Probiotics, immunity and exercise: a review.

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ABSTRACT

Nutritional practices that promote good health and optimal athletic performance are of interest to athletes, coaches, exercise scientists and dietitians. Probiotic supplements modulate the intestinal microbial flora and offer promise as a practical means of enhancing gut and immune function. The intestinal microbial flora consists of diverse bacterial species that inhabit the gastrointestinal tract. These bacteria are integral to the ontogeny and regulation of the immune system, protection of the body from infection, and maintenance of intestinal homeostasis. The interaction of the gut microbial flora with intestinal epithelial cells and immune cells exerts beneficial effects on the upper respiratory tract, skin and uro-genital tract. The capacity for probiotics to modulate perturbations in immune function after exercise highlight their potential for use in individuals exposed to high degrees of physical and environment stress. Future studies are required to address issues of dose-response in various exercise settings, the magnitude of species-specific effects, mechanisms of action and clinical outcomes in terms of health and performance.

Keywords: probiotics, immunity, gastrointestinal illness, intestinal microbial flora, exercise immunology.

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INTRODUCTION

Preventing illness during heavy training and competition is a high priority for athletes, coaches and exercise scientists. A perception exists that some athletes, especially elite athletes in sports such as rowing, cycling, swimming and triathlon, undertaking prolonged intense exercise, may suffer from an increased incidence of upper respiratory tract illness (URTI) during heavy training and competition (33, 61, 82). Anecdotal observations from our own work with athletes also indicate there is strong interest in minimising gastrointestinal (GI) disturbance, particularly diarrhoea, during international travel. The increased incidence of URTI may relate to exercise-induced perturbations in immunity that provides pathogens increased opportunity to establish infection (32, 35). Investigations into the issue of athletes and URTI indicates that the aetiology of URTI may also be non-infectious in nature, with mechanical damage to epithelial cells in the respiratory tract from hyperventilation and air-borne particles initiating inflammation (95). The underlying causes of GI disturbances in athletes relate to changes in diet and sanitary conditions associated with travel in foreign countries. Illness during training and competition may negatively affect athletic performance (83). Given the considerable personal and economic investment in preparing athletes for competition, a need exists to identify strategies aimed at improving host resistance and minimizing the risk of illnesses that may compromise athletic performance.

Nutrition is known to influence immunity and host resistance to illness. Widespread interest exists in the use of probiotics to enhance host resistance to illness. Probiotics are live micro-organisms that transiently alter the intestinal microbial flora, which is the diverse bacterial population that inhabits the GI tract. Probiotics modulate immunity in the GI tract by interacting with a range of receptors on intestinal epithelial cells (IEC), M-cells and dendritic cells (Figure 1). Probiotics also enhance immunity beyond the GI tract through interactions with the common mucosal immune system (CMIS). The CMIS is an interconnected system that links inductive sites in the GI tract (Peyer's patches) to diverse effector sites within the GI tract itself, and other mucosal surfaces such as the upper respiratory tract and uro-genital tract (55, 96).

Probiotics may benefit athletic performance indirectly by maintaining GI function and health, preventing the immunosuppressive effects of intense exercise, and reducing susceptibility to illness (6, 73). Substantial evidence exists indicating that probiotics can reduce susceptibility to acute infectious diarrhea and its associated symptoms (36, 81). Evidence from population studies indicates that probiotics enhance host resistance to URTI (22); however, initial investigations in athletes have thus far not been convincing (48).

Examining the efficacy of probiotics in athletes also provides an opportunity to advance our understanding of the relationship between changes in immunity and susceptibility to infection. There remains uncertainty about the way changes in immunity in healthy people relate to risk of illness (41). Exercise as an experimental model for studying the relationship between stress, health and immunity is

well established (39, 108). The increased incidence of illness in athletes undertaking prolonged intense exercise may result, in part, from exercise-induced transient perturbations in cellular and humoral aspects of immunity, with acute and chronic changes in secretory IgA (SIgA) (34), antimicrobial proteins (3, 21, 107), and granulocyte activity (71) all described. Mechanical changes in GI barrier function also occur with exercise. Exercise increases GI permeability through several mechanisms related to reduced blood flow and hyperthermia in the gut. One study has reported that GI permeability increases after treadmill running at 80% VO_{2max} (but not 40% or 60% VO_{2max}), and that GI permeability correlates with core temperature (78). Many of the exercise-induced changes in immunity are similar to those changes associated with other forms of stress, such as sleep deprivation, ageing and psychological stress (39). If probiotics can improve immunity in athletes, then they may also be suitable for improving immunity in other individuals exposed to regular stress, such as the military and shift workers. This review examines the role and efficacy of probiotic supplements in enhancing gut immunity and attenuating exercise- or training-induced perturbations in immunity and health.
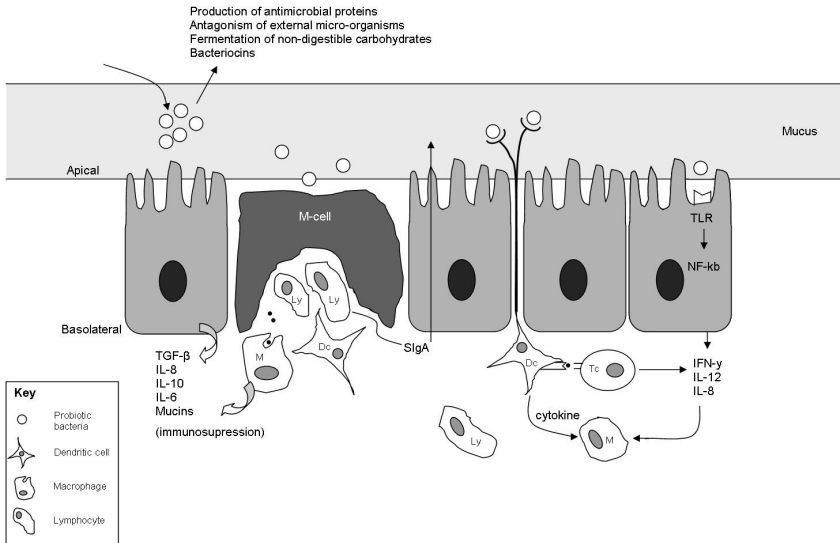

Figure 1. Probiotics modulate immune function in the mucosa through interaction with M-cells in the Peyer's Patch, intestinal epithelial cells and dendritic cells. Macrophages (M) or dendritic cells (DC) below M cells process probiotics and trigger immune responses (suppressive or inflammatory). DC extend their dendrites between intestinal epithelial cells and sample probiotics in the gut lumen to regulate immunity. Probiotic interaction with intestinal epithelial cells induces secretion of antimicrobial factors and cytokines that regulate the activity of DCs, T cells and B cells in the gut-associated lymphoid tissue. Abbreviations: TLR – Toll like receptor; NF-κb, nuclear factor – kappa B; IFN-γ – interferon gamma; IL- interleukin; TGF-β – transforming growth factor- beta; SIgA – secretory immunoglobulin A.

Probiotics

A large range of bacteria are used as probiotics, with the most common strains belonging to the *Lactobacilli* and *Bifidobacteria* species. Specific criteria have been establishing for classifying bacteria as genuine probiotics (Table 1). No consensus currently exists on the optimum dosage required for probiotic supplements to induce beneficial effects. Current dosages range from 10^8 through to 10^{11} colony forming units (CFU) per day. Measurement of the number of CFU recovered in feces is a marker commonly used to determine whether the ingested bacteria can survive gastric transport and colonize the GI tract (72, 86). Fecal recovery of probiotic bacteria, however, varies substantially. A dose response study utilising doses of 10^8, 10^9, 10^{10} or 10^{11} CFU per day of *B. animalis ssp. lactis* (BB-12) and *L. paracasei ssp. paracasei* (CRL-431) in healthy subjects reported no recovery of L. paracasei for any of these doses administered. Fecal recovery of *B. animalis,* however, was dose-dependent [17]. Another study utilising dosages of 10^8 CFU *L. paracasei* DN-114001 per day in 12 healthy subjects indicated that fecal recovery increased 1000-fold after 10 days in all subjects (86). There is debate whether or not adherence and colonisation of the GI tract is essential for probiotics to exert biological activity (53). Interactions between probiotics and antigen-detecting cells, such as M-cells and dendritic cells, which sample luminal contents, play an important role in the regulation of mucosal homeostasis and the induction of a mucosal immune response. As research uncovers the mechanisms by which probiotics exert their effects the criteria determining their definition will continue to evolve.

Table 1: Essential criteria for bacteria to be classified as a probiotic

1. Viability during processing, transport and storage;
2. Ability to survive gastric transport;
3. Ability to adhere and colonise the GI tract;
4. Ability to antagonize pathogenic bacteria; and
5. Demonstrated clinical health outcomes

The use of probiotics to enhance host resistance is a strategy aimed at exploiting the symbiotic relationship between enteric bacteria and their host. Enteric bacteria colonise the GI tract in increasing numbers from the stomach to the colon, where they are estimated at numbers in the order of 10^{14} in the colon. Only a small number of these bacteria can be cultured, and the identity of the vast majority of intestinal micro-organisms remains unknown (105). Enteric bacteria play a number of roles in human health and physiology. They are essential for the developing immune system following birth. Comparisons of the immune system between germ-free and wild type animals show that germ-free animals have fewer dendritic cells, cytotoxic T cells and plasma cells within the mucosa (44, 93), and greater abnormalities in immune function (45). Enteric microbial flora contribute to host resistance by creating ecological niches throughout the mucosa to prevent pathogenic micro-organisms from colonising mucosal surfaces (49). Enteric microbial flora achieve this "competitive exclusion" by limiting the surface area available

for attachment, reducing the availability of nutrients to exogenous pathogens, and secreting antimicrobial substances, such as bacteriocins. Interactions between enteric bacteria and host cells also stimulate the host immune system (56). Experimental evidence from animal models suggests that dendritic cell sampling of the luminal microbial flora and bacterial products such as LPS stimulate poly-specific IgA plasma cells in the mucosa (12, 62, 79). Enteric microbial flora also induce the secretion of multiple innate antimicrobial factors from paneth cells (103). SIgA and antimicrobial proteins are key effectors of mucosal immunity against invading pathogens (9, 10, 83).

Enteric bacteria are also involved in metabolic functions, specifically by producing substrates from the fermentation of non-digestible starches. These starches are the primary source of energy for enteric microbial flora. *In vitro* and *in vivo* studies show that when bacteria ferment these carbohydrates, short chain fatty acids (SCFA) such as butyrate, propionate and acetate are formed as by-products (99). Degradation of peptides and amino acids by bacteria also generates vitamins and enzymes (7, 68). These substances benefit the host in a variety of ways (51). Host cells use SCFAs to reduce DNA damage from bile salts (89), scavenge free radicals (90), and regulate cell activity and differentiation (42). The fermentation of non-digestible carbohydrates by the microbial flora releases energy that otherwise would not be available for absorption, storage and use of energy (25). The composition of the microbial flora differs between obese and non-obese individuals (101), and these differences may determine individual responses to diet and pharmacological agents (57). Given the diverse roles of enteric bacteria in human physiology, consuming probiotics is proposed as a safe and natural method that could be used prophylactically and therapeutically to improve host resistance to illness.

Probiotics and immune function
Interest in the use of probiotics to improve health focuses largely on their potential ability to modulate various factors of the immune system. To function as effective prophylactic agents against common illnesses, probiotics must enhance innate and acquired elements of the mucosal immune system (88). As the body's first-line-of-defence the mucosal immune system is central to protection against invading pathogens. The mucosal immune system consists of physical (mucus), molecular (antimicrobial proteins) and cellular components that act synergistically to prevent microbes invading the body. Dys-regulation or impairment of the mucosal immune system is implicated in a range of inflammatory illnesses, such as ulcerative colitis and celiac disease, as well as increased susceptibility to cancer and infection (20).

Physical Barriers
In vitro studies show that the probiotics *L. plantarum 299v* and *L. rhamnosus GG* increase the expression of MUC2 and MUC3, and mucin secretion from intestinal epithelial cells. In turn, this mucin inhibits the adherence of pathogenic microbes (59, 60). The mucus barrier, along with the periciliary fluid layer, is composed of a variety of substances including proteins, fats, sugars and water that physically trap and remove pathogens as they come into contact with the mucus

layer (58, 104). The effect of probiotics on mucin secretion is not uniform, however. *B. infantis* and *L. salivarius* do not alter MUC3 expression in HT-29 human colonic epithelial cells (74). Antimicrobial proteins and secretory immunoglobulins further enhance the protective function of mucus. Antimicrobial proteins are a constituent component of mucosal surfaces that are secreted by respiratory tract epithelia and glands (27). Probiotics can increase the concentration of human β-defensin at mucosal surfaces. Dietary supplementation with several *E-coli* genotypes in 23 healthy individuals increased fecal human β-defensin-2 content by up to 80% for several weeks (69). Other probiotic strains produce similar effects *in vitro* (69).

Experimental evidence suggests that probiotics can modulate GI permeability. The GI barrier is comprised mainly of individual epithelial cell membranes that are impermeable to hydrophilic solutes except at sites of specific transporters (102). The space between epithelial cells is controlled by intercellular junctions that regulate the movement of solutes through the paracellular space. The permeability of these tight junctions regulates the overall barrier function of the intestinal epithelium (102). Damage to enterocyte membranes and tight junctions, coupled with alterations in mucous secretion and immune cell activity can increase the permeability of the GI barrier to molecules >150 kDa in size. Increased GI permeability permits the translocation of luminal antigens, including enteric microbial flora, into the systemic circulation. This translocation induces immune responses within the mucosa, and may subsequently lead to chronic inflammation.

Probiotics can enhance epithelial barrier function *in vitro* and animal models (63). Probiotics protect human epithelial cell cultures (HT29) and T84 cells against damage resulting from exposure to *E. coli* (84, 111). Probiotics also protect these cell types against damage from TNF-α, IFN-γ and non-steroidal anti-inflammatory drugs by maintaining ion pump activity and the integrity of tight junctions, and activating mitogen-activated protein kinases and heat shock protein-70 (29, 70, 80, 84, 97). Animal models provide further evidence that probiotics can reduce GI permeability. Dietary supplementation with *L. paracasei NCC2461* (Lpa) (but not *B. lactis NCC362* or *L. johnsonii NCC533*) restored GI permeability following restraint stress and maternal separation stress in rats (28). A number of clinical studies, however, have reported that that probiotics do not alter gut permeability. One to two weeks of supplementation with various formulations of probiotics and prebiotics did not alter GI permeability in critically ill patients (1, 46) or burns patients (75). Further research is needed to investigate whether probiotics enhance barrier function in healthy people.

Cellular Changes
Probiotic interaction with intestinal epithelial cells and immune cells elicits changes in cell phenotype, the secretion of cytokines and the activation/suppression of intracellular signaling pathways, all of which modulate host resistance. A key function of the mucosal immune system is controlling responses to luminal antigens to limit inflammation. This state of control is achieved by regulating the balance of Th1:Th2 cytokines in favour of Th2 cytokines, which

include IL-2, TGF-β, IL-6 and IL-10 (24, 91). Probiotic bacteria induce the secretion of cytokines from intestinal epithelial cells in a strain-specific manner (88). One study found that two of five *L. plantarum* strains (BFE 5759 and BRE 1685), and one of two *L. johnsonii* strain (BFE 6128) stimulated HT29 intestinal epithelial cells to secrete IL-8 (106). In contrast, *L. rhamnosus GG* and *L. paracasei* down-regulate IL-8 secretion (106), whereas *B. infantis, L. salivarius, B. longum* Bar33 and *L. acidophilus* do not alter IL-8 secretion from HT-29 cells (74). Further to this, an investigation of the effect of non-pathogenic bacteria on cytokine responses of Caco-2 cells, an *in-vitro* human adenocarcinoma enterocyte-like cell model, reported that *L. johnsonii* increased expression of transforming growth factor (TGF-β) mRNA (38). These data suggest that probiotic interaction with intestinal epithelial cells play an essential role in shaping the mucosal immune environment.

Immune cells resident in the mucosa, such as dendritic cells and mononuclear phagocytic cells, also strongly influence the mucosal milieu. Mucosal dendritic cells sample luminal contents and stimulate Th1, Th2 or Th3 responses (50). *Ex vivo* studies indicate that probiotic supplements induce anti-inflammatory cytokines from mucosal dendritic cells, and down-regulate co-stimulatory molecules. One study incubated human intestinal dendritic cells with the cell wall components of the bacterial strains in the probiotic VSL#3 (VSL#3 Pharmaceuticals, Fort Lauderdale, FL, USA). VSL#3 combines four Lactobacillus strains, three *Bifidobacteria* strains and a *Streptococcus* strain. Following exposure to these bacterial strains (at a dose of 10^8 CFU/ml) the dendritic cells produced IL-10 and expressed fewer CD80 receptors (40). Individual strains within VSL#3 elicited different effects. All *Bifidobacteria* strains stimulated the dendritic cells to produce IL-10, with a dose-response effect evident from amounts of 10^6 to 10^8 CFU. In contrast, no *Lactobacillus* strains altered IL-10 production (40).

Obtaining tissue from the GI mucosa is highly invasive. Animal models are therefore utilised to further understand the way probiotics affect intestinal immune cells. Studies of murine dendritic cells indicate that probiotics stimulate dendritic cells to produce IL-12, TNF-α, IL-6 and IL-10 in a strain-specific manner, and with dose-dependent effects (14). These studies demonstrate that probiotics act directly on immune cells within the mucosa to modulate immunity.

Substantial evidence exists that probiotic bacteria modulate various aspects of mucosal immunity through their interaction with intestinal epithelial cells and immune cells (38, 50, 79, 106). Determining probiotic contribution to mucosal homeostasis and host resistance is, however, complex given the number of other mechanisms that regulate immunity in the mucosa. The roles of various cells in the mucosal immune system, such as dendritic cells, regulatory T cells and macrophages, and the way these cells interact to influence mucosal homeostasis, is not yet fully understood (17, 85). The complexity surrounding mucosal homeostasis is evident when considering the interaction of immune cells with dendritic cells. A recent study identified a new subset of NK cells that secrete IL-22, a cytokine that has anti-inflammatory effects on epithelial cells *in vitro* (11). The secretion of IL-22 to down regulate epithelial activity contrasts with earlier data

indicating that lymph node NK cells secrete the inflammatory cytokine IFN-γ (11). Determining the role probiotics play in the mucosa is further complicated by evidence that bacteria-bacteria interactions influence the way in which probiotics affect the mucosa, with bacterial strains from the same species having inhibitory effects on each other (14). The complexity of the mucosa, and its inaccessibility for sampling, makes it to difficult to determine the effects of probiotics on mucosal immunity.

Systemic Immunity

Extensive research has examined the effect of probiotic supplementation on systemic immune parameters in healthy subjects to determine whether probiotics enhance systemic immunity. Probiotics can enhance the number and activity of granulocytes and lymphocytes, and the secretion of cytokines in response to stimulation. Granulocytes constitute about 60% of white blood cells, and are an important non-specific component of immune defenses. Studies in healthy humans provide a mixed picture of how these cells respond to probiotic supplementation, even when examining the same species of probiotic.

Supplementation with different doses (10^8–10^{11} CFU per day) of the bacteria *B. lactis* BB-12 (BB-12) and *L. paracasei* CRL-431 (Chr Hansen A/S, Horsholm, Denmark) in healthy adults did not alter total blood phagocytic activity, or the phagocytic activity of the granulocyte and monocyte cell fractions of blood to opsonised *E. coli in vitro* (15). Other studies report that supplementation with *L. acidophilus* and *B. lactis* HN019 in healthy subjects significantly increased the phagocytic activity of polymorphonuclear cells (4, 52, 87). Whether the difference in outcomes between these studies is related to different methods of measurement, antigens used to stimulate cells or the strain and viability of the probiotic is uncertain. Probiotic consumption may also enhance anti-viral protection. Consuming a low fat milk drink with *B. lactis* HN019 increases natural killer cell activity (13). Consumption of a yoghurt with the added probiotics *L. gasseri* CECT5714 and *L. coryniformis* CECT5711 resulted in a significant 21% increase in the number of NK cells from pre- intervention to post-intervention. NK cell numbers were 36% higher following supplementation than in a control group consuming yoghurt without added probiotics (76). The effect of probiotics on NK cells is not uniform. A study examining the consumption of milk fermented with a total of 3×10^9 *L. casei Shirota* by 20 healthy male subjects over a four week period reported no effect on natural killer cell activity (94). Further randomized controlled trials are required to clarify which strains and doses of probiotic bacteria are most effective for stimulating non-specific cellular immunity.

Probiotics can also positively affect specific immunity. In one study, 33 healthy young women consumed 100 g of either yoghurt with starter cultures, or yoghurt with added probiotics for two weeks. All of the women then consumed 200 g of each preparation for another two weeks. In the probiotic group, the number of circulating cytotoxic T lymphocytes increased by 31% after the first two weeks, by 22% after the second two weeks on 200 g of yogurt, and by a further 33% after a two week washout period (67). Whether these responses are physiologically and clinically beneficial is questionable. Only the group consuming the conventional

yoghurt, rather than the probiotic-enriched yoghurt, showed a higher percentage of T cell activation (both CD4+ and CD8+) following stimulation with pokeweed mitogen (67). Probiotic supplementation also enhances antibody responses to immunisation. In a study of 477 healthy men and women vaccinated against influenza, subjects receiving a probiotic supplement presented with increased proportions of CD4+T and CD8+T cells, as well as higher leukocyte counts than the placebo group (110). Plasma cell activity and serum (but not secretory) IgA concentration also increased (110). Finally, consuming a probiotic supplement with several bacterial strains of the *Bifidobacteria* and *Lactobacillus* species also increases serum IgG concentration (77).

Enhanced cellular activity with probiotic supplementation may involve increased production of constitutive cytokines released from the interaction of probiotic bacteria and host cells in the mucosa and lymph nodes. *In vitro* studies indicate that probiotic bacteria stimulate immune cells to produce cytokines, but evidence for this stimulatory effect *in vivo* is less consistent. An investigation of the effects of three milk-based probiotics (*L.rhamnosus, B. lactis* Bb12 and P. *freudenreichiii ssp. Shermanii JS*) in healthy adults reported no substantial differences in the serum concentrations of TNF-α, IL-6, IFN-γ and IL-10 after 21 days of supplementation compared with a placebo group (47). This finding is consistent with evidence from our laboratory showing that 77 days of probiotic and placebo supplementation in a cohort of 80 athletes did not substantially alter the resting serum concentrations of IL-6, IL-8, IL-10, IL-12, IFN-γ, TNF-α or granulocyte macrophage colony stimulating factor between the groups (West et al, unpublished observations). Probiotic supplementation alters cytokine production *ex-vivo* in response to antigen-challenge. This effect appears to be strain- and antigen-specific. Following 21 days of supplementation with *B. lactis* Bb12, IFN-γ secretion was significantly lower in peripheral blood lymphocytes stimulated with lipopolysaccharide (LPS), but not phytohaemagglutin-P (PHA-P) or a mixture of LPS/PHA-P (15). In contrast, consumption of conventional yoghurt or probiotic-enriched yoghurt (*L. casei* DN 114 001) stimulated cytokine production in cultured blood cells from 33 healthy women. TNF-α production increased by 24% in the conventional yoghurt group, and by 63% in the probiotic group. IL-1β increased by 40% in the conventional yogurt group, whereas INF-γ increased by 108% in the probiotic group only (66). IL-10 production decreased by 26% in the probiotic group, and then increased 129% two weeks after supplementation. Furthermore, another study reported stimulation of peripheral blood mononuclear cells with *S. pyogenes* significantly increased TNF-α production in response to *Streptococcus* challenge, but not in response to influenza or LPS challenge in healthy adults who consumed *L. rhamnosus GG* and not *B. lactis Bb-12* or *P. freudenreichii* ssp.*shermanii* JS. IL-2 production increased significantly in response to influenza (but not *Streptococcus* or LPS) only in those individuals who consumed *B. lactis Bb-12* (47). Collectively these results indicate that probiotics alter serum cytokine responses to infectious challenge, but some strains are more effective than others.

Probiotics can counteract stress-induced perturbations in immunity, which suggests they may benefit athletes. Consumption of milk containing yoghurt cultures

with added *L. casei* DN-114001 ($2x10^8$) by students undergoing academic examinations significantly increased the number of lymphocytes in peripheral blood and prevented a fall in the number of NK cells compared with a control group who did not consume probiotics (64). Exercise reduces circulating NK cell numbers, which may increase susceptibility to illness (37, 43). Probiotic supplementation can provide greater benefit to those individuals with a poor pre-intervention immune status (31). In one study, 30 healthy elderly volunteers consumed 200 ml of low fat milk enriched with either 5×10^9 or 5×10^{10} for three weeks. The greatest changes in CD4+ helper cells, CD8+ T cells, NK cells and *ex vivo* granulocyte phagocytic activity occurred in those individuals with the poorest pre-supplementation immune status (31). These findings may provide justification for athletes to use probiotics if they suffer from recurrent illness, or they have lower immune function (16, 18).

Probiotics and illness in athletes

To date, only three studies have examined the effects of probiotics in athletes. A double-blind, placebo-controlled cross over trial investigated the use of *L. fermentum* VRI-003 (Progastrim, Probiomics Ltd) in 20 elite male runners over a four month winter training season. Athletes taking the probiotic supplement reported less than half the number of days of respiratory symptoms during the supplementation period (30 days) compared with the placebo group (72 days). Illness severity was also lower for episodes occurring during the supplementation period (19). In contrast, two studies, one in athletes and one in military cadets, report mixed findings. A randomised double-blind intervention study in which 141 runners took either a placebo or *L. rhamnosus* for three months leading into a marathon reported no significant difference in either respiratory tract illness, or GI symptom episodes, in the two weeks after the marathon (48). There was, however, a trend toward shorter duration of GI symptom episodes in the probiotic group. Probiotic supplementation with *L. casei* DN-114001 by 47 French commando cadets during a three week training course, followed by a five day combat course, had little effect on the incidence of respiratory tract illness (98). These initial studies in athletes do not provide compelling evidence that probiotics are of substantial clinical benefit in highly active individuals.

Positive results with probiotic supplementation in a number of non-athlete settings may justify their use by athletes under certain conditions. Initial interest in the use of probiotics evolved from efforts to prevent diarrhea, particularly in children. In developing countries, diarrhea is a cause of high mortality in children under five (8). In developed countries, acute diarrhea often precedes irritable bowel conditions and the development of Crohn's disease. Strong interest also exists in preventing other forms of acute diarrhea, particularly traveler's diarrhea. A meta-analysis of the available data from 34 masked, randomised, placebo-controlled trials, indicated that probiotics significantly reduced antibiotic-associated diarrhea, the risk of traveler's diarrhea and acute diarrhea from diverse causes (92). The probiotic strains *S. boulardii, L. rhamnosus GG, L. acidophilus, L. bulgaricus* all produced positive benefits. Traveler's diarrhea during international competition is a concern for elite athletes. A meta-analysis of 12 trials indicated that probiotics reduced the occurrence of traveler's diarrhoea (65). *S. boulardii*

and a mixture of *L. acidophilus* and *B. bifidum* were identified as providing particular benefit. Probiotics may prevent stress-induced gastrointestinal illness. An 80 day investigation comparing the use of a probiotic supplement (Probio-Stick containing 3×10^9 of *L. acidophilus* Rosell-51 and *B. longum* Rosell-175) on GI illness in 75 employees found that probiotic supplementation reduced abdominal pain and nausea/vomiting (26). A recent Cochrane Review has supported these findings and concluded that probiotics reduce the risk of diarrhoea as well as the mean duration of diarrhoea (2).

Several placebo-controlled clinical studies have examined the efficacy of probiotics alone (19, 23) and in combination with other purported preventative agents, such as prebiotics (81), in relation to common illnesses, such as URTI and GI illness. These studies generally indicate that probiotic supplementation may reduce the number, duration and severity of illness compared with placebo supplements. The specific effect of probiotics is obscured by the inclusion of other additives, many of which have not been studied as anti-infective agents in their own right. A randomised, double-blind, placebo-controlled intervention trial of 479 healthy adults taking vitamins and minerals alone, or in combination with 5×10^7 of *L. gasseri, B. longum and B. bifidum* over two winter/spring periods, reported a significant 22% reduction in the duration of illness episodes, and a 10% reduction in severity score in the probiotic group (23). A study of 721 healthy adults over three winter periods that compared a range of synbiotic formulations containing probiotics, also reported favourable results. Each stage utilised a combination of probiotic bacteria (*L. plantarum, B. lactis* and *L. rhamnosus*) with additional gut health nutrients (lactoferrin, fructooligosaccharides and galactooligosaccharides). The synbiotic supplements reduced the number of illness episodes (acute respiratory, flu and colds) by 30–45%, and also reduced the illness episode duration, days of illness per person, and the severity of illness (81). Unfortunately, none of the preparations in this study utilised probiotics as a supplement on their own. These studies indicate that probiotics may reduce the pattern of URTI in healthy populations. Further research involving athletic groups is needed before definitive recommendations can be made about the efficiacy of probiotics for preventing illness in athletes.

Considerable disagreement exists as to whether immunological or clinical endpoints should determine efficacy of immuno-nutrition trials (30, 54). Ultimately the decision to take probiotics should be based on their ability to reduce susceptibility to illness, or to alter the pattern of illness (duration and severity). A number of confounding factors may obscure clinical outcomes in research projects, however, meaning that both clinical and physiological endpoints must be considered. These confounders include the multi-factorial etiology of infectious illness, the unpredictable nature of exposure to pathogens, the low prevalence of URTI and GI episodes that necessitate large subject numbers, and human subject compliance to the dosing regimen (30, 54). Utilising only clinical endpoints may result in unnecessarily discounting the value of probiotics for improving host resistance. A change in physiological endpoints, such as cytokine production, phagocytic activity and natural killer cell activity, provides insight into the mechanism by which probiotics may enhance host resistance to infection and should be a factor

in justifying their use. Furthermore, patient value-benefit, which refers to the smallest important benefit from a supplement user's perspective, will also determine the use of probiotics. A study examining the smallest important benefit from a treatment for the common cold indicated that a reduction of 25–75% in illness severity is required to justify costs, risks and harm (5). Evidence from studies of probiotics and URTI indicates benefits in the range of 20–40% for differences in episodes of respiratory illness, number of days of illness per person and average duration of an illness episode. In the case of athletes, the smallest worthwhile effect may be substantially less, considering that compromised performance may be the cost of illness.

Future directions

Further *in vivo* studies are required to clarify the mechanistic and clinical effects of probiotics in athletes. These studies need to clarify the most appropriate strain, dosage, duration and timing of supplementation. Issues regarding route of administration also deserve attention. The use of a spray or probiotic-enriched drink formula to localise the probiotic effect to the upper respiratory tract may be more effective in preventing URTI. Clarifying these issues will require a series of well-designed placebo-controlled and comparative studies in laboratory and field settings. With the increasing use of molecular biology techniques, these studies also need to examine the composition of the intestinal microbial flora at the individual level. This consideration is important given the effectiveness of probiotic treatment may depend on the composition of an individual's microbial flora (100, 109). This approach may provide support for personalising probiotic use. It is not feasible to undertake individual profiling on each and every athlete, yet this level of investigation may be appropriate for athletes who experience recurrent illness.

An extensive amount of information exists on the effects of probiotics on immune factors *in vitro*, from experimental animal models and from non-healthy populations (particularly critically ill populations and those suffering chronic illness such as irritable bowel disease). Currently there is a lack of information on the effects of probiotics on the mucosal milieu of healthy individuals. A need exists to examine whether changes in intestinal microbial flora with probiotic supplementation enhance basal mucosal immune status. Markers of host resistance worth investigating include GI permeability, salivary immune markers (SIgA) and circulating immunoregulatory cytokines (IL-6, IL-8, IL-10, TGF-β, IFN-γ).

CONCLUSIONS

Symbiotic interactions between the microbial flora inhabiting the GI tract and the mucosal environment are essential for normal growth and development, host resistance and metabolism. The interactions between the microbial flora and the host immune system have generated interest in investigating whether modulating various bacterial populations of the microbial flora can improve health and reduce susceptibility to illness. Athletes undertaking prolonged intense exercise may be more susceptible to illness from exercise-induced immunosuppression. There is

strong interest among the sporting community in the potential benefits of probiotics to reduce susceptibility to URTI and GI illness. Some probiotics strains may confer benefit in the GI and respiratory tracts in both athletic settings and in the general population. However, before definitive recommendations for athletes can be developed, further studies are required to determine the efficacy of probiotics on illness, and also to elucidate the mechanisms by which probiotics enhance immunity.

ACKNOWLEDGEMENTS

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