food and nutrition can be protective, and the use of therapeutic dietary measures is increasing, especially during competition seasons. The relationship between probiotic supplementation and exercise-induced disorders should be actively discussed with specifics regarding the most appropriate bacterial strains [94]. Moreover, gut microflora and food consumption should be analyzed and compared to better understand the effects of probiotic supplementation on symptoms, well-being and performance in athletes.

In conclusion, studies investigating the effects of probiotics in athletic populations and on sports performance are limited and it is still premature to issue definitive practical guidelines. Nonetheless, it is becoming clear that regular consumption of specific probiotic strains may assist with immune function and may reduce the number of sick days an athlete experiences when training or during competition. It should be pointed out that probiotics may not directly influence the performance of athletes, but they are hugely important for supporting indirectly. Taking probiotics to help prevent illness and protect against the symptoms of overtraining or digestive complaints can be hugely influential on an athlete’s career. Probiotic benefits are strain-specific and dose-dependent and include improved gut-barrier function, nutrient absorption, recovery and performance in athletes. Athletes are encouraged to use clinically researched strains with validated benefits, matching the athletes’ desired health benefits [96].

Conflict of Interest

The author(s) declare(s) no potential conflicts of interests.

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