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Original Article

Probiotic effects as therapeutic anti-stress treatment for athletes under the influence of stress and anxiety on gut microbiota: A Brief Review

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Abstract:
The aim of this study is to review and discuss the probiotic effects as therapeutic treatment under the influence of stress on gut microbiota. A comprehensive search of the literature was electronically performed in the PubMed/Medline, ISI Web of Knowledge, Scopus and The Cochrane Library databases from their inception up to June 2018, using various combinations of the following keywords: 'Probiotic', 'Prebiotics', 'Gut microbiota' combined with 'sport psychology', 'athletes', 'stress' and 'anxiety'. Furthermore, references cited in these articles were considered in the case of limited information regarding specific topics. In addition, one book chapter on sport psychology dealing with sports medicine aspects as well as prebiotics and gut microbiota were also considered. An additional study using specialized terminology associated to the keywords 'psychology', 'mood states' or 'anxiety disease' was performed for particular chapters of this review.
Key words: microbiome; microbiota; sport psychology; prebiotics; nutritional sciences.

Introduction

Competitive sport has the potential for high levels of anxiety and stres (Hanton, Mellalieu, & Williams, 2015). Anxiety is defined as an unpleasant psychological state in reaction to perceived stress concerning the performance of a task under pressure (Cheng, Hardy, & Markland, 2009). Cognitive stress and anxiety are common emotional states experienced by athletes at high performance levels. In general, stress and anxiety are effects of cognitive (i.e. apprehensions and worrying thoughts) or somatic (i.e. degree of physical activation) components. In a sporting context, anxiety and stress are often regarded as typical responses to a situation where an athlete's skills are being evaluated (Smith, Smoll, & Schutz, 1990). Stress and anxiety are characterized by a range of physiological (i.e. sweating, increased heart rate), behavioral (i.e. biting fingernails, fidgeting), and/or cognitive (i.e. negative thoughts, lack of focus) signs and symptoms. Various treatments may be suggested for reducing stress-related and anxiety-related disorders, especially those associated with nutrition, and particularly with the use of probiotics.

Probiotics are defined as live microorganisms that exert health properties on the host through microbial actions when ingested (Bravo et al., 2012; Luyer et al., 2005). These properties may be of assistance in preserving gut barrier integrity after training and stress situations (Cryan & Dinan, 2012). The 'healthy' gut microbiota is perceived to be a constant community, there are moments and situations within the training plan in which changes can occur in the structure and function of this microbiota, considering that gut microbiota contain up to 100 trillion micro-organisms in an adult, including at least 1,000 different species of known bacteria, with more than 3 million genes (Cryan & Dinan, 2012; Qin et al., 2015).

These live microorganisms are a heterogeneous group of microbes in which a variety of *Lactobacillus* spp. and *Bifidobacterium* spp. are used in probiotic therapy models, which inhibit the inflammatory response of bowel disease and pouchitis (Luyer et al., 2005; Madsen, 2001; Osman, Adawi, Ahrne, Jeppsson, & Molin, 2004). A novel insight was provided by a study showing that probiotic DNA plays a crucial role in the observed protection of probiotic therapy in experimental colitis via a Toll-like receptor 9 (TLR9) signaling pathway (Rachmilewitz et al., 2004). Other studies have shown inhibition of pathogen adhesion and production of antimicrobial metabolites, which are believed to be important characteristics of viable probiotic strains, and selection of those strains is often based on these properties (Luyer et al., 2005; Rolfe, 2000).

Mental health and psychological factors such as mood state of elite athletes impact sport participation (Rice et al., 2016). Mood disorders are linked with gastrointestinal disease (Forsythe, Wang, Khambati, & Kunze, 2012) and several studies have specifically indicated that stressful events are associated with anxiety and

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depression depending on the genetic background (Caspi et al., 2014). Chronic administration of *Lactobacillus rhamnosus* (JB-1) alters GABAR expression in the brain, and reduces anxiety-like and depressive behavior (Bravo et al., 2011). There is emerging evidence from preclinical studies of a role for gut microbiota on the central nervous system function (Bharwani et al., 2016; Mayer, Tillisch, & Gupta, 2015).

Neural increase and function is extensively discussed in previous contributions indicating the systemic role of the microbiome (Bharwani et al., 2016; Mayer et al., 2015). Studies on probiotic treatments are very recent (Luyer et al., 2005; Rachmilewitz et al., 2004) and a well-established summary of the available literature to deliver clearer insight into the implications of probiotic effects as therapeutic anti-stress treatment also requires extensive knowledge about the functional relationship between the microbiota and stress-induced alterations. Therefore, the aim of this study is to review and discuss the probiotic effects as therapeutic treatment under the influence of stress on gut microbiota.

Material & methods

Procedure

A comprehensive search of the literature was electronically performed in the PubMed/Medline, ISI Web of Knowledge, Scopus and The Cochrane Library databases from their inception up to June 2018, using various combinations of the following keywords: 'Probiotic', 'Prebiotics', 'Gut microbiota' combined with 'sport psychology', 'athletes', 'stress' and 'anxiety'. Furthermore, references cited in these articles were considered in the case of limited information regarding specific topics. In addition, one book chapter on sport psychology dealing with sports medicine aspects as well as prebiotics and gut microbiota were also considered. An additional study using specialized terminology associated to the keywords 'psychology', 'mood states' or 'anxiety disease' was performed for particular chapters of this review. It provides a summary of the available literature examining the influences of stress and anxiety and their relationship with the intestinal microbiota. The benefit of probiotics for athletes shown in this article generates the need for future research to test different probiotics and to evidence cause and effect relationships on stress and anxiety of athletes.

Results

Sports psychology defines stress and an anxiety state as the effect of an organism which is feeling an environmental demand or pressure (Aguilera, Vergara, & Martinez, 2013; Pusceddu et al., 2015). There is representative evidence showing the adverse impact of stress anxiety on physiology and neurocognitive correlations throughout development of the mental and physical responses (Bharwani et al., 2016; Gilbert, 2015). Acute and chronic factors of stress such as anxiety, depression, trauma and abuse through early life increase the risk of psychiatric conditions (Dinan, Cryan, Shanahan, Keeling, & Quigley, 2010), and inadequate behavior have effects on the development of diseases such as gastrointestinal disorders (Dinan & Cryan, 2012). Preceding reports have shown an essential symbiosis to this host function, which has been observed as multi-species organisms or "holobionts" (Gilbert, 2015).

Anxiety and stress processes accomplish communication between the brain and the other organs as essential functions through the coordinated activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis and the Sympathetic Adrenergic-medullary (SAM) nervous system, which is responsible for hormones modulation which play a key role in the development and expression of physiological functions and behaviors (Rivière, Gagnon, Weckx, Roy, & De Vuyst, 2015). The HPA axis is activated by a range of stressors on neurons located in the hypothalamus, and it stimulates the synthesis of Corticotropin-releasing hormone (CRH) and arginine vasopressin. Once these hormones are secreted through the pituitary portal system, they reach the pituitary gland by promoting the synthesis and systemic release of adrenocorticotropic hormone. In the adrenal cortex this hormone induces biosynthetic pathway activity glucocorticoids (Münch et al., 2012; Rivière et al., 2015).

The production of glucocorticoids, which are the end product of the HPA axis, has a regulatory effect on its own axis, especially by acting on the basal activity of the hypothalamus and pituitary gland. This activity creates negative feedback circles on secretion of CRH, adrenocorticotropic hormone, and mitigates the stimulation of the SAM nervous system, ending the stress response and promoting maintenance of the homeostasis organism (Münch et al., 2012). Preceding reports have shown that human intestinal flora may influence the development of the HPA axis and thus be linked to the stress response. Animal experiments have recently shown that germfree adult male BALB/c mice had an elevated basal level of CRF gene expression in the hypothalamus and over-reacted to an acute restraint stress by a hyper-secretion of adrenocorticotropic (ACTH) and corticosterone (CORT) hormone compared with specific pathogen-free (SPF) counterparts (Sudo et al., 2004). Such a maladaptive response in GF mice to stress was partly corrected by gut microbiota reconstitution with fecal bacteria from SPF mice. Interestingly, this treatment was only effective when the GF mice were colonized at six weeks but not at 14 weeks of age, suggesting the existence of a critical period for programming the reactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Sudo et al., 2004).

GABA is the main CNS inhibitory neurotransmitter and is significantly involved in regulating many physiological and psychological processes. Alterations in central GABA receptor expression are implicated in the pathogenesis of anxiety and depression, which are highly comorbid with functional bowel disorders (Fow & Grossman, 2007). Preceding reports have shown that chronic treatment with *L. rhamnosus* (JB-1) induced region-dependent alterations in GABA(B1b) mRNA in the brain with increases in cortical regions (cingulate and

prelimbic) and concomitant reductions in expression in the hippocampus, amygdala, and locus coeruleus in comparison with control-fed mice (Bravo et al., 2011). In addition, *L. rhamnosus* (JB-1) reduced GABA(Aα2) mRNAexpression in the prefrontal cortex and amygdala, but increased GABA(Aα2) in the hippocampus (Cryan & Dinan, 2012). Importantly, *L. rhamnosus* (JB-1) reduced stress-induced corticosterone and anxiety- and depression-related behavior (Cryan & Dinan, 2012). Together, these findings highlight the important role of bacteria in the bidirectional communication of the gut-brain axis, and suggest that certain organisms may prove to be useful therapeutic adjuncts in stress-related disorders such as anxiety and depression in athletes (Bravo et al., 2011; Dinan & Cryan, 2012). During competitive moments and hard training, exposure to stress alters the structural composition of the intestinal microbiota, where previous authors have shown that early life stress increased the number of fecal boli in response to a novel stress, where the plasma corticosterone was increased in the maternally divided animals and an increase in the systemic immune response was noted in the stressed animals after an in vitro lipopolysaccharide challenge (O'Mahony et al., 2009). These findings demonstrated an altered brain-gut axis and an important model for future investigations about potential mechanistic insights into stress-related disorders including depression and irritable bowel syndrome. The current model to explain the mechanism of irritable bowel syndrome includes both central and end-organ components which may be combined to create an integrated hypothesis incorporating psychological factors (stress, distress, affective disorder) with end-organ dysfunction (motility disorder, visceral hypersensitivity), and therapeutic approaches include anti-inflammatory agents, antibiotics, probiotics, antagonists of CCK1 receptors, tachykinins and other novel neuronal receptors (Farthing, 2004). For instance, relevant contributions on the usefulness of probiotic preparations in the treatment and prevention of pouchitis have been published (Cabré & Gassull, 2003).

However, the etiology of inflammatory bowel disease probably involves a combination of genetic predisposition and environmental factors that may be channeled through an abnormality in gut-barrier function, with a loss of antigen tolerance. Some genetic markers that predispose the intestines to inflammatory disease have been identified (alleles DR2, DRB1*0103, DRB1*12 and mutations in the NOD2/CARD15 gene on chromosome 16) (Thompson-Chagoyán, Maldonado, & Gil, 2005). Alterations in the cytokine production pattern by T cell subclasses leading to a loss of tolerance to oral antigens have been documented. Moreover, a number of environmental factors (e.g., the use of non-steroid anti-inflammatory drugs, psychological stress and the presence of the caecum appendix) have been postulated as a trigger of inflammatory bowel disease. It has also been suggested that the gut microbiota plays a major role in the development and persistence of inflammatory bowel disease, and numerous modifications in intestinal microbiota composition have been identified (Thompson-Chagoyán et al., 2005). Manipulation of the microbiota with antibiotics is a current therapeutic strategy for athletes; however, more recently a number of studies have reported promising results when using probiotic organisms to manipulate gut microbiota composition in order to restore tolerance to microbial antigens of the host's own microbiota (Thompson-Chagoyán et al., 2005).

Therefore, it has long been recognized that the intestinal microbiota affects human behavior modulation (Bailey et al., 2011), as an alteration to intestinal microbiota have been linked to inflammatory diseases such as asthma, suggesting that the microbiota interact with the immune regulation (Huffnagle, 2010). Altered immunity is associated with psychosocial stress in sports (Ramirez et al., 2015). Remarkable progress has been made in characterizing the bidirectional interactions between the central nervous system, the enteric nervous system, and the gastrointestinal tract, and a series of provocative preclinical studies have suggested a prominent role for the gut microbiota in these gut-brain interactions (Mayer et al., 2015).

The digestive tract mechanism works over a multifaceted network of integrative utilities. At the gut level, this combination happens between the immune, neuromotor and enteroendocrine systems, managing the physical and chemical factors of the intestinal barrier in order to simplify digestion while defending the gut from unwelcome elements of the luminal contents. However, the central nervous system ensures effective motility, secretion, absorption and mucosal immunity controlling and coordinating this gastrointestinal function. Lately, attention has been aimed at the emerging suggestion that gut luminal content contributes to regulate normal GI function and on the beneficial chances rising from modulating its influence on gut physiology and immunity of athletes using probiotic bacteria (Verdu, 2009). In this issue of Neurogastroenterology and Motility, recent findings have indicated effects of specific probiotic bacteria on spinal neuronal activation and in vitro muscle (Dalziel et al., 2017).

Probiotic effects as therapeutic anti-stress treatment for athletes

In the early 1900s, Eli Metchnikoff, a Russian scientist of the Pasteur Institute in Paris associated longevity of rural Bulgarians to their consumption of fermented milk products, indicating that the *lactic acid* bacteria in the fermented milk products ingested by these peasants provided an antiaging effect; it was called *Lactobacillus bulgaricus* (Cresci & Bawden, 2015). Pioneer reports showed that fermenting flora (probiotic flora) was deranged owing to disease or antibiotic treatment and a new concept of ecoimmune nutrition was presented for enteral supply of mucosa-reconditioning ingredients with new surfactants, pseudomucus, fiber, amino acids such as arginine, and mucosa-adhering *Lactobacillus plantarum* 299 (Bengmark & Gianotti, 1996). The increasing interest in a healthy diet is stimulating innovative development of novel scientific products in the food industry. Viable *lactic acid* bacteria in fermented milk products such as yoghurt have been associated with

increased lactose tolerance, a well-balanced intestinal microflora, antimicrobial activity, stimulation of the immune system and antitumor, anticholesterolaemic and antioxidative properties in human subjects.

Therefore, intestinal microbiota comprises a varied group of functional microorganisms, including candidate probiotics or viable microorganisms that benefit the host. Positive effects of probiotics contain enhancing intestinal epithelial cell function, protecting against physiologic stress, modulating cytokine secretion profiles, influencing T lymphocyte populations, and enhancing antibody secretion (Thomas & Versalovic, 2010). Accumulating evidence demonstrates that probiotics communicate with the host by modulating key signaling pathways such as NFkappaB and MAPK to either enhance or suppress activation and influence downstream pathways (Thomas & Versalovic, 2010). Advantageous microbes can intensely alter the physiology of an athlete's gastrointestinal tract, and it may be considered that these mechanisms may have a consequence on new performances (Thomas & Versalovic, 2010).

Regarding physical stress, preceding authors have studied a human *Lactobacillus spp*. strain that possesses antioxidant activity (Kullisaar et al., 2003). The aim of their pilot study was to develop goat milk fermented with the human antioxidative lactobacilli strain, *Lactobacillus fermentum* ME-3, and to test the effect of the fermented probiotic goat milk on oxidative stress markers (including markers for atherosclerosis) in human blood, urine and on the gut microflora (Kullisaar et al., 2003). Twenty-one healthy subjects were assigned to two treatment groups: goat milk group and fermented goat milk group (150 g/d) for a period of 21 d, in which the consumption of fermented goat milk improved anti-atherogenicity in the healthy subjects: it prolonged resistance of the lipoprotein fraction to oxidation, lowered levels of peroxidized lipoproteins, oxidized LDL, 8-isoprostanes and glutathione redox ratio, and enhanced total antioxidant activity (Kullisaar et al., 2003).

Probiotic therapy modulates the composition of the intestinal flora and inhibits the inflammatory response. These properties may be of benefit in preserving the gut barrier integrity after injury or stress. Preceding authors examined the effect of two *Lactobacillus strains* selected for their pathogen exclusion properties on intestinal barrier integrity following hemorrhagic shock (Luyer et al., 2005). Additionally, the responsiveness of the macrophage cell line RAW 264.7 to combined exposure to *Lactobacillus* DNA or oligodeoxynucleotides containing CpG motifs (CpG-ODN) and endotoxin was assessed by measuring tumor necrosis factor alpha (TNF-alpha) release. Rats were administered *lactobacilli* (5 x 10(9) CFU) or vehicle for 7 days and were subsequently subjected to hemorrhagic shock by withdrawal of 2.1 ml blood/100 g tissue. Plasma endotoxin levels, bacterial translocation to distant organs, and filamentous actin (F-actin) in the ileum were determined 24 h later. Rats treated with *Lactobacillus rhamnosus* showed reduced levels of plasma endotoxin (8 +/- 2 pg/ml versus 24 +/- 4 pg/ml; $P = 0.01$), bacterial translocation (2 CFU/gram versus 369 CFU/gram; $P < 0.01$), and disruption of F-actin distribution following hemorrhagic shock compared with non-treated control rats. In contrast, pretreatment with *Lactobacillus fermentum* had no substantial effect on gut barrier integrity. Interestingly, DNA preparations from both *lactobacilli* reduced endotoxin-induced TNF-alpha release dose dependently, whereas CpG-ODN increased TNF-alpha release (Luyer et al., 2005). The pathogen exclusion properties of both *Lactobacillus strains* and the reduction of endotoxin-induced inflammation by their DNA in vitro were not prerequisites for a beneficial effect of probiotic therapy on gut barrier function following hemorrhagic shock. The lack of genetic tools has hindered the development of functional genomic studies in bifidobacteria, like the identification of molecular mechanisms underlying their survival under different environmental challenges. Some of these experimental obstacles have been successfully overcome with the use of proteomics, a set of techniques which are directed to identify all the proteins produced by the cells under a given physiological condition when applied to microorganisms (Ruíz García et al., 2008).

Athletes often increase rates of stress and anxiety because of the need for weight control. Data recently indicated that prebiotic-treated mice showed a lower plasma lipopolysaccharide (LPS) and cytokines, and reduced hepatic expression of inflammatory and oxidative stress markers (Cani et al., 2009) This decreased inflammatory tone was related with lower intestinal permeability and improved tight-junction integrity. Prebiotics amplified the endogenous intestinotrophic proglucagon-derived peptide (GLP-2) production, but the GLP-2 antagonist abolished most of the prebiotic effects (Cani et al., 2009). In addition, preceding reports indicated that pharmacological GLP-2 treatment reduced gut permeability, systemic and hepatic inflammatory phenotype associated with obesity to a similar extent as that observed following prebiotic-induced changes in gut microbiota (Cani et al., 2009).

Physical stress and anxiety and mental stress can alter an athlete's immune system and specific probiotic strains have been identified as beneficial to influence the composition and/or metabolic activity of the endogenous microbiota, and some of these strains have also been shown to inhibit growth of a wide range of enteropathogens and the *Lactobacillus johnsonii* NCC 533 (La1) probiotic bacteria could modulate the cutaneous immune homeostasis altered by solar-simulated UV exposure in humans (Guéniche et al., 2009). Later, with UV exposure to twice 1.5 MED, these authors demonstrated that La1 intake facilitated earlier allostimulatory function recovery of Epidermal cells (Guéniche et al., 2009).

Since *Lactobacillus farciminis* suppresses stress-induced hyperpermeability, previous study examined whether *L. farciminis* affects the HPA axis stress response, as stress induces changes in LPS translocation and central cytokine expression which may be reversed by *L. farciminis* and if the prevention of "leaky" gut and LPS

upload are involved in these effects. The attenuation of the HPA axis response to stress by *L. farciminis* depends on the prevention of intestinal barrier impairment and decrease of circulating portal blood LPS levels (Afifa Ait-Belgnaoui et al., 2012), and of a low pH for *lactobacilli* function as probiotics in nutritional strategies in athletes (Moslehi-Jenabian, Gori, & Jespersen, 2009).

Preceding authors showed effects of a probiotic formulation composed by a combination of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175, called Probio'Stick((R)), which displayed anxiolytic-like activity and reduces apoptosis in the lymbic system in animal models of depression, being based on the hypothesis that modulation of gut microbiota by this probiotic formulation has beneficial effects (A. Ait-Belgnaoui et al., 2014). These authors suggested that chronic stress-induced abnormal brain plasticity and reduction in neurogenesis can be prevented by a pretreatment with the Probio'Stick((R)) formulation, suggesting that probiotics modulate neuroregulatory factors and various signaling pathways in the central nervous system involved in stress response (Dash, Clarke, Berk, & Jacka, 2015). In addition, it has been recognized that the gut microbiota bidirectionally interacts with these elements in anxiety and mental health disorders, as well as with environmental factors such as diet, anxiety, and stress, thereby suggesting promise in the development of interventions targeting the gut microbiota for preventing and treating common mental health disorders in athletes (Dash et al., 2015).

Combinations of probiotic cultures have proven to be more effective than the use of single cultures for treatment and prevention of heterogeneous diseases. A previous study investigated the effect of pre- adaptation of probiotics to multiple stresses on their stability under simulated gastrointestinal conditions and the effect of their singular as well as their synergistic antagonistic effect against selected enteric pathogens (Mathipa & Thantsha, 2015). Probiotic cultures were inoculated into MRS broth adjusted to pH 2 and incubated for 2 h at 37 °C. Survivors of pH 2 were subcultured into 2% bile acid for 1 h at 37 °C. Cells that showed growth after exposure to 2% bile acid for 1 h were finally inoculated in fresh MRS broth and incubated at 55 °C for 2 h. The surviving cells were then used as stress adapted cultures. The adapted cultures were exposed to simulated gastrointestinal conditions and their non-adapted counterparts were used to compare the effects of stress adaptation. The combination cultures were tested for their antipathogenic effects on *Escherichia coli* and *Staphylococcus aureus*. The authors showed that acid and bile tolerances of most of the stress-adapted cells were higher than of the non-adapted cells. Viable counts of all the stress-adapted *lactobacilli* and *Bifidobacterium longum* LMG 13197 were higher after sequential exposure to simulated gastric and intestinal fluids. However, viability of non-adapted cells was higher for *B. longum* Bb46 and *B. bifidum* LMG 13197 than for adapted cells after exposure to these fluids. A cocktail containing *L. plantarum* + *B. longum* Bb46 + *B. longum* LMG 13197 best inhibited *S. aureus*, while *E. coli* was best inhibited by a combination containing *L. acidophilus* La14 150B + *B. longum* Bb46 + *B. bifidum* LMG 11041. Lastly, a cocktail containing the six non-adapted cultures was the least effective in inhibiting the pathogens (Mathipa & Thantsha, 2015).

Lactobacillus ruminis is a motile *Lactobacillus* that is autochthonous to the human gut, and which may also be isolated from other mammals. A recent study suggested that *Lactobacillus ruminis* isolates have potential to be used in functional food (O'Donnell, Harris, Lynch, Ross, & O'Toole, 2015).

Exposure to environmental pollutant 1,2-dimethylhydrazine (DMH) is attributed to systemic oxidative stress and is known to cause neurotropic effect by altering brain neurotransmitter status. Probiotics are opted for as a natural therapeutic agent against oxidative stress and also have the ability to modulate gut-brain axis. Pyrroloquinoline quinone (PQQ) is water-soluble, heat-stable antioxidant molecule. Preceding reports aimed to evaluate the antioxidant efficacy of PQQ-producing probiotic *E. coli* CFR 16 on DMH-induced systemic oxidative damage and altered neurotransmitter status in rat brains (Pandey, Singh, Chaudhari, Nampoothiri, & Kumar, 2015). The results showed that blood lipid peroxidation levels exhibited a marked increase, while antioxidant enzyme activities of superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase and glutathione peroxidase were found to be reduced in DMH-treated rats. Likewise, brain serotonin and norepinephrine levels displayed a significant decrease, whereas epinephrine levels demonstrated a marked increase in the brains of these rats. PQQ-producing *E. coli* CFR 16 supplementation reduced systemic oxidative stress and also restored brain neurotransmitter status. However, *E. coli* CFR 16 did not show any effect on these parameters. In contrast, *E. coli* CFR 16:: vgb-gfp and *E. coli* CFR 16:: vgb-gfp vector exhibited some degree of protection again oxidative stress, but they were not able to modulate neurotransmitter levels. In conclusion, continuous and sustained release of PQQ by probiotic *E. coli* in rat intestine improved systemic oxidative stress and restored brain neurotransmitter levels (Pandey et al., 2015).

In a recent work, the probiotic properties of *Lactobacillus* and *Bifidobacterium strains* were screened, and their in vitro effects were evaluated (Presti et al., 2015). They were screened for probiotic properties by determining their tolerance to low pH and to bile salts, antibiotic sensitivity, antimicrobial activity and vitamin B8, B9 and B12 production, and by considering their ability to increase the antioxidant potential and to modulate the inflammatory status of systemic-miming cell lines in vitro. Three out of the examined strains presenting the most performant probiotic properties, as *Lactobacillus plantarum* PBS067, *Lactobacillus rhamnosus* PBS070 and *Bifidobacterium animalis* subsp. *lactis* PBSO75, were also evaluated for their effects on human intestinal HT-29 cell line (Presti et al., 2015). The findings aimed to verify whether *Lactobacillus paracasei* B21060-based

symbiotic therapy could prevent or repair colon damage in a mouse colitis model, highlighting the beneficial effects of this symbiotic formulation in acutely colitic mice, suggesting that it may have therapeutic and possibly preventive efficacy in human colitis (Simeoli et al., 2015).

Discussion

Such a focus would have both theoretical and applied value, providing researchers and general athletes with advice on optimizing their lives with probiotic use and reducing stress and anxiety inducing situations. Consequently, a summary of the available literature examining the influences of stress and its relationship to intestinal microbiota and probiotic information should stimulate future investigations with strong observational or experimental designs to produce better theoretical implications. Studies on intestinal microbiota are very recent, so systematic errors occurring in testing by selecting or encouraging one outcome or answer over others could easily occur; however, bias is not a dichotomous variable and this kind of subjective research has limitations to be controlled. Interpretation of bias cannot be limited to the question: is bias existent or not in stress situations and microbiota analysis? For instance, the present review indicated recent findings, which revealed that stress situations engage similar microbiota effects, whereas probiotics could reduce possible damage (Bharwani et al., 2016). However, the quantified effect of an amount and intensity of specific stress situations on the microbiota is not yet known. Researchers in the area must consider the degree to which bias was prevented by proper study design and implementation. As some degree of bias is nearly always presented in an experimental study, the authors must also consider how bias might influence a stress or probiotic effect for an anti-stress intervention. Table 1 provides a summary of different effects and probiotic treatments used; it is designed to minimize bias in future study designs. Bias may complicate efforts to establish a cause-effect relationship between procedures of microbiota analysis and the use of probiotic outcomes, as preceding studies have rarely been standardized and study design variation occurs between investigations, but maybe standardization could be performed in the future studies. Nonetheless, research on the impact of probiotic treatments on stress and hormonal adaptations will further advance the microbiota literature. In addition, the majority of the research conducted so far has only used samples drawn from specific populations. It is not clear whether the results observed in these groups can be generalized to healthy athletes. Researchers are encouraged to compare different probiotic intervention methods, and to examine the effects of these interventions for populations in everyday situations. The present review recommends to improve the internal validity referring to the reliability or accuracy of the protocol used in probiotic treatment studies. A study with internal validity reflects the confidence that the study design, implementation and data analysis have minimized the bias, and that the findings are representative of the true association between probiotic treatments and stress reduction.

Conclusions

This brief review synthesized the peer-reviewed studies that have been published to date which identified the effect of stress and anxiety on intestinal microbiota, and the potential moderators of probiotic use to anti-stress action in athletes were also examined. It provides a summary of the available literature examining the influences of stress and anxiety and their relationship with the intestinal microbiota. The benefit of probiotics for athletes shown in this article generates the need for future research to test different probiotics and to evidence cause and effect relationships on stress and anxiety of athletes.

Conflicts of interest - none.

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