

**EXPLORING LONGITUDINAL AND CROSS-SECTIONAL STUDIES
ASSOCIATING PHYSICAL ACTIVITY WITH INTESTINAL MICROBIOTA
CHANGES**



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BOGOTÁ, 06 DE AGOSTO DE 2020

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**TESIS PARA OPTAR AL TÍTULO DE MAESTRÍA EN ACTIVIDAD FÍSICA Y
SALUD**

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Para ti Joaquín, todo es por ti hijo mío.

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ABSTRACT

The current review aimed to elucidate the modulatory effects of physical activity and sports activities on the gastrointestinal microbiome. Twenty cross-sectional and longitudinal studies were analyzed, using metagenomic and various omics measures. Studies across gut microbiota and exercise have recently been published, mainly examining the effects of training programs in subjects who did not previously perform regular physical activity. These studies suggest that exercise does not cause structural changes in the microbiota, with species diversity remaining unchanged after 6 weeks or less of engaging in an exercise program. Gut ecosystem adaptation to exercise in sedentary subjects has been reported to respond to the host phenotype (body mass index and age). Results suggests that cross-sectional studies can provide insights into differences between highly adapted athletes with active lifestyles and sedentary adults, particularly in the abundance and function of gut microbiota. Interestingly, comparing healthy adults with different levels of cardiorespiratory fitness has revealed similar results, indicating a lack of important differences in the abundance of taxonomic groups and species richness. Metabolomic, metagenomic and transcriptomic approaches allow the identification of possible mechanisms between the intestinal microbiota and the host physiology in physical exercise, mainly through functional groups of bacteria such as *Veillonella*, *Prevotella*, *Akkermansia* and archaeon *Methanobrevibacter*. Most of the reviewed research focused on endurance training. Thus, the potential effects of resistance training and high-intensity training on the gastrointestinal microbiome need to be explored in future studies.

KEY WORDS: Gastrointestinal Microbiome, Exercise, Athletes, Active Lifestyle, Physical inactivity.

INTRODUCTION

The field of human microbiota has evolved rapidly, and literature increases exponentially through years. Moreover a consensus about good practices and definitions is necessary in order to standardize concepts and research outcomes [1] also to understand the difference between microbiota and microbiome. The intestinal microbiota is the set of microorganisms that symbiotically inhabit the digestive system, comprising bacteria, fungi, archaea, protists and viruses. However, because of the lack of information on eukaryotes and the high numbers of bacteria, most previous studies have focused on the diversity of the bacteriome [2,3]. Actually gut intestinal microbiota is thought to be dominated by five bacterium phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [3,4]. Various international consortia and research groups, including the Human Microbiome Project, have coordinated efforts to generate databases and relevant information to define the composition of a healthy microbiota [6]. This question remains largely unanswered because of the high variability between age groups, gender, inflammatory states, metabolic diseases, pregnancy, and differences in events at birth and the early stages of life [5]. Moreover, there is high variability in microorganisms between segments of the gastrointestinal tract, with various factors, including pH, oxygen availability and temperature, affecting colonization by different groups of bacteria [7]. The term microbiome refers to the sum of the microbes and their genomic elements in a particular environment [8] nonetheless exists a variety of definition from different point of since the microbiome comprises all of the genetic material within a microbiota, also referred as the metagenome [9] but can also be defined as an “ecological community of commensal, symbiotic and pathogenic microorganisms within a body space or other environment” [1,10]. For this review the gut microbiome will be defined as collective genome of microbes inhabiting the gut including bacteria, archaea, viruses and fungi [11]

To understand the microorganisms that make up this complete set molecular, bioinformatic and biostatistical techniques are required, beginning with the collection of a sample, extraction of genetic material from the microorganisms and sequencing of the 16S (for bacteria) or 18S ribosomal gene (for eukaryotes), usually using the V3–V4 regions of the 16S rDNA gene. Once data is obtained, phylogenetic assembly and reconstruction processes are conducted to identify the largest number of genes within a specific niche, such as the

human gut. However, it should be noted that the total number of reads obtained per sample does not reflect the absolute number of microbes present in the intestine because a sample obtained from fecal matter represents only a fraction of the original environment [12].

To understand the composition of the microbiota and establish significant differences or relationships, ecological concepts such as diversity and abundance are used. Because composition does not float freely in Euclidean space, standard methods of analysis are not applicable [12]. Beta (β) diversity metrics provide a measure of the degree to which samples differ from each other, revealing aspects of microbial ecology that are not apparent when examining the composition of individual samples [13]. Alpha (α) diversity indices summarize the structure of microorganism communities with respect to richness (number of taxonomic groups), uniformity (group distribution of abundance), or both, depending on the index used. α diversity is considered a first approach to evaluate the differences between environments (in this case, hosts) [14]. In addition, omics techniques, in which the sequencing of associated metabolites (metabolomics), protein quantification (proteomics) or mRNA sequencing (transcriptomics) is used, have enabled researchers to obtain a wide spectrum of relevant information, including the impact of the meta-community of organisms in the host, and how the different members of the microbiota interact.

Numerous studies aiming to establish relationships between changes in the microbiome and health-disease processes [15], lifestyles and changes at different stages of the life cycle [16] have provided evidence regarding the roles of microorganisms in human health. However, the causal relationships and mechanisms have not been fully explained. The intestinal microbiota in healthy individuals has been shown to be stable, particularly in the absence of clinical and in general healthy lifestyle habits, such as eating a diverse diet and meeting physical activity recommendation [17,18]. Changes in diversity can lead to a reduction in the abundance of beneficial bacteria with an increase in the prevalence of potentially pathogenic microorganisms, known as “dysbiosis”. Dysbiosis is reported to be related to various diseases, ranging from early childhood to old age. In obesity, for example, changes in the abundance of Bacteroides and Firmicutes, also known as the B/F ratio, might promote the storage of adipose tissue in the host, facilitating the collection of energy from diet and decreased energy expenditure [19]. It has been proposed that everyone has a unique signature

of microorganisms, which is more stable in middle age [9]. However, endogenous factors have direct impacts on this niche [20].

The composition and activity of the human intestinal microbiota evolves from birth and is subject to complex networks that depend mainly on the host genome, nutrition and lifestyle [21–23]. During the last two decades, studies have attempted to identify whether an active lifestyle can modulate the intestinal microbiota regardless of diet [24], or if the sum of both factors enables a healthy intestinal environment, animal models have led researchers to hypothesize that higher oxygen consumption improves the metabolic response of the microbiome and alters the diversity of the bacteria that colonize the intestine [25]. Because fully elucidating the effects of physical activity on the composition and structure of the intestinal microbiome has not yet been achieved, the role of the microbiome in adaptation to exercise is not completely understood. Given that human studies in this field have increased in recent years, especially observational and longitudinal studies, the aim of this systematic review was to summarize the results of all human studies comparing the microbiome composition of healthy individuals with different physical activity level (PAL).

Longitudinal and cross-sectional study designs, analysis of different populations, and variation in interventions emphasize the need to synthesize the obtained results regarding whether the communities of microorganisms inhabiting the intestine are susceptible to change with physical exercise, not only in composition but also function. The current review explores longitudinal and cross-sectional studies investigating the association between physical activity and intestinal microbiome in humans without reported pathology and without diet modifications, published between January 2008 and March 2020. Articles published before 2008 were excluded since no data was found.

MATERIALS AND METHODS

Search strategy: This review was based in Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement guidelines [15]. Supplemental figure 1 shows the search process using various electronic databases, and the selection of articles after review by title and abstract. A computerized search was conducted between June 2019 and February 2020 using the standardized English search terms assigned by the MeSH and EMTRE indexes using the Boolean operators OR/AND: “exercise” AND “human” AND “gastrointestinal microbiome” OR “gut microbiota” OR “gut microbiome”. The databases consulted included ProQuest, EMBASE, MEDLINE, PubMed, CENTRAL, HINARI, INASP, Central BioMed, and Public Library of Science. A total of 20 articles were included in the current review, including 12 cross-sectional studies and eight longitudinal studies. The results are shown independently.

Study selection

All human controlled comparisons and observational studies were including in this review assuming the risk of bias due to the possibility of finding little or no information due to perceived limitations in randomized trials.

Data extraction:

The criteria for including studies in this review were: (a) Cross-sectional studies focused on comparing the gut microbiome among subjects with different physical activity level, from athletes to inactive individuals, to define inactive status guidelines of American College of Sports Medicine (ACSM) were used [26] (b) Studies focusing on describing gut microbiome responses to any type of exercise stimulus, taking account of acute and chronic effects (c) Studies containing healthy middle-aged and older adults (d) Studies written in English. Studies containing diet modification, probiotic and prebiotic consumption were excluded, as were studies focused on diabetes, hypertension, cancer, hormonal dysfunction, or related since evidence suggest these conditions may lead to significant changes in the composition of gut microbiota. Reviews, comments, letters, interviews, and book chapters were not included since the aim of this review was to explore research on apparently healthy human.

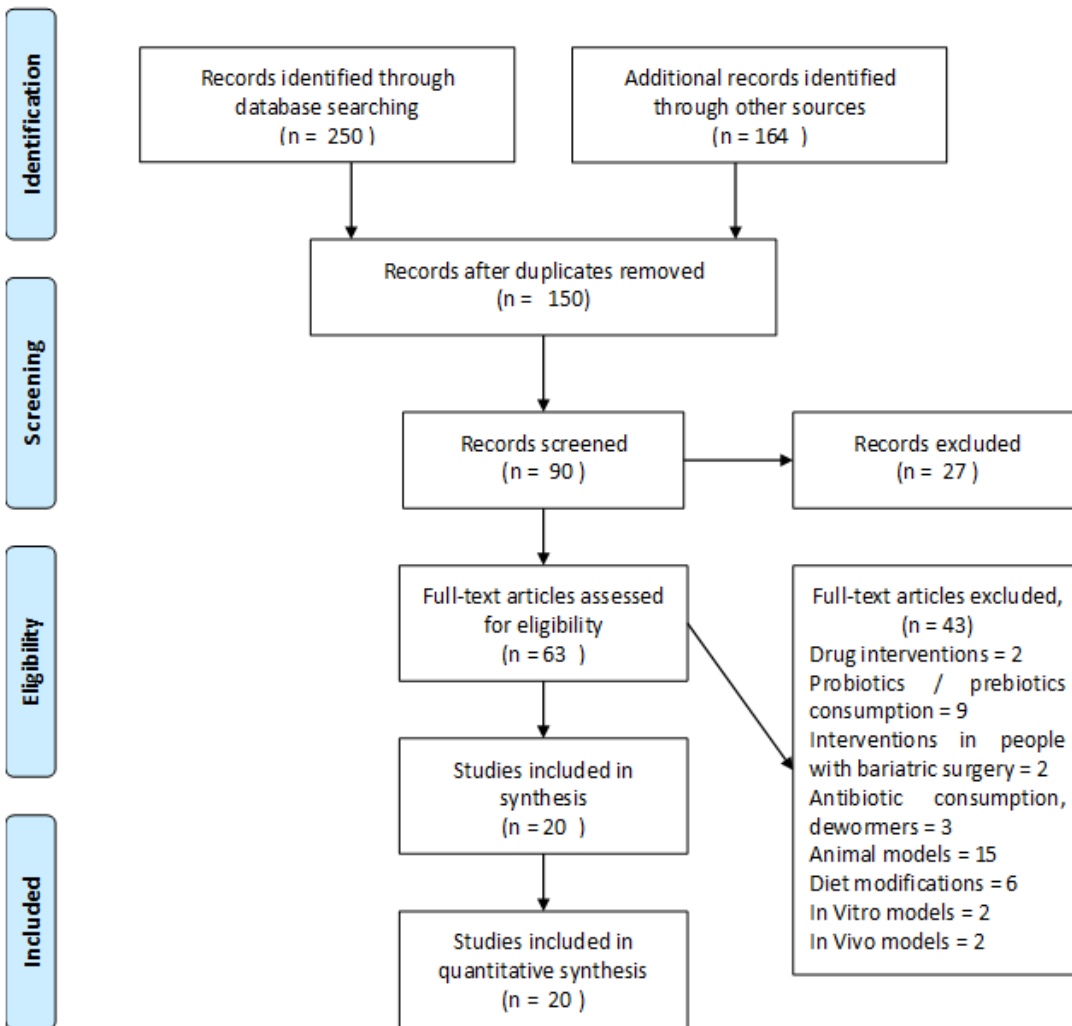
As shown in the PRISMA based workflow the initial search process by key words described previously allowed to identify 250 articles in the databased selected and 164 articles from other sources, mainly secondary references from the search in databases; after eliminate duplicated texts a total of 150 articles related to the inclusion criteria for this review were register. Once records were screened by abstract a total of 27 studies were excluded since they referred to pilots, letters to editor and similar communication papers.

After full reading of methodology section of the remaining 63 potential eligible articles, exclusion criteria were applied:

- a) Diet modifications
- b) Supplement consumption (Vitamins - proteins - amino acids)
- c) Drug interventions (metformin)
- d) Consumption of probiotics / prebiotics
- e) Population with known pathology or chronic diseases (IBS, DT2, HTA, Cancer, etc.)
- f) People with bariatric surgery or surgical interventions for weight loss
- g) Antibiotic consumption, deworming drugs and similar treatments
- h) Animal models
- i) *in vitro* models
- j) Models *in silico*

Finally, a total of 20 studies were included in this review. Recorded outcome measures included differences for alpha and β diversity and relative abundance ($p < 0.05$), also transcriptional and metabolomic data extracted from feces samples was included. Measured or description PAL was taking account in order to classified results based on inactive, active and athletic subjects.

PRISMA-based workflow:



RESULTS

High performance sport individuals have a differential composition of the intestinal microbiota

To determine whether the composition of the microbiome exhibits a metabolic advantage in high-performance athletes, previous studies examined athletes from different sporting disciplines [27–32]. One study examined a sample of 37 elite athletes engaged in different sporting disciplines at a similar level, investigating differences related to the type of activity [28]. The findings revealed no significant differences in α or β diversity indexes in functional groups categorized by energy demand, with the whole sample exhibiting abundance of the species *Eubacterium rectale*, *Polynucleobacter necessarius*, *Faecalibacterium prausnitzii*, *Bacteroides vulgatus* and *Gordonibacter massiliensis* [28]. In addition, between 19.8–11.6% of the microbial species belonged to the phylum *Bacteroidetes* [28]. A similar study of 31 Wushu martial artists identified significant differences in α diversity of athletes performing at a high level compared with those performing at a lower competitive level (Shannon index $p = 0.019$; Simpson index $p = 0.001$) [31]. In addition, researchers reported a greater abundance of the genus *Parabacteroides*, *Phascolarctobacterium*, *Oscillibacter*, *Bilophila* and a lower abundance of *Megasphaera* ($p < 0.05$) among high-performing individuals [31]. Gut microbiota from other athletes (Table I) also exhibited a higher diversity of species, particularly among professionals and competitive cyclists [32].

Descriptive studies in high-performance athletes and the composition of the microbiome are limited (Table I). Although some relationships between the training level and diversity/abundance of taxa have been established, it is important to examine information relevant to the phenotype as well as physiological adaptations, which can vary substantially from one discipline to another. In addition, because of the relevance of diversity in foods and supplements to the composition and function of the microbiome, diet composition should not be overlooked. There is currently insufficient information to determine whether there is a modeling effect of the microbiota as a consequence of adaptation to high levels of athletic performance, actually probiotics and prebiotics consumption has become recurrent in athletic population since recovering and immunologic benefits are attached to *Lactobacillus*, *Bifidobacterium*, and *Bacillus* genera, nonetheless further research is needed considering that

differences in gut microbiota composition affect probiotic efficacy [33]. Similarly, it is unclear whether there is a common group of microorganisms across different categories or sports disciplines, or, conversely, if performance at a highly competitive level generates specific and individual modifications in the intestinal environment regardless of the sporting activity performed.

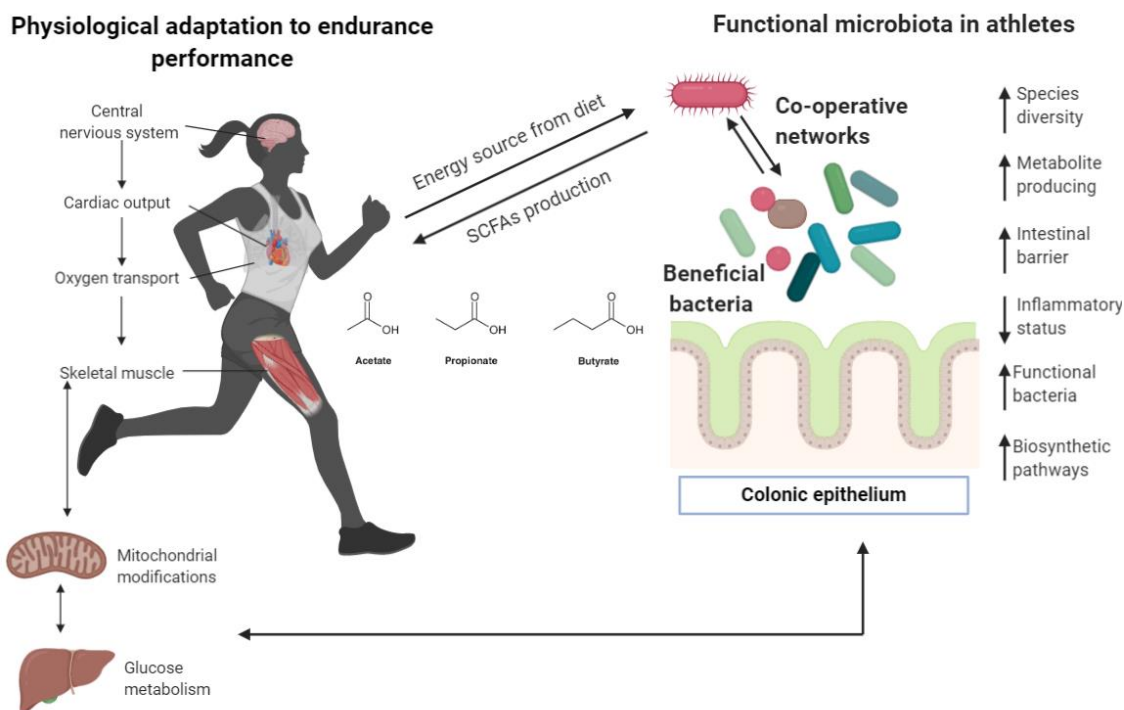


Figure 1: Insights about meta-community adaptation among gut microbiota in athletes. Most human physiological systems adapt to performance endurance in elite and competitive athletes, and clear differences between physically inactive and athletic individuals have been described previously, particularly physiological adaptations in the cardiovascular and pulmonary systems due endurance activities require major oxygen capacity to transport oxygen to different organs, including the muscles and liver. In addition, efficient glucose and fatty acids metabolism is required to provide substrates that are finally transformed into energy by mitochondria, the function and capacity of which are also modified. Recent research has indicated that an efficient and gut microbiota may be present in elite sportspeople, and special and unique bacteria can positively impact the host, providing substrates from the diet. Here, it is proposed that modifications of the gut microbiota are similar to a major ecosystem because gut ecosystem needs to create co-operative networks to improve metabolic functions, particularly the production of biometabolites that can be used for the host (in this case, during highly demanding performance activities). Arrows denote increase (↑) or decrease (↓).

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The gut microbiota of active people differs from that of inactive people

Table I shows a summary of abundance and diversity outcomes from cross-sectional studies.

Three results indicated that microbiota from athletes significantly differs from physically inactive adults [27,32,34]. One study reported a clear difference between rugby players and

two control groups of non-athletes with distinct body mass index (BMI) characteristics, particularly in phylogenetic diversity ($p < 0.0001$), Shannon index ($p = 0.0064$), Simpson index ($p = 0.0185$) and observed species ($p = 0.0098$), indicating greater α -diversity in the athlete group [34]. These results were not reproduced in a comparison of healthy young people (18–45 years old) with similar characteristics but different levels of cardiorespiratory fitness (CRF), measured as the maximum capacity of an individual to consume oxygen; subsequently, no significant differences in α or β diversity indices were found [27,35–39]. However, one study with a sample of 270 older individuals (> 62 years) without associated pathology reported different clusters (i.e., β -diversity) in the composition of the microbiome according to physical state ($p = 0.01$).

Regarding the abundance of taxa, which reflects the groups of bacteria at different phylogenetic levels (genus, family, species), the results were found to vary considerably between groups, with athletes and active adults exhibiting a greater abundance of representative species from the *Lachnospiraceae* [28,40], *Akkermansiaceae* [34,36] and *Faecalibacterium* [27,36] families, and a lower abundance in bacteria belonging to the phylum *Bacteroidetes* [34,36]. However, the gut microbiome of women with a high level of CRF was reported to exhibit a greater abundance of the genus *Bacteroides*, similar to high-level Olympic athletes [28,36]. Comparison at the species level revealed that Olympic athletes had a higher abundance of the butyrate producer *Eubacterium rectale*, whereas this microorganism was found to remain low in women with high CRF [28,36].

One study compared the microbiota of subjects from two different sporting disciplines, bodybuilding and athletics [27]. Differences in the abundance of observed species indicated that bodybuilders exhibited a significant decrease in *Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Lactobacillus sakei*, *Blautia wexlerae* and *Eubacterium hallii*, whereas people engaged in athletics and healthy adult controls exhibited an abundance of these taxa [27].

Abundant microorganisms in people with an active lifestyle and elite athletes are classified as “beneficial bacteria” (Figure 1) for the intestinal ecosystem, particularly its metabolic activity and production of short chain fatty acids (SCFAs). Table I and II show results related to potential members of gut microbiota from elite and competitive athletes in different

sporting disciplines that have revealed an increased abundance of important butyrate-producing bacteria like *Eubacterium rectale*, which principally consumes starch from the diet and successfully captures the products of resistant starch (RS) degradation by other bacteria [41,42]. However, a greater abundance of this species has also been found in obese people and is linked to inflammatory status and dysbiosis [2]. Similar findings have been reported for *Akkermansia muciniphila*, an abundant intestinal bacterium from the *Verrucomicrobia* genus that has been linked to a healthy intestinal microbiota due to this capacity to colonize the mucosal layer and improve host metabolic immune response by increasing mucus thickness. *A. muciniphila* also plays a key role in metabolic activity, leading to the production of beneficial SCFAs for hosts and other microbiota members [20,43,44]. *Faecalibacterium prausnitzii* is another habitual intestinal microorganism related to healthy gut microbiota that exhibits increased abundance in active individuals. This *Firmicutes* member is associated with immunomodulatory properties, leading to reduced levels of IL-2 and the production of Interferon gamma. Increased abundance of *F. prausnitzii* is associated with higher secretion of anti-inflammatory cytokine IL-10 [45]. Some evidence suggests a link between depletion of *F. prausnitzii* and the onset of certain inflammatory diseases, such as inflammatory bowel disease and Crohn's disease. This bacterium is also considered to produce butyrate [46,47].

Another microorganism with notable abundance among sportspeople is *Eubacterium hallii*, a commensal bacteria species that contributes to the formation of the SCFAs propionate, butyrate, acetate and formate, mainly from substrates like lactose and galactose; *E. hallii* has trophic interactions with other bacteria from the *Bifidobacterium* family, which is beneficial for host metabolism, particularly in colonization of the infant gut [48,49]. Results from other research in the pathobiont *Bacteroides vulgatus* are contradictory, being found in healthy gut microbiota but also in patients with insulin resistance [50]. Therefore, its abundance is associated with the pathogenesis of Crohn's disease and ulcerative colitis [51]. Moreover, this *Bacteroidetes* member is thought to play an important role in the biosynthesis of branched chained amino acids (BCAAs).

Recently culturomics techniques have allowed the identification and sequencing of genomes from bacteria that have not been reported before in individuals with active lifestyles, including *Gordonibacter massiliensis* [28] a gram-positive, motile non-spore forming and

obligate anaerobic coccobacillus [52] . Similar members of the *Eggerthellaceae* family have the capacity to metabolize polyphenols from diet into urolithin [53] This bioavailable metabolite has been tested in cellular lines and murine models and identified as a new regulator of muscle mass, as well as being implicated in androgen pathways, possibly via AMP-activated protein kinase to enhance the growth of myotubes and protein synthesis that is finally reflected in muscle hypertrophy [39]. Several biological activities have been related to urolithin B, including anti-inflammatory and antioxidant effects that exert protective effects against neuroinflammation [47,48] . To date, it has not been possible to ensure that *G. massilensis* can promote these effects via sports or physical activity, and further research is needed to clarify this issue.

Some species detected in healthy and athletic samples are widely considered to be candidates for probiotics because of their beneficial effects, such as *Bifidobacterium adolescents*, which exhibit strong RS degrading activity. *B. longum* has been extensively researched because of its relationship with brain activity via the vagal nerve, stimulating the pathways between the gut and brain. Studies of *B. longum* reported an anxiolytic effect and neural modifications that normalized anxiety-like behavior and hippocampal brain-derived neurotrophic factor in mice with infectious colitis, as well as helping modulate resting neural activity, reducing mental fatigue, and improving activation of brain coping centers to counter-regulate negative emotions [57][58]. *Roseburia hominis* is another flagellated gut anaerobic bacterium found in women with an active lifestyle and has been reported to exhibit immunomodulatory properties that could be helpful for treating gut inflammation, with beneficial properties for the intestinal barrier [59,60]. *R. hominis* has been considered as a potential probiotic treatment [59,60].

At the genus level, several species from *Parabacteroides* have been linked to the amelioration of obesity and the production of succinate and secondary bile acids [61,62]. Among the *Firmicutes* phylum, the *Phascolarctobacterium* genus has the capability to produce SFCAs like acetate and propionate, and *Phascolarctobacterium* species have been reported in healthy individuals [63], with positive relationships with insulin sensitivity and secretion [64].

Functional activity of the intestinal microbiota is related to high levels of physical activity

Outcomes analyzed in the current review include information about possible functional activity of the intestinal microbiota related to high levels of physical activity. To further characterize the microbiota of professional and amateur cyclists, Petersen et al. used a metatranscriptomic approach involving sequencing of RNA from fecal samples, enabling identification of the diversity of microorganisms' active genes [32]. Unlike the results of complete genomic sequencing, a lower relative abundance of *Bacteroidetes* mRNA ($p < 0.001$) was identified. Conversely, a higher abundance of *Firmicutes* ($p < 0.001$) was found. Importantly, the use of this tool expanded knowledge to other domains, such as *Euryarchaeota*, where a greater abundance of transcripts from archaea were found compared with DNA sequencing ($p < 0.001$), indicating that the expression of *Methanobrevibacter smithii* genes was highly variable among professional cyclists. It should be noted that presence of this archaeon was positively correlated with methane metabolism ($\rho = 0.88$, $p < 0.01$) and numerous pathways involved in transcription and translation, synthesis of secondary metabolites, and lipid metabolism ($\rho \geq 0.5$, $p < 0.01$). To determine whether the presence of archaea can confer any metabolic benefit to athletes, subsequent analyses were performed on samples with an abundance $\geq 8\%$ of mRNA. The results confirmed that the 50 most expressed genes are involved in the biosynthesis of methane, in addition to the presence of an important cluster of genes in which rubreritritin is found with several rubredoxin genes that are tolerant to oxidative stress. Archaea methanogens are common members of the gut microbiota, and *M. smithii* has been reported to play a role in weight development in childhood [65] also differentiating healthy subjects compared with IBD patients [66] and a healthy cohort of 116 Japanese individuals with a significant depletion of this archaeon [67]. Furthermore, metabolism related to methanogens is a risk factor for colon health, particularly in the presence of an abundance of different archaeon methanogen products. However, this factor is more closely related to gut dysbiosis because both are related to altered conditions in the host and its microbiome. Nevertheless, methanogen metabolism has also been described as potentially beneficial for gut health in normal and symbiotic conditions [68]. Subsequent analyses performed on a sample selected by Clarke et al. [34], included SCFA quantification using directed gas chromatography-mass spectrometry (GC-MS) directly from the feces of rugby players and individuals who did not exercise regularly, revealing a

significantly higher abundance of acetate ($p < 0.001$), propionate ($p < 0.001$), butyrate ($p < 0.001$) and valerate ($p < 0.001$), in rugby players [29]. These SCFAs have been described in many studies because of their beneficial effects on gut integrity and appetite control, as well as their functional impact on other systems (Figure 1) [69].

Table I: Summary of cross-sectional studies and comparison of groups according to their PAL.

Reference	Publication year	N	Sample	Comparison axis	Results	Country
Clarke et al. [34]	2014	86	Rugby players (29 ± 4) and two control groups with different BMI (29 ± 6)	Athletes and non-athletes with distinct BMI ≤ 25 – > 28	Higher diversity in athletes (Shannon index, $p = 0.0064$) Relative abundance Athletes vs IMC ≤ 25 \uparrow 40 taxa \downarrow <i>Bacteroidetes</i> \downarrow <i>Lactobacillaceae</i> \downarrow <i>Lactobacillus</i> Athletes vs IMC > 28 \uparrow 48 taxa \uparrow <i>Akkermansiaceae</i> (family) \uparrow <i>Akkermansiaceae</i> (genus) \downarrow <i>Bacteroidetes</i> (genus)	Ireland
Estaki et al. [35]	2016	39	Healthy young men and women (26.2 ± 5.5)	Categories of cardiorespiratory fitness: High – Avg – Low	No differences in α and β diversity	Canada
Bressa et al. [36]	2017	40	Middle-aged women (ACT 30.7 ± 5.9 – SED 32.2 ± 8.7)	Physical activity level and sedentary behavior	No differences for α and β diversity among groups Sedentary women \uparrow <i>Barnesiellaceae</i> (family & genus) \uparrow <i>Odoribacteraceae</i> (family & genus) \uparrow <i>Bifidobacterium</i> (genus)	Spain

					<p>↑<i>Turicibacter</i> (genus) ↑<i>Clostridiales</i> (genus), ↑<i>Coprococcus</i> (genus) ↑<i>Ruminococcus</i> (genus) Women with an active lifestyle ↑<i>Faecalibacterium prausnitzii</i> (spp) ↑ <i>Roseburia hominis</i> (spp) ↑ <i>Akkermansia muciniphila</i> (spp)</p>	
Petersen et al. [32]	2017	71	Cyclists (women and men) with ≥2 years participating in competitive events	Performance level: professional vs amateurs	<p>Higher diversity in cluster 3 (11 professional and 3 amateur cyclists) Shannon index $p = 0.0004$ Higher abundance of the genus: <i>Bacteroides</i>, <i>Prevotella</i>, <i>Eubacterium</i>, <i>Ruminococcus</i>, and <i>Akkermansia</i></p>	United States
Yang et al. [37]	2017	71	Premenopausal women age between 19 and 49 years	Cardiorespiratory fitness (CRF): High – Low	<p>High CRF ↑<i>Bacteroides</i> ↓ <i>Eubacterium rectale</i></p>	Finland
Whisner et al. [38]	2018	82	University students (men and women) (18.4 ± 0.6)	Physical activity level and sedentary behavior	No differences in α and β diversity	United States
Durk et al. [39]	2019	38	Apparently healthy men and women (25.7 ± 2.2)	Comparison between gender and oxygen consumption (VO_{2peak})	No differences in α and β diversity	United States
Jang et al. [27]	2019	45	Bodybuilding (n = 15), athletes (n = 15), non-	Differences among sporting activity	Relative abundance in bodybuilders ($p < 0.05$)	South Korea

			athlete control group (n = 15)		↑ <i>Faecalibacterium</i> ↑ <i>Sutterella</i> ↑ <i>Clostridium</i> ↑ <i>Haemophilus</i> ↑ <i>Eisenbergiella</i> ↓ <i>Bifidobacterium</i> ↓ <i>Parasutterella</i> Athletes and control group (p < 0.05) ↑ <i>Bifidobacterium adolescentis</i> ↑ <i>Bifidobacterium longum</i> ↑ <i>Lactobacillus sakei</i> ↑ <i>Blautia wexlerae</i> ↑ <i>Eubacterium hallii</i>	
O'Donovan et al. [28]	2019	37	International level athletes	Differences among sports classification groups	↑ <i>Eubacterium rectale</i> ↑ <i>Polynucleobacter necessarius</i> ↑ <i>Faecalibacterium prausnitzii</i> ↑ <i>Bacteroides vulgatus</i> ↑ <i>Gordonibacter massiliensis</i>	Ireland
Castro Mejia et al. [70]	2020	270	Healthy older men (≥ 65 years old)	Physical test result high fitness (HF) – low fitness (LF)	β diversity (p = 0.01)	Denmark

* Reported findings refer to the composition of the microbiome in terms of diversity (α and β) and species abundance where arrows denote increase (↑) or decrease (↓). Only significant results are shown (p < 0.05)

CRF = cardiorespiratory fitness

Changes in composition and function of the intestinal microbiota after an exercise program and sporting events

Starting an exercise program can induce changes in specific groups of bacteria. However, the effects are diverse and may respond to the phenotypic characteristics of the host and the stimulus exposure time (Figure 2). BMI appears to be a determining factor in the response of the microbiota to exercise. In apparently healthy people with a BMI ≥ 25 kg/m², discrete

changes in the relative abundance of groups belonging to the phylum *Actinobacteria*, *Bacteroides*, *Firmicutes*, *Proteobacteria* and *Verrucomicrobia* have been reported after 6 weeks of supervised aerobic training (Table II). Microbiota from lean subjects apparently responds to aerobic exercise by increasing the abundance of species from *Faecalibacterium spp* and *Lachnospira spp*, and by reducing *Bacteroides* members [40,71], whereas α - β diversity indices remain unchanged in this timeframe, discrete or any changes were registered for BMI. Likewise, changes in the microbiota of older people (≥ 62 years old) are discrete and respond mainly to increases and/or decreases in the relative abundance of groups such as *Clostridium* and *Bacteroides* [72,73]. Kern et al. [74] reported a significant difference in Shannon index values after 3 months ($p = 0.02$) and 6 months ($p = 0.059$) of vigorous intensity physical activity (70% of peak VO₂) in subjects aged between 20 and 40 years who were overweight or obese. There was a significant reduction of fat mass ($p < 0.001$) and increased of CRF ($p < 0.001$) in both groups meanwhile diet values remain similar before and after intervention.

Although previous studies have reported contradictory outcomes in subjects undertaking exercise programs, these differences could be due to differences in exposure time, which varied from 6-week to 6-month interventions. In addition, BMI and risks associated with physical inactivity could be determinants for gut microbiota responses to exercise. The main type of exercise used in this research is endurance training (aerobic), in which the oxidative system is the most important pathway for energy production, particularly in the mitochondria of active muscles where the Krebs cycle and respiratory chain promote efficient production of adenosine triphosphate per mmol of glycogen. Once a physically inactive subject initiates an aerobic training program it typically takes 4 to 6 weeks to exhibit acute adaptation to exercise in the cardiovascular system, depending on cardiac output and the capacity of active muscle to extract oxygen from arterial blood [75,76]. Figure 2 represents a possible progressive modulator effect that exercise exerts on gut microbiota once a physically inactive subject begins endurance training and enhances his CRF.

Progressive increase of physical activity level generates changes in the intestinal microbiota

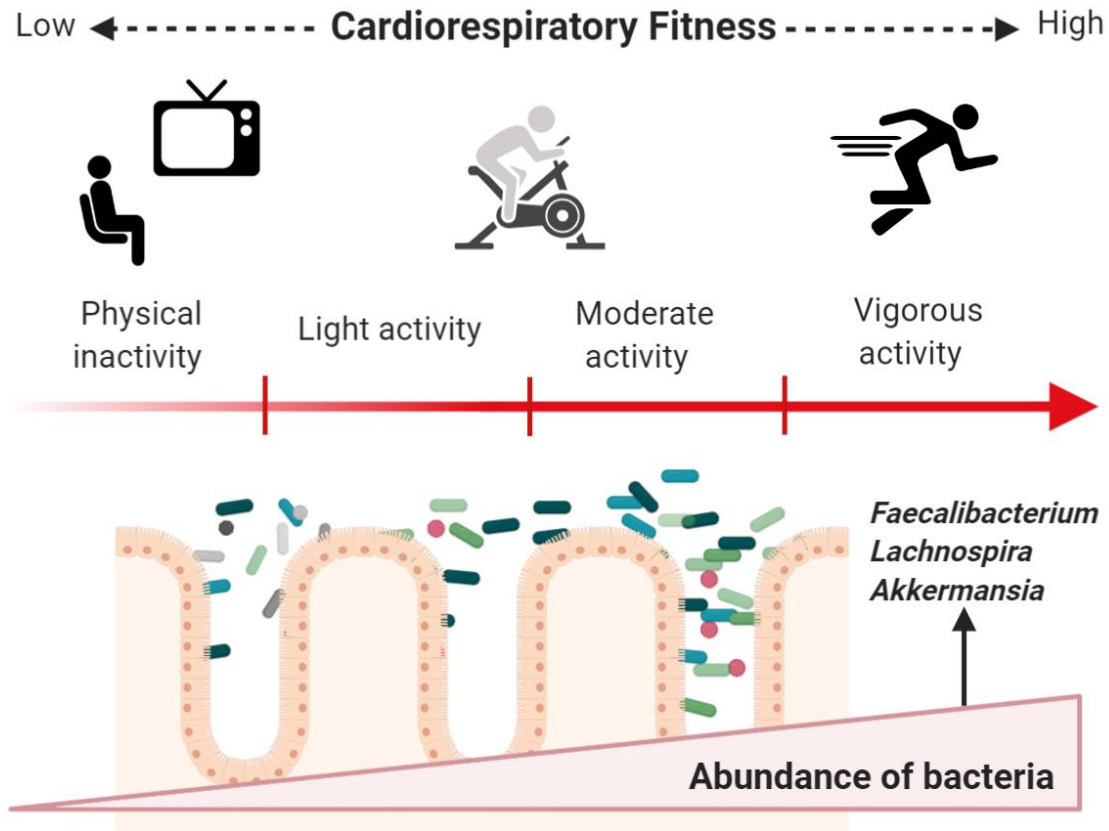


Figure 2: Exercise induces changes in gut microbiota through enhanced CRF in previously physically inactive subjects. Once a subject increases PAL, a series of beneficial molecular adaptations is induced, allowing enhancement of CRF. Major oxygen consumption is related to lower cardiometabolic risk, which may occur by augmenting progressively more energy demanding activities based on endurance training. Physiological modifications occur, and the gut microbiota do not appear to play a role in this process. Recent research has provided insight into a progressive increase of helpful members from different genus of bacteria. However, this change could depend on BMI status, energy demand and exposure time to exercise. Figure created with Biorender.com

Intestinal microbiota is susceptible to changes in events involving high energy demand

To identify whether there are changes after an endurance event, two previous studies collected samples before and after a half-marathon and a marathon, respectively. The athletes had similar characteristics in terms of body composition, training level (amateurs and professionals), diet and age. The most representative changes in the microbiome composition

were related to relative abundance. In the case of the athletes who ran 21 km, the presence of *Pseudobutyrvibrio*, *Coprococcus_2*, *Collinsella* and *Mitsuokella* was significantly greater at the end of the race [77]. Similarly, the abundance of *Veillonella* (genus) was significantly greater in the feces of professional runners after the Boston marathon, even 1 week later [30]. Using a different timeline, Hampton-Marcell and collaborators followed the intestinal microbiota of university swimmers for 1 year to track differences in the diversity or composition of the microbiome and their relationship with training volume during the season. Interestingly, the microbiota remained stable throughout the follow-up period, with no significant differences in α or β diversity, or in the relative abundance of species.

Physical activity produces substantial improvements in metabolites associated with intestinal microbiota

Exercise in physically inactive individuals not only produces changes in the composition of the microbiome, it can also improve the synthesis of metabolites associated with the intestinal microbiota. Allen et al. [40]. used gas chromatography to quantify SCFAs from feces of people who started a 6-week program of mainly aerobic exercise, participants were differentiated mainly by BMI (lean and obese). Only samples from lean subjects (BMI <25.0 kg/m²) exhibited a significant increase in SCFA concentration after the training period ($p < 0.05$), whereas the concentrations of acetate, propionate and butyrate in the obese group (BMI >30 kg/m²) remained unchanged. Likewise, the significant increase in butyrate-producing bacteria in people with BMI <25.0 kg/m², such as *Roseburia* spp, *Lachnospira* spp, Lachnospriaceae, Clostridiales and *Faecalibacterium* (Figure 2) was positively related to changes in butyrate concentrations and butyryl-CoA:acetate CoA-transferase (BCoAT) genes.

Performing a highly energy-demanding activity (e.g., a half marathon) has also been associated with modifications in metabolites related to gut microorganisms. One study using metabolomic analysis with liquid chromatography of samples before and after a half marathon event in Shanghai reported an increase in the concentration of approximately 40 metabolites (VIP score > 1), mainly of organic acids [77]. Results also revealed a decrease in 19 of these compounds (fold change > 0). The metabolic routes with the greatest modification after the race were pentose phosphate (more enriched with a value of $q =$

0.0071), pyrimidine metabolism, and biosynthesis of phenylalanine, tyrosine, and tryptophan [77].

Another study included repeated samples from athletes 1 week before and after running the Boston Marathon [30]. The results revealed a significant increase in *Veillonella*. Later, the presence of this genus from the phylum Firmicutes was confirmed in a subsample of 84 rowing athletes [30]. Metagenomic sequencing was performed, revealing an overrepresentation in the methylmalonyl-CoA pathway. Isolation and subsequent treatment in mice with the strain *Veillonella atypica*, were carried out to test whether mice inoculated with the bacteria or with *Lactobacillus bulgaricus* (control) exhibited altered responses after endurance training. The results revealed that animals treated with *V. atypica* showed a significant reduction in post-training proinflammatory cytokine levels, as well as better performance. However, no changes in GLUT4 glucose transporters were observed. To determine whether the richness of *Veillonella* causes functional changes in the emission of messengers (SCFA), propionate of the sample isolated directly from three athletes was quantified using mass spectrometry, showing that there was not only increased abundance of *Veillonella* but also an improvement in the lactate metabolism pathway. However, subsequent tests in animal models failed to demonstrate that resulting lactate could cross the lumen barrier and impact other tissues, such as muscle, brain or liver. However, it could cross the barrier to the intestinal lumen. Gram negative cocci *Veillonella* is a genus of anaerobic bacteria that commonly inhabit gut and oral microbiota, possessing unique physiology with an inability to utilize glucose and the capability to derive energy for growth through the fermentation of lactate [30].

Athletes typically have exceptional CRF [29,78] VO₂max in competitive athletes can be up to twice as high as that of sedentary subjects, leading to a higher capacity for oxidative metabolism in muscles, better neural connections, and improved general metabolism [18] (Figure 1). The capacity to perform endurance activities, such as a marathon, depends on many factors, particularly muscle-buffering capacity and lactate metabolism that could affect individual performance [78]. Findings from longitudinal studies may be helpful for understanding the link between enhanced microbiota and high-performance activities. Identification and isolation of *Veillonella* in endurance athletes suggests the existence of

communication between gut microbiota and muscle. The potential impact of anaerobic bacteria on propionate production via the Cori cycle should be further examined in future studies [30].

Table II: Longitudinal studies containing two or more gut microbiota samples, before and after exercise activities.

Reference	Study type**	Year	Sample	Observation/intervention time	Comparison axis	Findings*	Country
Allen et al. [40]	Intervention	2018	N = 32 Women and men with different BMI: Lean n = 18 (\bar{X} 25 years old) Obese n = 14 (\bar{X} 31 years old)	Six weeks of aerobic exercise, duration 30 to 60 min and moderate – high intensity (60 – 75% HR)	Before and after exercise intervention	LEAN ↓ <i>Bacteroides</i> ↑ <i>Faecalibacterium</i> spp. ↑ <i>Lachnospira</i> spp. OBESE ↓ <i>Faecalibacterium</i> spp. ↑ <i>Bacteroides</i> ↑ <i>Colinsella</i>	United States
Munukka et al. [71]	Intervention	2018	N = 17 Sedentary middle age women BMI >27.5 kg/m ² .	Six weeks of aerobic exercise	Before and after exercise intervention	↑ <i>Dorea</i> ↑ <i>Anaerofilum</i> ↑ <i>Akkermansia</i> ↓ <i>Porphyromonadaceae</i> ↓ <i>Odoribacter</i> ↓ <i>Desulfovibrionaceae</i> ↓ <i>Enterobacteriaceae</i>	Finland
Taniguchi et al. [79]	Intervention	2018	N = 33 Healthy older men (≥ 62 years old)	Five weeks of aerobic exercise	Before and after exercise intervention	↓ <i>Clostridium difficile</i>	Japan
Morita et al. [72]	Intervention	2019	N = 29 Sedentary women	Twelve weeks of supervised exercise	Before and after exercise	AE:	Japan

			(\bar{X} 65 years old)	AE = aerobic exercise MT = muscular tone	intervention	↑ <i>Bacteroides</i> ↑ <i>Clostridium subcluster XIVa</i> MT: ↑ <i>Clostridium subcluster IX</i>	
Kern et al. [74]	Intervention	2020	N = 88 Overweight and obese people age between 20 – 45 years old	Six weeks of intervention with different types of activities CON: No exercise (n = 14) BIKE: Active transport (n = 19) MOD: leisure time exercise (n = 31) VIG: vigorous supervised exercise (n = 24)	Control group vs rest of groups	Three months intervention VIG α diversity p = 0.012 6 months intervention VIG α diversity p = 0.059	Denmark
Zhao et al. [77]	Observational	2018	N = 20 Amateur athletes (\bar{X} 31 years old)	Gut microbiota before and after running a half marathon, distance → 21 km BEF: Feces analyzed before half marathon AFT: Feces analyzed after half marathon	NA	BEF: ↓ <i>Bacteroides coprophilus</i> AFT: ↑ <i>Pseudobutyrvibrio</i> ↑ <i>Coprococcus_2</i> ↑.- ↑ <i>Mitsuokella</i>	China
Scheiman et al. [30]	Observational	2019	N = 15 Professional marathonists (\bar{X} 27.4 years old)	Feces recollected during one week before and after the Boston marathon, distance → 42 km	NA	↑ <i>Veillonella</i>	United States
Hampton-Marcell et al. [80]	Observational	2020	N = 13 University swimmers aged between 18 and 24 years old	Feces recollected at different points in the season (beginning, maintenance and end of the season) Training volume was an important criterion	NA	Gut microbiota remained stable during the follow-up season	United States

* The reported findings refer to the composition of the microbiome in terms of diversity (α and β) and species abundance. Arrows denote increase (\uparrow) or decrease (\downarrow). Significant results are shown ($p < 0.05$). ** Types of studies included: Intervention: The selected sample carried out an exercise program for a certain time period. Observational: The selected sample was followed before and after a sporting event. **HR** = heart rate

CONCLUSIONS

The results of physically inactive subjects examined in the current review suggest that it could take more than 6 weeks to exhibit differences in α diversity indices. It is likely that acute responses to exercise are related to functional modifications in gut microbiota. Furthermore, studies have reported an abundance of butyrate-, propionate- and acetate-producing bacteria specially in chronic adaptation to exercise, this was associated to improvements in species diversity, CRF and intestinal diversity [35,37,39].

Further exploration in this field is required mainly by using controlled clinical trials that include different types of exercise (i.e endurance and HIT), distinct age groups, larger samples and incorporation of multiomics approaches, as well as detailed information about diet. Few studies have expanded the results to other types of microorganisms, such as viruses [81] and archaea [32]. In addition, the role of parasites and fungi has not yet been identified. Exploration of other domains could elucidate the role of gut microbiome on exercise adaptation, particularly muscle growth and signaling. One recent study reported the use of adeno-associated virus 9 (AAV9) as a vector to deliver the protein follistatin to improve muscle performance and mitigate the severity of osteoarthritis sequelae, including inflammation and obesity, in mice [82]. Although this is a gene therapy approach, the possibility that viruses could mediate signaling pathways in short- and long-term adaptations to exercise should not be excluded.

To date, it is not yet possible to draw firm conclusions about whether the metabolic activity of these microorganisms can impact various tissues via SCFAs during exercise or sporting activities, resulting in an improvement of performance activities. Nevertheless, the results reported by Scheiman et al. [30] appear to be conclusive, identifying a stable relationship between efficient microbiota members and the production of propionate, impacting lactate production, at least in the colonic lumen. Similarly, Allen et al. [40], reported an increase in

acetate, butyrate and propionate concentrations after 6 weeks of aerobic exercise only in normal weight subjects.

Further research is needed to determine whether these metabolites can cross the intestinal barrier and impact other tissues, such as muscle, brain or liver in endurance and other types of training activities like resistance or high intensity training (HIT), and to determine whether there are mechanisms for efficient use of nutrients from diet via gut microbiota members that could improve pathways both in bacteria and the host. In the current review, we propose that exercise modulates the intestinal microbiome by increasing the abundance of beneficial and metabolite-producing bacteria (Figure 1 & 2). In addition, long term adaptation to endurance training in athletes appears to enhance aerobic physical abilities as a result of planned training volume, as shown in Figure 2. Thus, previously physically inactive subjects can improve CRF by performing exercise from medium to vigorous intensity ($\geq 60\%$ of HR max), indicating that enhancement of VO₂max is related to the abundance of certain groups of metabolites producing bacteria. We recommended exploring other groups of bacteria such methane metabolizing or those that may improve BCAAs synthesis for further research in this field.

Exercise may not only have benefits in human systems but could also contribute to communication pathways within the same microbiota members, which constitute metacommunities that need to function in symbiotic environments. In addition, organ-specific adaptations induced by exercise may help to improve human health and general life quality. The outcomes analyzed in the current study suggest the importance of the presence of symbiont bacteria, which can influence and enhance the function of other members of the ecosystem inhabiting the intestine. The application of omics approaches may be crucial for identifying other possible microorganisms with potential functional impacts, as well as for verifying possible relationships or crosstalk with systems and organelles proposed in past reviews [24] (Figure 2) especially with the mitochondria [83].

This research area is still in its infancy. Although numerous important studies have provided approaches for elucidating possible mechanisms, the effects of individual characteristics and

the numerous factors that can influence the composition of the microbiome are important to consider. Research examining the microbiota-exercise association should aim to test homogeneous groups to characterize the possible aspects of lifestyle as well as possible, including diet, the level of training, and sedentary behavior. Nevertheless, it is important to emphasize that all studies included in this review were performed in relatively developed high-income countries. There is a lack of information from developing regions, particularly South America and Africa. Future studies should examine populations in these geographic areas to elucidate the effects of substantial variations in diet [84]. Similarly, the use of biomarkers that reflect inflammatory and metabolic status could provide useful information regarding the metabolism and physical condition of the host. Finally, possible relationships within the same microbiota should not be neglected; the meta-community in the intestinal environment emphasizes the potential connections between bacteria, fungi, parasites and viruses, which could directly influence the response to exercise through functional improvement [85,86].

Mainly limitation for this review is the lack of information about diet and the influence of certain foods on gut microbiota composition and function, since diet may lead to major changes it is important to explore these two variables together.

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