



Review Article

Probiotics, gut microbiota and physical activity: A close relationship

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ABSTRACT

Background: The topic of this review is the study of the gut microbiota (GM), and the use of probiotics, especially in humans, as a new frontier in the field of prevention and health in general. The beneficial effects and functions performed by probiotics in the GM are increasingly at the centre of both scientific, medical, and pharmaceutical interest. It is now known that diet and probiotics can modify the GM, although in these situations there is a need for greater and more in-depth research regarding the methods and timing of treatment. However, the relationship between physical activity, GM, and probiotics is still largely unclear, as regards certain mechanisms between physical exercise and probiotics in humans.

Discussion: In this study, we tried to demonstrate whether and how physical exercise was able to alter the composition of the microbiota and how probiotics can facilitate it. Therefore, alteration of the microbiota was considered in terms of both diversity and composition.

Conclusions: The ones examined propose vastly different physical exercises, both in terms of timing and type of intervention itself, and the use of probiotics.

1. Introduction

The World Health Organization has defined probiotics as ‘living microorganisms that can provide health benefits to humans when administered in adequate amounts. Several species such as *Lactocaseibacillus casei*, *Lactopantibacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Streptococcus thermophilus*, *E. coli* strains, have been shown to be involved in immunomodulation and intestinal barrier function.^{1,2} These, such as other species, have been marketed for the treatment of certain diseases, such as irritable bowel syndrome and antibiotic-associated diarrhoea. The main concept in the use of this treatment is to provide an armamentarium that can mimic the physiological function of the ‘friendly’ microbiota in promoting health and modulating gut microbiota (GM).³ The administration of prebiotics can enhance the effects of probiotics. Prebiotics are defined as nutrients that contain non-digestible oligosaccharides, such as galactooligosaccharides (GOSs) and inulin. Prebiotics and probiotics,

when combined, are named synbiotics or symbiotics.⁴ The GM selectively ferments these fibres, producing short-chain fatty acids (SCFAs), leading to positive effects on the health of the host. The GM consists of bacterial communities that have metabolic, immune, and regulatory functions. The relationship between the GM and the health of the body is characterized by interdependence, as a possible change in its homeostasis can cause negative consequences and diseases.^{5,6}

At the same time, living conditions and other environmental factors can lead to changes in the GM. This “vicious” cycle between the balance of GM homeostasis and the health of the body has led to the search for foods that will help maintain this uniformity.⁷

Understanding the structure and environment of the GM is a necessary step for the subsequent evaluation of foods that can potentially favour the healthy conditions of the GM. GM varies depending on the given reference point along the gastrointestinal tract.⁸

Research shows that the microbiota has enormous diversity, and 2 172 species were identified, of which 93.5% belonged to the bacterial phyla: Pseudomonadota, Bacillota, Actinomycetota, and Bacteroidota,

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Abbreviations list

ACSM	American College of Sports Medicine
ANS	autonomic nervous system
CAVI	cardio-ankle vascular index
CFUs	colony-forming units
CNS	central nervous system
ENS	enteric nervous system
GBA	gut-brain axis
GIT	gastrointestinal tract
GLP-1	glucagon-like peptide-1
GM	gut microbiota
GMA	gut/muscle axis
GOSs	galactooligosaccharides
GPRs	G protein receptors
GRAS	Generally Recognized as Safe
HbA1c	glycated haemoglobin
HPAA	hypothalamic-pituitary-adrenal axis
LABs	lactic acid bacteria
ROS	reactive oxygen species
SCFAs	short chain fatty acids
$\dot{V}O_2$	oxygen volume

with many of them being obligate anaerobes.⁷

Among these, the phyla that usually dominate the microbiota are Bacteroidota and Bacillota, while Actinomycetota, Pseudomonadota, and Verrucomicrobia are usually secondary. The number and type of microorganisms vary along its length, with their distribution determined by pH, oxygen, and nutrient availability, digestive flow rates, and secreted enzymes.⁹ For example, the concentration of bacteria in the stomach is relatively low (10 colony-forming units [CFU] per gram), while it steadily increases to (1×10^7) CFU/g in the ileum and (1×10^{12}) CFU/g in the colon. The concentration of microorganisms in the stomach and proximal small intestine is less than (1×10^4) CFU/mL due to the adverse conditions in the GIT (gastrointestinal tract). The bacterial density in the distal small intestine and colon ranges from (1×10^{11}) to (1×10^{12}) CFU/mL and hosts the most microorganisms, respectively.^{10,11}

At the same time, in combination with the modern lifestyle, the health of the average person can be degraded without even realizing it. Excessive intake of antibiotics and the stress that people experience daily become crucial factors that promote intestinal dysbiosis. This disruption of the GM, since its homeostasis is directly related to the normal functioning of the body, can cause various diseases and adverse conditions. Probiotics play a key role in intestinal homeostasis. When the consumption of probiotics is combined with the maintenance of a healthy diet and a corresponding lifestyle, it can contribute to solving these problems at their foundations.¹² However, for probiotics to be able to perform their beneficial actions on the GM, they must survive the adverse conditions that prevail in the intestinal tract to adhere to and colonize the intestinal epithelium. In vitro methods are useful tools through which the changes that occur during gastrointestinal digestion can be studied and understood. They are rapid and economical methods that can predict the survival of probiotic cells immobilized on selected food matrices during gastrointestinal transit.¹³

2. Probiotics functions

As we mentioned, probiotics are defined as “live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host”. They are live microbial food supplements that benefit the health of consumers, with effects aimed at improving the intestinal microbial balance. They are considered Generally Recognized as Safe

(GRAS), i.e., generally recognized as safe for consumption. Probiotics include live cultures, mainly of lactic acid bacteria (LABs) such as *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, and bifidobacteria that have been isolated from natural environments.¹⁴

The most common probiotics belong to the bacterial genera *Lactobacillaceae* family and the genera *Bifidobacterium*, *Bacillus*. Thus, most of them, such as *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc* belong to the group of lactic acid bacteria (LAB). They are important physiological components of the human GM and produce lactic acid as a major metabolic product.^{15,16}

That is, they can convert hexose sugars to lactic acid, reducing the pH and causing an acidic environment. The *Lactobacillaceae* family includes rod-shaped, Gram + bacteria, facultative anaerobes, or microaerophilic. They are selected as probiotics because they are characterized by high tolerance to acids and bile, can adhere to intestinal surfaces, tolerate low pH, and have antimicrobial activity.¹⁵

The genus *Bifidobacterium* includes Gram +, non-motile, anaerobic bacteria. Their habitat is the gastrointestinal tract and vagina of humans. They are selected as probiotics because they effectively resist bile salts. The genus *Lactococcus* includes Gram + lactic acid bacteria to produce fermented products. The physicochemical properties of food carriers affect the growth and survival of probiotics during gastric transit.¹⁷

Metabolically, LABs produce high amounts of lactic acid and other metabolites (metabolome) from various carbon sources such as glucose, fructose, lactose, and galactose. Regarding glucose metabolism, they are classified into homozygotes and heterozygotes. Homozygotes produce exclusively lactic acid while heterozygotes produce many other metabolites such as ethanol, acetic acid, and carbon dioxide. LABs can also produce secondary metabolites such as bacteriocins, exopolysaccharides, and enzymes. The above can contribute to improving the quality and shelf life of fermented foods.¹⁸

Probiotic-enriched foods are defined as products that carry enough viable microorganisms, i.e. at least 10^9 colony-forming units (CFUs) per serving. They must be capable of altering the GM to create healthy effects in the host.^{19,20}

3. Food matrices for probiotics

Probiotic bacterial strains are living organisms that require specific conditions for their survival and growth. For their consumption to provide benefits to the body, it is necessary that it be conducted in the correct way. The consumption of probiotics in an inappropriate manner or after incorrect treatment and storage can kill the microorganisms and thus make them unable to act in the intestine. Therefore, for probiotics to survive during their consumption, they must be in the appropriate carrier.²¹ It is important to determine which physicochemical factors of foods constitute a protective medium for probiotics. The nature of these factors (acidity, protein composition, etc.) must aim at the most important and decisive point, that of the survival of probiotics. Survival is the most important factor, as it prevails over antimicrobial properties, acid and enzyme tolerance, and adhesion to the epithelium.²²

The primary purpose is to ensure the survival of probiotics. The survival of probiotics can be compromised by the initial stage of production and storage of the products, the conditions of preservation, and their interaction with other microorganisms and components contained in the product up to the passage through the gastrointestinal tract.²¹ The content and nature of fats, the type of proteins, the pH, and the sugar content seem to be some of the most crucial factors that can influence the survival of probiotic bacteria. Understanding these variables has led to the search for methods of adapting them so that foods can accommodate microorganisms even more effectively. Foods are used as carriers of probiotics. The physicochemical matrix of foods affects the growth and survival of probiotics during their passage through the gastrointestinal tract. One of the most important mechanisms of foods for the protection of probiotics is the provision of a favourable pH, resulting in their stability.^{13,22}

4. Effects of probiotics on the host's health

Consumption of probiotic products can cause a multitude of positive effects on the human body. Probiotics, thanks to their antimicrobial action, can combat intestinal and other diseases, reduce serum cholesterol levels, and stimulate the immune system. In addition, the consumption of probiotics promotes the growth of desirable microorganisms, overcoming potentially harmful bacteria and strengthening the body's defences.²³ In several studies, it appears that the native starter cultures of yogurt, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, did not affect enhancing the immune system during rotavirus, nor on faecal enzymes. Most scientific papers commenting on these results refer to studies using cultures of the species *L. acidophilus* and *Bifidobacterium*. Fuller (1989), lists some beneficial effects and therapeutic applications of probiotic bacteria.^{24,25} Among other things, it appears that the consumption of probiotics contributes to the maintenance of a normal environment in the GM, reduces lactose intolerance and exhibits anticancer effects. Regarding therapeutic applications, probiotics can contribute to the prevention of urogenital infection, the relief of constipation, and the prevention of hypercholesterolemia and osteoporosis.²⁶

Disruption of the balance (dysbiosis) between GM and the host's health can cause Chron's disease and ulcerative colitis. This disruption can lead intestinal bacteria to alter the balance of pro-inflammatory and anti-inflammatory cytokine levels in the intestine, mainly resulting in a predisposition to intestinal diseases and disorders.²⁷

Thus, dysbiosis, that is, the alteration of the GM, may contribute to obesity. This fact has increased interest in identifying probiotics that will manage this condition. Obesity can have various causes. To find a probiotic capable of combating it, it is important to determine the mechanisms by which it is caused. One of the main reasons is the abnormalities that appear in the lipid profile, a condition known as dyslipidaemia. Therefore, finding strains of probiotic bacteria able to limit the accumulation of lipids in fat cells may have optimistic results for contributing to the treatment of obesity through the consumption of probiotics.²⁸

Hypercholesterolemia is an important pathogenic factor for cardiovascular diseases, metabolic syndrome, and type 2 diabetes mellitus. Statins drug therapy is commonly used to combat hypercholesterolemia; however, it is associated with adverse effects such as headache, diarrhoea, and constipation. The occurrence of these side effects has led many patients to prefer functional foods to combat hypercholesterolemia. Probiotics have been widely shown to favour the reduction of cardiovascular disease.²⁹ One of the most interesting effects of probiotics on the body focuses on the presence of the gut-brain axis (GBA). The GBA is the connection and two-way communication between the enteric (or intestinal) nervous system (ENS) and the central nervous system (CNS). This axis is a complex bidirectional pathway essential for metabolic homeostasis, the influence it has on emotions, mood, and generally higher cognitive functions.³⁰

It is a complex system in which the CNS, the autonomic nervous system (ANS), the brain, the spinal cord, and the hypothalamic-pituitary-adrenal (HPAA) axis participate. The GBA axis indicates the two-way relationship and interdependence between the CNS and the ENS. It is important to understand this interaction and how a healthy GM can influence the nervous system of the body and vice versa during physical activity.³¹

Finally, the ability of probiotics to control intestinal infections lies in their antimicrobial properties. Probiotic bacteria such as bifidobacteria and those from *Lactobacillaceae* family have antimicrobial properties, and bacteria such as *L. acidophilus* and *B. bifidum* show inhibitory activity on food-borne pathogens.³² Research shows that *Lactobacillus rhamnosus* GG (ATCC 53103) acts by reducing the activity of enzymes in the stool, reducing antibiotic-associated diarrhoea in children, and contributing to the regulation of the immune response. The proliferation of intestinal pathogens may play a role in the incidence of infections in athletes after prolonged physical exercise. In this case, however, no problems of

infection or inflammation at the gastrointestinal level, during and after the competition, have been reported.³³

5. Gut-brain axis (GBA) during physical activity

Exercise positively affects the biodiversity of the gut microbiota and is positively correlated with protein intake and creatine kinase levels. Athletes have lower levels of Bacteroidota and higher amounts of Bacillota phyla.³⁴

This is because athletes follow a lifestyle that favours the eubiosis, not only of the gut microbiota. Intrinsic bio-changes due to systematic training, such as reduced blood pressure and tissue hypoxia, lead to changes in the gut microbiota.³⁵

Indeed, athletes have lower inflammatory markers and better metabolic markers. Exercise is thought to be associated with reduced morbidity due to the reduction of chronic inflammation. Thus, bacteria such as *Bifidobacterium* spp., *Akkermansia* spp., and those from the *Lactobacillaceae* family increase due to exercise, while the Pseudomonadota phylum, *Turicibacter* genus, and *Rikenellaceae* family do not.³⁶

On the other hand, extreme exercise leads to physiological stress and reversible changes in immune defence that are related to the number and functionality of T and B cells, the activity of natural killer cells, neutrophil function, salivary IgA concentration, and the oxidative capacity of granulocytes. It also increases the release of stress hormones, pro- and anti-inflammatory cytokines, and reactive oxygen species (ROS).³⁷

In fact, increased cortisol secretion, together with small repetitive muscle injuries and excessive consumption or lack of energy, negatively influences immunomodulation. The altered function of the immune system has a negative effect on the intestinal mucosa. Therefore, these conditions can lead to a severe dysbiosis not only of the gut microbiota but generalized dysregulation not only of the gut-brain axis but also of others, such as the gut-liver, gut-lung, gut-oral, and others. This contributes and is responsible for both a state of malaise that leads to mediocre performance but also a further cause of pathological disorders and diseases.³²

Thus, through a number of mechanisms, hundreds of substances and neurotransmitters, the relationship between the two complex systems is constantly changing and can have both positive and negative effects. Acute stress (physical or/and mental) can increase the permeability of the colon, leading to the overproduction of Interferon- γ . So, how does exercise alter the microbiota community? Several studies indicate the presence of a bidirectional interaction between the gut and the musculoskeletal system, called the gut/muscle axis (GMA), that interacts with GBA. The existence of this axis is based on evidence that musculoskeletal contractions have anti-inflammatory effects through the release of myokines.^{33,38} Myokines appear to play an important role in mediating the secretion of glucagon-like peptide-1 (GLP-1), a key incretin involved in metabolism throughout the body, during exercise or physical activities. For example, Interleukin-6 is involved in the secretion of GLP-1 by L-cells in the ileum. Further evidence for the existence of the axis comes from the production by the GM of SCFAs, mediators of metabolic energy in the mitochondria of muscle cells, which in part help regulate glucose metabolism.³⁹ Furthermore, SCFAs interact with specific G protein receptors (GPR41 and GPR43) in intestinal L-cells, stimulating the secretion of GLP-1. Several bacteria produce SCFAs, the best-known being *Bifidobacterium* (phylum Actinomycetota) that produces acetate, which can be transformed into butyrate, and *Akkermansia* (phylum Verrucomicrobia) that produces propionate, which can be transformed into acetate. Acetate and butyrate both increase the oxidation of muscle fat, changing the oxidative state of muscle fibres. Therefore, acetate and butyrate influence metabolic flexibility, increasing the ability to use lipids or carbohydrates as energy reserves.⁴⁰ Butyrate also inhibits histone deacetylase, protecting muscle proteins from catabolism and, therefore, preventing age-related muscle loss. Furthermore, it has been documented that physical exercise makes SCFAs more available to the bacterial community. These testimonies, as well as those collected in the

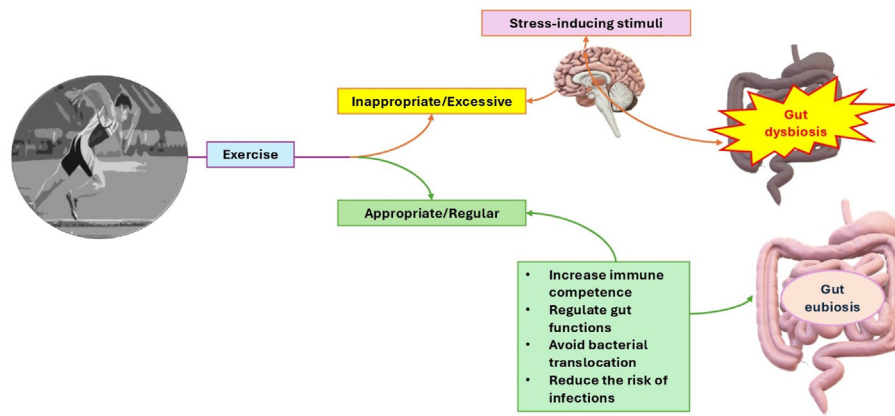


Fig. 1. The figure shows the hypothesis of the connection between physical activity and gut microbiota. GM: gut microbiota.

selected studies, lead us to think that a GMA connecting muscles and intestines does, in fact, exist and that it is partly controlled by the GBA.

The exact modalities with which this occurs, however, have yet to be established.⁴¹ The above negative effects of unpleasant psychological states mentioned indicate this strong dependence and relationship between the CNS and the ENS. It is worth focusing on the reverse course, that is, how the health of the body's GM can give, through probiotic bacteria, the appropriate signals to the brain to influence potential diseases.³⁰ It has also been observed that a high blood concentration of catecholamines, adrenaline and noradrenaline corresponds to the phases of greater lymphocyte activation, while the post-exercise phase, more cortisol, corresponds to the reduction of lymphocyte concentration. There is interindividual variability in immunocompetence, linked to age, nutrition, recovery, exercise capacity, presence of stressors, and even unrelated to training and personal tolerance to stress.⁴² This multitude of parameters explains the different vulnerability of athletes to disease. Most athletes should be able to train with high loads (both intensity and work) if their program includes strategies designed to control overall effort and stress. Athletes and coaches should be alert to periods of increased risk of stress-related disease, according to the American College of Sports Medicine (ACSM).^{43,44}

The immunomodulatory effects of physical activity, regular physical exercise increase immune competence and reduce the risk of infection compared to a sedentary lifestyle. In contrast, acute prolonged bouts of exercise or repeated periods of intensive training are followed by a temporary increase in the risk of infection (Fig. 1).³³

The conclusions state that the effects of physical activity on the immune system are strongly dependent on the mode and intensity of exercise or training. The GM can, in turn, influence the pathophysiology of several distant organs, including skeletal muscle. A gut-muscle axis can indeed regulate muscle protein deposition and muscle function. The immune system plays a fundamental role in these processes, being influenced by the composition of the GM and, at the same time, helping to shape the microbial communities.^{45,46}

6. Physical activity and GM modulation

Generally, physical activity or extreme exercise can modify the composition of the GM.³³ Although other factors (sedentary, diet, and others) may have influenced these results, no changes in diet or mood were reported during data collection. However, further studies with planned training programs in healthy and pathological populations with an interdisciplinary team are needed to better understand the interaction between physical activity and GM.

In a clinical study, the effects of exercise intervention on the composition of the GM were examined in 32 healthy sedentary older women aged 65 years and older. In this study, a 12-week program of daily brisk walking was shown to increase the relative abundance of

Bacteroides, while also contributing to cardiorespiratory fitness and without changes in diet type and nutrient supplementation. Previous studies have already shown that exercise positively modulates the composition of health-promoting *Bifidobacterium* and *Lactobacillus* in animals, and negatively modulates that of *Turicibacter* spp., associated with immune system dysfunction and irritable bowel syndrome.⁴⁷ Another animal study (mice) showed that a 6-week treadmill running program increased the abundance of *Bacteroides*. Overall, it can be argued that aerobic exercise can modify the GM in healthy older women. Therefore, concerning other studies, maintaining a healthy GM can be considered a preventive factor for various pathologies. The mechanism by which aerobic exercise can alter the microbial composition may be due to the variation of the transit time in the colon and the consequent modification of the pH in the lumen. Specifically, a prolonged transit time leads to a limited diversity in the composition of the GM, which coincides with an increase in pH during the transit from the proximal to the distal colon.⁴⁸ In another randomized crossover study, they evaluated how resistance exercise can modulate the GM in older adults and whether these changes are associated with cardiometabolic phenotypes in a population of 33 older Japanese men, between 62 and 76 years old. It was noted that when comparing changes in GM between individuals, the results revealed that a short-term exercise program did not appreciably influence the diversity and composition of the microbiota, although slight changes were associated with a decrease in cardiometabolic risk factors. In addition, preliminary analyses indicated some changes in metagenomic functions during the intervention period. These findings highlight real and hypothetical functions of GM and point to the microbiota as a mediator of cardiometabolic well-being. Further studies, both large-scale and long-term, are needed to clarify the relationship between exercise and gut microbiota and reveal the beneficial role of the microbiome in preventing cardiometabolic diseases.⁴⁹ Furthermore, the changes occurred in the absence of improved cardiorespiratory fitness. These findings have improved our understanding of the mechanisms by which the GM responds to sustained aerobic exercise and how this may have implications for athletes competing in high-intensity events. It has been noted that an increase in the genus *Bacteroides*, highlights an increase of *Oscillospira* and a decrease is noted in the *Clostridium* subcluster XIVa and *Clostridioides difficile*.⁴⁹ The ambivalent role of the genus *Bacteroides* is known for these species, which may be positive or negative depending on the host, and on the intestinal environment in which it is found. *Oscillospira*, on the other hand, was positively correlated with a lean metabolism. A decrease in its relative quantity was demonstrated in individuals affected by Crohn's disease and non-alcoholic steatosis hepatitis. The results indicate that *Oscillospira* has a beneficial role on the health of the host, specifically it could be linked to cardiometabolic health.⁴⁹ Regarding *C. difficile*, which is the main cause of infectious diarrhoea in humans, the results indicate that physical exercise can decrease the production of toxins by this bacterium. Furthermore, a

decrease of this in GM was associated with an increase in peak oxygen volume ($\dot{V}O_2$) and a decrease in cardio-ankle vascular index (CAVI) and glycated haemoglobin (HbA1c) during the intervention. Finally, the bacterial species most increased were *Roseburia hominis* and members of the genus *Subdoligranulum*, both butyrate producers. The latter is an SCFA, which plays a significant role in regulating the intestine, reducing inflammation, oxidative stress, as well as strengthening the epithelial barrier and moderating visceral sensitivity.

In a case report, they observed the effects of prolonged and intense exercise on the GM of four well-trained athletes who completed an unassisted transatlantic crossing. They noted that high-intensity exercise increased microbiota diversity, the proliferation of butyrate-producing species, and positively influenced the metabolic potential of some genes with specific functions in well-trained athletes.⁴⁶ These changes were evaluated in a context where the diet differed in composition, but remained similar in terms of energy, macronutrients, and fibre content. During the crossing *Dorea longicatena*, *Roseburia hominis*, and the genus *Subdoligranulum* increased, while *Bacteroides finegoldii* decreased. Finally, the levels of *Pseudomonadota* phylum, *Veillonella* and *Streptococcus* spp. increased and those of *Alloprevotella* and *Subdoligranulum* decreased. *Dorea longicatena*, capable of improving intestinal metabolism, as well as being positively correlated with insulin sensitivity, was also increased. Also, in the last study, butyrate-producing genera, such as *Faecalibacterium*, increased the most. Furthermore, *Veillonella* increased and was also shown to be involved in the lactic acid cycle. All these results show that, although in a very heterogeneous way, physical activity has influenced the acquisition of an eubiotic GM, moreover decreasing, in some cases (*Clostridium* spp.), the level of pathogenic bacteria. It is interesting to note how, in high-intensity physical activity, species capable of improving the performance of athletes have increased. The microbial species that have undergone alterations in several articles are diverse and heterogeneous.⁵⁰ Finally, in another case report, they explored the effects of intense exercise on the GM in a 32-year-old male ultramarathoner. Manipulating the GM has health implications and therapeutic potential in humans. This study demonstrated the most rapid and consistent change in the microbiota after a high-intensity exercise intervention in the human literature to date. These GM further highlight the importance of exercise on health. Richness in taxonomy (alpha diversity) is often considered an indicator of a healthy microbiota, which in this case, decreased after 19 weeks of training in preparation for the race. This could be due to the proliferation of beneficial bacterial species, such as those involved in butyrate production (e.g. *Faecalibacterium*, +40% from baseline to pre-race), with the concomitant decrease of less relevant microorganisms and the increase in the amount of *Veillonella*. Recent studies have indicated that *Veillonella* plays a role in the lactic acid cycle. Other post-competition dynamics have also been observed, with the emergence of *Haemophilus*, a genus of bacteria with several pathogenic species, and *Streptococcus* spp. The proliferation of intestinal pathogens may play a role in the incidence of infections in athletes after prolonged physical exercise. In this case, however, no problems of infection or inflammation at the gastrointestinal level, during and after the competition, have been reported.⁵¹

7. Exercise and probiotic supplements

In recent years, the use of probiotic supplements by professional athletes to optimize their health, training and ultimately their performance in their sport has gained increasing interest from the scientific community. As already mentioned, probiotic supplements contain many bacteria, especially lactic acid bacteria, and after colonizing the gastrointestinal tract, they provide a series of beneficial effects on the health of the individuals taking them. However, according to the scientific research on the effect they have on the specific group of athletes (and especially high-performance athletes) lags significantly behind the corresponding research that has been conducted both in animals and on the

various pathological conditions that affect the general population.⁵² It is often difficult to study athletes during their training or competitive activities, as a wide range of interactions between diet, physical activity, and the many stressors and stimuli they experience come into play. In all cases, successfully managing the combination of training, lifestyle, and dietary habits is a significant challenge for high-performance athletes, who, in addition to sports, have multiple activities in their social and family lives, work, and travel.⁵³

Therefore, according to the above, athletes should give high priority to maintaining their health in excellent condition to achieve high performance in their sport. This purpose is also served using probiotic food supplements, which are mainly taken by them to promote the health of their intestines, as even mild disorders can have negative effects on their training and athletic achievements.⁵⁴ From an immunological point of view, both the respiratory system and the gastrointestinal system have a primary role in mediating the defence mechanisms of the human body and regulating the homeostasis of its mucous membranes. These specific defence mechanisms, located on the surfaces of the mucous membranes of the respiratory tract (especially in the oropharynx) and gastrointestinal system, largely protect the athlete from attack by common pathogenic microorganisms.⁵⁵ However, during intense or prolonged exercise (or their combination), in many of cases the integrity of the mucosa is disrupted, resulting in the appearance of a series of unpleasant symptoms for the athlete, such as nausea, flatulence, colicky abdominal pain, diarrhoea or even bleeding from the gastrointestinal system. Therefore, health professionals specializing in sports medicine have always sought to implement a series of nutritional practices, including various nutritional supplements, to minimize, as much as possible, the risk of weakening the respiratory and gastrointestinal systems of athletes during their intensive training and exercise.^{56,57}

It is precisely in this context of strengthening the defence mechanisms of the athlete's body, and especially the upper respiratory and gastrointestinal systems, that the use of probiotic nutritional supplements comes into play, the use of which, as well as the corresponding research in relation to their mechanisms of action and effectiveness, has increased over the last decade. Intense exercise and training, on an almost daily basis, as performed by long-distance runners and especially marathon runners, is associated in many cases with an increased incidence of upper respiratory tract infections. At the same time, gastrointestinal disorders in runners are quite common.⁵²

In a randomized, blinded study with a control group, a total of 141 marathon runners participated to investigate the effectiveness of the supplement *L. rhamnosus* GG. This supplement was administered for a period of three months, and immediately after the athletes competed in a marathon road race. The results of the study showed that there were no statistically significant differences in terms of upper respiratory tract infections and the total number of healthy days of athletes in the intervention group and the control group (placebo). Furthermore, no statistical difference was found in terms of the number of events from the athletes' gastrointestinal system, however. Athletes who received the probiotic supplement had a statistically significant faster recovery from the discomfort that arose during their training from the gastrointestinal system, compared to the control group (2.9 days average recovery period compared to 4.3 days), which clearly constitutes. The effect of probiotic supplements on the health of athletes is a significant advantage as even one extra day of training can be effective for athletes competing at a championship.⁵⁸

Another clinical study is the randomized double-blind controlled trial which investigated the efficacy of the probiotic supplement *Lactobacillus salivarius* about possible changes in the immune response of athletes (male and female endurance athletes). A total of sixty-six athletes participated, with those in the intervention group receiving the specific probiotic organism ($[2 \times 10^{10}]$ CFU) daily for 16 weeks. The results of the study showed no statistically significant difference between the two study groups in several outcome criteria, which included a) number, severity and duration of upper respiratory tract infections, b) levels of

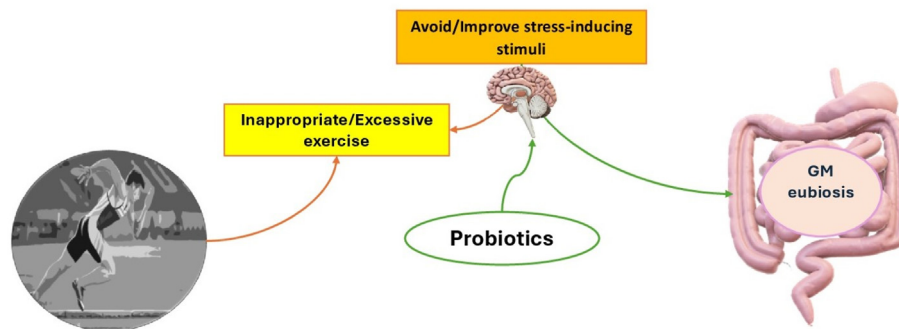


Fig. 2. The figure summarizes the hypothesis of the connection between unbalanced physical activity, intestinal microbiota, and probiotics. GM: gut microbiota.

white blood cells, neutrophils, monocytes and lymphocytes, c) levels of IgA in the saliva of the athletes, and d) levels of salivary lysozyme. It therefore appears that this probiotic supplement does not offer statistically significant benefits and advantages to endurance athletes.⁵⁹

In another research, the effectiveness of administering a probiotic dietary supplement containing the combination of the microorganisms *Lactobacillus rhamnosus* and *Lactobacillus paracasei* was studied concerning oxidative stress in athletes during four weeks of intensive training. More specifically, the intervention group was administered a supplement containing these two microorganisms in a 1:1 ratio (approximately 1×10^9 CFU/day), while the control group was not administered any supplement. The outcome criteria studied were the plasma levels of a series of metabolites and antioxidant factors, immediately before and after the administration of the specific supplements, while at the same time, an analysis of the athletes' faeces was performed before the start and after the end of the study. A total of 24 volunteers participated (12 in the intervention group and 12 in the control group), with the main results of the study showing that, a) the intense athletic activity and training of the athletes resulted in the induction of oxidative stress, b) administration of the specific combination of probiotic microorganisms resulted in an increase in the plasma levels of antioxidant agents, which neutralized oxidative radicals and the specific combination of the microorganisms *L. rhamnosus* and *L. paracasei* had a strong antioxidant effect on the athletes in the intervention group.⁶⁰

According to the data reported in such a review, physical activity is a factor capable of modifying the GM, although it is not possible to state this with certainty given the limitations mentioned above. If any new evidence confirms this hypothesis, the execution of a specific exercise modality could prove capable of developing a microbiota capable, for example, of preventing some pathologies in elderly subjects or improving the performance of athletes and sportsmen (Fig. 2).

8. Conclusions

This review aimed to investigate the literature supporting the effectiveness of physical activity as a factor capable of altering the composition of the GM also in healthy individuals, and how probiotics can help to maintain an eubiotic GM. The results suggest that physical exercise could have effects on the composition of the GM, and vice versa. In particular, the variables of intensity and duration could be the key to a significant modification. Furthermore, the acquisition of a 'friendly' microbiota with a positive impact on the health of the participants is also a good omen, and probiotics administration may be a key element to reach the goal of both a good gut microbiota and improved physical performance.

CRediT authorship contribution statement

Ioannis Alexandros Charitos: Writing – original draft, Validation, Resources, Methodology, Conceptualization. **Marica Colella:** Writing – review & editing, Validation, Investigation, Funding acquisition. **Domenico Maria Carretta:** Validation, Data curation. **Luigi**

Santacroce: Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Data availability statement

All the data are contained within the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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