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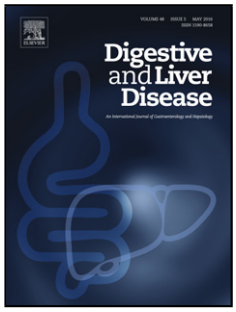

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- Review Article -

Exercise has the guts: how physical activity may positively modulate gut microbiota in chronic and immune-based diseases

Running head: *Exercise and gut microbiota*

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Abstract

Limited animal and human research findings implied that exercise might have a beneficial role on health gut. Cardiorespiratory fitness has been correlated to health-associated gut parameters such as taxonomic diversity and richness. Physical exercise may augment intestinal microbial diversity through several mechanisms; the promotion of an anti-inflammatory state is one of these. The expression of disease-associated microbial functions was linked to distinct taxa in previous studies of familial type 1 diabetes mellitus (T1D). An integrated multi-approach in the study of T1D, including physical exercise, is advocated. The present review explores how exercise might modulate gut microbiota and microbiome characteristics in chronic and immune-based diseases, considering the ascertained relationship between transversal gut functions and human health.

Abbreviations:
AMPK, phosphorylated adenosine monophosphate-activated protein kinase; BDNF, brain-derived neurotrophic factors; BF, *Bacteroides fragilis*; BMI, body mass index; Cox-2, cyclo-oxygenase 2; DIO, diet-induced obesity; FFAR, free fatty acid receptor; Fiaf, fasting-induced adipose factor; GF, germ-free; GIT, gastrointestinal tract; GLP-1, glucagon-like peptide; HbA_{1c}, glycosylated hemoglobin; HFD, high fat diet; HIIT, high-intensity interval training; HPA, hypothalamic-pituitary adrenal; IFN, interferon; Ig, immunoglobuline; IL, interleukin; LPS, lipopolysaccharide; MAMP, microbe-associated molecular pattern; NK, natural killer cells; NOD, non-obese diabetic; SCFAs, short chain fatty acids; SPF, specific pathogen free; T1D, type 1 diabetes; T2D, type 2 diabetes; TNF, tumor necrosis factor; TLRs, toll-like receptors; URTI, upper respiratory tract infections; WHO, World Health Organization; 5-HT, 5-hydroxytryptamine

Keywords:
Exercise, gut microbiota, type 1 diabetes

1. Background | the multiform protection offered by physical exercise to health

Nowadays, the appeal of practicing regular physical activity has become redundant as multiple international agencies advocate for the protective, curative, and revertive effects of exercise in a myriad of metabolic and psychological disturbances [1,2].

Increasing physical activity emerges as a mandatory forefront to abate the burden associated with longevity and expanded life expectancy of present days. In fact, exercise covers a broad spectrum of health benefits, from boosting mental wellness by enhancing mood states and neuroplasticity via augmented brain-derived neurotrophic factors (BDNF) levels [3], to help preventing excess weight gain or maintaining weight loss [1,4–6], mastering a cascade of favorable events in the metabolic equilibrium of the human body. A substantial number of both cross-sectional and longitudinal studies have indicated that regular physical exercise exerts diversified anti-inflammatory actions [7,8]. An exercise-mediated organ crosstalk orchestrates a pattern leading to the increase of anti-inflammatory cytokines, and/or the decreasing of pro-inflammatory cytokines. In contracting muscles, myokines play a major role in lowering markers of chronic inflammation and protecting against accumulation of abdominal fat [7]. Particularly, muscle-derived interleukin (IL)-6 has been recognized as a pleiotropic myokine in the modulation of immuno- and inflammatory metabolism: it inhibits tumor necrosis factor (TNF) production [9] and stimulates, at the same time, the release of the anti-inflammatory cytokines IL-1ra and

IL-10 [10,11]. Furthermore, IL-6 mediates the release of cortisol from adrenal gland and it may have systemic effects on the liver and adipose tissue [12]. IL-6 increases insulin secretion by upregulating glucagon-like peptide 1 (GLP-1) [13]. It increases insulin-stimulated glucose uptake both in skeletal muscle and adipose tissue and, in fact, IL-6 stimulates, through the activation of AMPK, both lypolisis and fat oxidation, peripherally and whole-body [14,15].

Regular and adequate levels of physical activity protect against all-cause mortality: the World Health Organization (WHO) ranked physical inactivity as the fourth leading risk factor for global mortality, i.e. 6% of deaths globally [16]. It has been estimated that 21-25% of breast and colon cancers, 27% of diabetes, and approximately 30% of ischaemic heart disease burden can be attributed to physical inactivitivy. Exercise training has been clinically proven, cost-effective, in the treatment and cure of several of these diseases [17]. Certainly, exercise enables these extraordinarily beneficial actions by affecting energy balance and by lowering atherogenic profiles [18]. Nevertheless, alternative and novel explanations covered by exercise are gaining momentum in the current literature. One of these hypotheses is related to a favorable modification of the human gut microbiota in health and disease.

2. Materials and Methods

2.1 Literature search strategy

A systematic literature search was carried out in the Cochrane Library and MEDLINE databases for studies published in English (2000 January to 2017 September) combining the terms “gut microbiota”, “gut microbiome”, “exercise”, “training”, “physical fitness”, “physical activity”, “diabetes”, “type 1 diabetes”. We examined reference lists in original articles, reviews, trials. Study search was

performed both electronically and by following up references quoted in relevant paper. Case reports, expert opinions, were excluded.

2.2 Study selection

The articles were extracted and read, and relative findings were classified by: i) gut microbiota functions interacting with metabolism, immune system, hypothalamic-pituitary-adrenal (HPA)-axis; ii) exercise effects on gut microbiota in different models; iii) how exercise-interventions modulating microbiota might be therapeutically exploited to promote health.

Periods of data collections ranged from 2000 and 2017, obtaining data from human and animal models (mice, rats). A synoptic table of the relevant studies analyzed is offered (Table 1).

3. Gut microbiota: the overcalled card

The gastrointestinal tract (GIT) is inhabited by trillions of microorganisms – approximately a hundred – composing the human gut microbiota [19,20]. By including roughly 9 million genes [21], this environment represents the human gut microbiome: a gene set 150 times larger than that of the human genome [22,23]. Gut bacteria composition may be influenced by both endogenous and exogenous stimuli. The type of birth (Caesarian versus vaginalis) and lactation (formula versus breast feeding) may differentiate the gut bacterial population during infancy. During childhood, gut microbiota assumes the composition of adulthood, comprising 5 phyla and 160 species in the large intestine [24], weighing up to 2 kg [25]. In the microbial community of the human intestine, the dominant bacterial phyla are the *Firmicutes* (~ 60%) and *Bacteriodetes* (~ 20%) whereas *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* are in relatively low abundance [26]. Although genetic, epigenetic,

and environmental factors may uniquely influence the community composition, one third of the adult gut microbiota is similar within most individuals [22]. Physical activity, diet, antibiotics, gender, genetics, age, ethnicity, health and diseases might characterize distinctively the human gut bacterial population.

Gut microbiota has been increasingly advocated because of its various functions (Figure 1). The possibility to manipulate the microbiota, on different fronts, for improving health outcomes, is being attractive. Primarily, the microbial communities contribute to the host's health through the fermentation of indigestible nutrients in the large intestine. The main products of the bacterial fermentation of carbohydrates and proteins, under anaerobic conditions of the large intestine, are short chain fatty acids (SCFAs). SCFAs such as acetate, propionate, butyrate, are secreted in the gut lumen (Figure 2), and their signaling to the multiple gut receptors (free fatty acid receptor 2-3, FFAR2, FFAR3) are implicated in the control of anorectic hormones – like peptide YY (PYY) [27] and GLP-1 [28]. Also, SCFAs promote the release of serotonine (5-hydroxytryptamine, 5-HT) [29], an aminoacid key regulator of GIT motility and secretion (Figure 2). Altogether, this involvement suggested a role of microbiota in the gut-brain axis, and appetite control. Furthermore, butyrate has been evoked for anticancer effects, anti-inflammatory properties and energy expenditure [30,31].

The gut microbiota harbours clusters of bacteria that are either innocuous, symbiotic, or potentially pathogens (pathobionts). This means that gut microbiota participates in a fine-tuning balance between both innocuous and harmful bacteria, by cross-talking with all immune system components [19]. The activation of the pattern recognition receptors, like the toll-like receptors (TLRs), is a consolidated mechanism by which the immune system discerns pathogens and non-harming elements [32].

Therefore the microbiota exerts a protective function to avoid GIT colonization by pathogens, via stimulation of the epithelial cell proliferation, and the production of various antimicrobial molecules. The gut microbiota may contribute to the development of the mucosal immune system either directly or indirectly (Figure 2). The microbiota *per se* modulates the expression of TLRs through the microbe-associated molecular pattern (MAMP), leading to the activation of the nuclear factor-kappa B pathway and activation of T-cells (Figure 2). Moreover, the gut microbiota may orchestrate B cell maturation, deciding also the fate of their immunoglobulin isotype (IgA versus IgE). On the other hand, metabolic by-products of the microbiota can be implicated in mucosal tolerance via induction of T-regulatory cells.

When the fine equilibrium between the immune system and the commensal bacteria is impaired, health is compromised, and several diseases may arise (Figure 1). A gut dysbiosis has been associated with inflammatory bowel diseases such as Crohn's disease and ulcerative colitis [33,34]. Particularly, Crohn's disease may be caused or aggravated by bacteria like *Firmicutes* (*Fecalibacterium prausnitzii*) and *Escherichia coli* [35]. Evidence exists that *Firmicutes*:*Bacteriodetes* ratio is elevated in irritable bowel syndrome, obesity, type 2 diabetes mellitus (T2D) and insulin resistance [36]. It is unascertained why the same ratio has been found increased in old age [37], however a greater diversity of microbiota was associated with a healthier status in elderly with respect to that found in younger adults [38]. A low diversity of gut microbiota has also been detected in allergic diseases, other than metabolic syndrome, type 1 diabetes mellitus (T1D) and T2D. Notably, an extensive body of literature has dealt with the etiopathogenetic role of intestinal microbiome in T1D (see § 5) [39,40]. Various microbiota profiles have been drafted for rheumatoid

arthritis, autism [41], and mood disturbances [25,42]. Excessive protein fermentation by pathogen bacteria within the colon has been putatively linked to colon cancer [43].

In this view, the metabolic capacity of gut microbiota is crucial. The gut microbiota may modulate host energy metabolism in different ways. Besides producing SCFAs, which may represent alternative energy substrates for hepatic gluconeogenesis (propionate), the intestinal microbiota may alter hepatic triglyceride production, lipid- and carbohydrate metabolism [44]. Several communities of the gut bacteria may synthesize vitamins (K, folic acid, biotin, thiamine), glycans, amino acids, or ferment indigestible fiber [23,37,45].

Interestingly, recent studies showed that exercise, as a homeostatic stimulus, might diversify the gut microbiota enhancing the number of benign microbial communities [25,46]. Notwithstanding, the underlying mechanisms behind this positive modulation remain undetetermined.

4. The impact of exercise on microbiota diversity

There is a conceptual framework in which exercise-microbiota studies are gaining momentum in the science community. Originally, the muscle-microbiota axis was legitimized by the seminal study of Bäckhed et al. [47], in which germ-free (GF) mice, in contrast to mice with a gut microbiota, were protected against diet-induced obesity showing a persistently lean phenotype with increased levels of phosphorylated AMP-activated protein kinase (AMPK) in muscle and liver. In the same study, GF mice with an inactivated expression of fasting-induced adipose factor (Fiaf $^{-/-}$), a circulating lipoprotein lipase inhibitor, tipically suppressed in the gut epithelium by the microbiota, were not protected from diet-induced obesity. Those findings suggested that manipulating microbial community impacted on muscle bioenergetics

(i.e. fatty acid oxidation) in a way that host energy metabolism was even protected against a high-calorie westernized-diet.

A further step was made when exercise-interventions showed a direct interaction with gut microbiota and intestinal microbiome. Not only GF mice exhibited a worse exercise performance with respect to littermates colonized by a single bacterial species (phylum), but also mice colonized by multiple non-harmful bacteria displayed the greatest endurance capacity [48]. In that study, different microbiota composition and structure affected exercise performance by modulating the anti-oxidant system activity [48]. Even under high-fat diet conditions, in obese mice, exercise seems capable to protect the gut integrity and morphology, for instance by reducing inflammatory markers such as cyclo-oxygenase 2 (Cox-2) in both proximal and distal gut [49]. Several exercise-triggered factors influencing gut microbiota can be enumerated: the modification of the bile acids profile (fecal bile acids increase as exercise-amount and -intensity increase) [50]; the elevated production of SCFAs via AMPK activation [47]; the suppression of TLRs signaling pathway in the liver, muscle, and adipose tissue by reducing lipopolysaccharide (LPS) serum levels [51]; the increase of immunoglobulin A (IgA) [52] production and a reduced number of B and CD4 + T cells; the weight loss [53], myokines (IL-6, IL-10, IL-1ra, TNF-R) [54]; the gut transit time [55] (exercise reduces intestinal transit time hence influencing microbiota composition).

Intriguing questions are open to be discussed in exercise immunology, specifically those concerning exercise as the nexus between the immune system and the microbiota. Several diseases manifest a disrupted signaling in host physiology: exercise was shown to positively participate in the host-microbe interactions. However, the extent by which exercise favorably alters innate immunity is only

partially understood. Regular, moderate physical acitvity is associated with an anti-inflammatory status in the intestinal lymphocytes post-exercise (TNF-α expression is suppressed while IL-10 is increased) [56]; it tempers intestinal barrier dysfunction; it preserves mucous thickness and intestinal permeability [57] (e.g. by SCFA-induced reduction of colonic mucosa permeability). A diminished bacterial translocation and an upregulated anti-microbial protein production have also been associated with moderate exercise [57]. SCFAs appear to modulate neutrophil function and migration in an anti-inflammatory fashion [58]. In young women, eccentric exercise was accompanied by blunted, TLR4-mediated, inflammatory responses [59]. Resistance exercise ameliorated the pro-inflammatory status of elderly subjects through an attenuation of TLR2 and TLR4 signaling pathways [60]. By contrast, strenuous, prolonged endurance exercise may reduce gastrointestinal blood flow [61], leading to hypo-perfusion [62,63], susceptibility to endotoxins ("leaky gut", endotoxemia) [64], and an overall increased expression of pro-inflammatory modulators (TNF-α, IL-1, IL-6, IL-1ra) [56,65,66]. In addition, excessively intense exercise may compromise mesenteric redox environment, therefore weakening the activity of antioxidant enzymes [67]. At the same time, the epithelial barrier disruption increases the TLR-mediated recognition of gut commensal bacteria [68].

In this perspective, a complete dose-response analysis between exercise levels and their beneficial alterations in microbial composition is yet to be fully explored.

Exercise was proven to restore impaired conditions (either involving the immune system or the microbiota) associated with inflammation. In fact, immunological system may contribute to the etiopathogenesis of several chronic

diseases, such as diabetes and obesity, both characterized by deteriorated microbiota profiles [69].

Regular exercise improves immunological profile in T2D [70], cardiovascular diseases [71,72], and obesity [73]. In T2D subjects, a combination of aerobic and resistance training was capable to lower pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IFN-γ) and increase anti-inflammatory cytokines (IL-4, IL-10) [70]. We have also shown decent anti-inflammatory profiles in subjects with T1D perfoming a regular schedule of moderate aerobic exercise [8,74].

However, whether exercise may play a causative-key role in the interactions between the microbiota and the innate immune system to promote health, remains to be addressed.

4.1 Animal studies

A vast number of animal models demonstrated that qualitative and quantitative changes in intestinal flora, under exercise-conditions, are able to influence the absorption of nutrients, the distribution of energy, the immunity, and the gut-brain axis (particularly involving the interactions between the HPA-axis and the microbiota).

Hsu et al. compared the endurance swimming performance of three groups of mice: GF mice lacking gut microbiota, GF mice colonized by one bacterium (BF, *Bacteroides fragilis*), specific pathogen-free mice (SPF, i.e. microbiota without pathogenic bacteria like *Helicobacter pylori*). The best time-to exhaustion was obtained by the complete microbiota group (SPF), followed by mice having one bacterium (BF), and the GF mice as the worst ones [48]. The absence of microbial colonization was accompanied by decreased levels of SCFAs and other critical antioxidant enzimes for reducing oxidative stress (serum and liver glutathione

peroxidase and serum catalase) [48]. Also, SCFA levels of butyrate were found significantly increased in male Wistar rats subjected to a free-wheel running protocol with respect to sedentary controls [75]. As said, SCFAs are one of the major end products of microbial fermentation and contribute to intestinal energy homeostasis by modulating immune system and reducing inflammation and oxidative stress. Matsumoto et al. hypothesized that cecal butyrate concentrations were inversely related to colon disease risks due to exercise [75]. Other animal studies supported the exercise-induced increase in butyrate-producing bacteria [76], however results might vary according to the protocol employed (voluntary- versus controlled exercise) [77]. In addition, Campbell et al. confirmed the unique exercise effects on microbiome, independently of dietary treatments [49].

The ratio *Firmicutes:Bacteroidetes* has been extensively analyzed in experimental animal studies. In a model of high fat diet-induced obesity (DIO mice), Evans et al. observed that voluntary wheel running prevented weight gain and altered microbiota, reequilibrating major-phylum levels. Furthermore, the *Firmicutes:Bacteroidetes* ratio was proportional to the total distance covered by the mice [78]. Voluntary wheel running was found to attenuate microbiome changes induced by a two-day exposure of polychlorinated biphenyls mixture, preserving the gut microbial richness in mice [79]. Also, in a male rat model of food restriction (anorexia), free access to exercise increased *Lactobacillus* genus and *Blautia coccoides-Eubacterium rectale* group, which were negatively correlated (together with *Bifidobacterium*) with serum ghrelin levels. Furthermore, serum ghrelin levels positivey correlated with *Bacteroides* and *Prevotella* [80]. On the other hand, serum leptin concentrations were directly proportional to the quantity of *Bifidobacterium* and *Lactobacillus*, and negatively correlated with *Clostridium*, *Bacteroides* and *Prevotella* [80]. Substantially, exercise

increased *Bacteroidetes* and reduced *Firmicutes*. Appetite-regulating hormones (therefore the nutritional status) and exercise importantly affected the gut microbiota composition [80].

Moderate treadmill training favorably modified bacterial communities at the genus level in obese and hypertensive rats, suggesting that exercise elicits distinctive microbiota profiles according to the host characteristics [81].

Cook at al. investigated both voluntary wheel- and forced treadmill training, resulting in a wide microbial taxonomy, possibly linked to gut immune cell homeostasis- and microbiota -immune interactions. Evidence was provided in support of a positive action played by exercise against the inflammatory insult occurring at the gut level [82].

Kang et al. analyzed the unrelated effects of diet and exercise on behavioral domains (anxiety and cognitive dysfunctions) through differently-impacted microbiota. Specifically, exercise resulted incapable of rescuing anxiety phenotypes determined by high fat diet (HFD). On the contrary, exercise ameliorated cognitive abilities without being affected by HFD [83]. Additionally, exercise caused substantial shifts in the gut micriobiome, and dinstictive bacterial abundances were orthogonally related to cognition and anxiety [83].

Denou et al. examined the effects of high-intensity interval training (HIIT) on microbiota of various segments of the gut, in HFD-fed mice [84]. HIIT ameliorated insulin tolerance with no changes in adiposity. Microbiota was modified in a segment-dependent manner. Exercise augmented bacterial diversity in diet-induced obesity (DIO), with an increased number of *Bacteroidetes*, therefore lowering the *Firmicutes:Bacteroidetes* ratio.

In a model of human menopause (ovariectomized female rats), 11 weeks of voluntary wheel running differently altered the gut microbial communities (taxa, families, genera) depending on low- rather than high-aerobic capacities [85]. Relative abundance of *Firmicutes* was decreased in low-capacity running rats, but increased in high-capacity running rats. Relative abundance of *Proteobacteria* and *Cianobacteria* resulted increased in low-capacity running rats, but decreased in high-capacity running rats. Nonetheless, no significant changes were detected in the quantity of *Bacteroidetes* and ultimately in the *Firmicutes:Bacteroidetes* ratio.

The effects of exercise on gut microbiota seem to be age dependent. Exercise induced more effective changes in juvenile rats than in adult rats [86]. When exercise was initiated at an early developmental stage, it produced modification in the gut microbiota composition (increase in phylum *Bacteroidetes* and decrease in *Firmicutes*) leading to a leaner body mass respect to what exercise entailed when practiced at an adult-stage. Juvenile-onset exercise altered more genera than adult-onset exercise. Futhermore, early-life exercise promoted brain function, metabolic- and psychological health, through health-enhancing gut microbes [87].

Increased LPS translocation has been postulated as another driver for several inflammatory conditions associated with insulin resistance, T2D, and obesity [88]. In the muscle, TLRs may be activated by LPS arising from the membrane of bacteria. LPS-injected mice developed chronic inflammation, obesity, insulin resistance, and muscle atrophy through TLR4 receptors [51]. Low and high doses of LPS induced activation of TLR4, resulting in enhanced glucose uptake and reduced fatty acid oxidation in different skeletal muscle models (TLR4 $^{-/-}$ mice) [89]. In HFD-fed mice, voluntary wheel running improved insulin tolerance, despite LPS injections (2 mg/kg ip) [90]. Intestinal immune response was found to be favorably modulated in the

studies by Hoffman-Goetz, in which exercise decreased TNF-α and proinflammatory cytokine IL-17, whereas it increased glutathione peroxidase, catalase, and the anti-inflammatory cytokine IL-10 [56,91,92]. Moderate exercise (swimming for 16 weeks) increased the levels of IgA in the duodenal lumen of Balb/c mice [93]. Similarly, it was found an increased expression of the genes encoding for IL-4, IL-6, IL-10, TNF-α and TGF-β, cytokines that regulate the synthesis of IgA – the inflammatory response, and the immune response in the intestine [52].

To a certain extent, exercise may thus elicit an ameliorated microbiota associated with diversity, richness (in health-promoting bacteria), and improved exercise performance.

Altogether, it seems that the microbiota changes induced by physical exercise depend on the physiological- [81], and above all, on the metabolic state [48,94].

4.2 Human studies

The lack of a decent number of properly designed and prospective controlled trials renders arduous to investigate the effects of exercise alone on the composition and function of human gut microbiota. Many studies, in fact, include dietary treatments, which represent another major confounding factor in altering the gut microbiota. The microbiota research embraces a multifaceted interplay, including energy metabolism and autoimmunity.

In the master study of Clark et al. [95], forty elite rugby players showed increased microbiota richness and diversity, and this latter correlated linearly with protein intake and creatine kinase concentrations. Dietary extremes and exercise were therefore recognized as crucial regulators of gut biodiversity. Further, not only athletes'microbiota was more diverse than controls'one in the *Firmicutes* phylum

(particularly, in *Faecalibacterium prausnitzii*), but also rugby players and low-BMI controls exhibited higher proportions of *Akkermansia muciniphila*, which is indeed negatively correlated with BMI, obesity, and metabolic disorders, possibly owing to an improved gut barrier function. Furthermore, the microbial changes ascertained in the elite rugby players were accompanied by lower inflammatory cytokines (IL-6, TNF-α, IL-1β) and higher anti-inflammatory cytokines (IL-10, IL-8) with respect to controls.

Liu et al. published a protocol to study the effect of aerobic exercise and low-carbohydrate diet on the microbiota of postmenopausal women and middle-aged pre-diabetic men with non-alcoholic fatty liver disease, throughout six months, and followed-up after 6 and 12 months. This approach explores the possibility to incorporate exercise-induced microbiota modifications to combat chronic diseases [96].

Higher abundance of health-promoting bacteria like *Faecalibacterium prausnitzii*, *Roseburia hominis* and *Akkermansia muciniphila* were found elevated in women regularly performing the minimum dose of exercise recommended by WHO. These women had a body fat/muscular mass percentage, significantly correlating with several gut microbial communities [97]. The authors suggested that those findings were supportive of a pattern of exercise inclined to break sedentary behaviours while beneficially modifying gut microbiota.

After normalizing BMI, diet, age, Estaki et al. analyzed fecal microbiota and fecal SCFAs in 39 healthy subjects (male and female) with different levels of cardiorespiratory fitness [98]. Independently of their diet, the higher the fitness level, the more diverse was their gut microbiome. Increased pruduction of butyrate, as a marker of gut health, and increased abundance of butyrate-producing taxa were also

measured in individuals with greater levels of aerobic fitness [98]. Regardless of age, carbohydrate- or fat intake, Yang et al. confirmed the association between cardiorespiratory fitness and gut microbiota composition in 71 premenopausal women [99]. Further, the changes in cardiorespiratory fitness (and also anxiety) were significantly associated with the gut microbial composition in breast cancer survivors [100].

Probiotics supplementation also seems to benefit intense endurance athletes. *Lactobacillus fermentum* was successfully used in highly trained distance runners to reduce URTI (upper respiratory tract infections) incidence and related-severity [101].

The exercise-intensity proposed in the various protocols remains a controversial issue. However, while moderate-intensity exercise reduces gut transit time, prolonged strenuous exercise may affect gut permeability, provoking diarrhea, bacterial translocation into the bloodstream, gastrointestinal bleeding and disorders [102,103]. A high volume of training (50 weeks) in elite America's Cup yacht racing athletes suppressed salivary levels of IgA, which were neatly correlated with intense training and competition load [104]. This restates the chance of an impaired immunity for those individuals undergone to strenuous exercise (as very intense exercise increases the prevalence of URTI and gastrointestinal problems in athletes [105]). Short-duration (5-min) running on treadmill enhanced lactulose catabolism, reporting a beneficial effect on colonic mucosa [106]. Instead, ultra-endurance runners may experience mucosal erosions and ischemic colitis [107]. A military training, consisting in 4-day cross-country ski march, augmented microbial diversity in 73 soldiers compared to controls. The intense training also increased intestinal permeability, which was associated with alterations in markers of inflammation. However military training enhanced the relative abundance of *Lactobacillus*, whereas

Streptococcus, *Aggregatibacter*, and *Sutterella* resulted lower with respect to pre-training levels [108].

Weight loss is another exercise-inducible factor that might be responsible for peculiar changes in gut microbiota [94]. As said, composition and diversity are divergent in obese and non-obese individuals [109]. It is yet unsolved whether weight loss causes gut microbiota modifications or *vice versa*. In obese individuals with sleep disorders, a 6-month individual progressive exercise program, combining Nordic walking, stretching, strength, relaxation, together with dietary suggestions, improved sleep quality and modified gut microbiota composition [110]. It has been speculated that even Tai-chi, as a form of moderate-intensity exercise involving deep breathing and meditation, might induce favorable changes in gut microbiota by mediating the HPA axis [111].

Certainly, the HPA axis is activated under diversified conditions, including exercise. A cross-talk between HPA axis and the gut microbiota has been recently postulated. By comprising the central nervous system, the autonomic nervous system, the stress system of the HPA axis, and the intestinal immune system, a brain-gut axis has been delineated. However, even exercise-induced stress modulates hormones, cytokines and gut microbial molecules, enlarging the network to a proper brain-gut-muscle axis (Figure 3). Regardless of the cause, hormonal changes drive modifications in gut microbiota composition and distribution, which in turn might affect host behavior.

The overall picture is complex, involving numerous interactions (host genetics, diet, exercise, individual habits) with environmental factors that play a central role in the gut microbiota modifications [20,112,113], and a major role in the development of diverse pathologies such as obesity and diabetes (Figure 1).

5. A possible role for exercise in the positive modification of the gut microbiome in T1D

Gut bacteria have been undoubtedly implicated in the development of autoimmunity for T1D, even in children [69,114,115]. Dysbiosis of the intestinal microbiota is known to affect the gut barrier, potentially playing a pivotal role in the onset of T1D [116]. Indeed, high levels of *Bacteroides* and a reduced abundance of *Prevotella* have been observed in children affected by T1D [115]. In one of the first studies comparing gut microbiota of T1D-children with healthy controls, the former showed increased *Firmicutes:Bacteroidetes* ratio as well as higher abundance of *Bacteroides* with respect to non-diabetic children [115]. More interestingly, the same bacterial profile was observed in pre-diabetic children [114]. In a European study, 28 children with newly-diagnosed T1D displayed a decreased fraction of butyrate-producing species compared to age-paired control children [114]. *Bacteroides* activity in mucine-synthesis and degradation may be involved in T1D development by reducing the mucus layer (weakening of tight junctions) and increasing gut permeability and inflammation. All these effects may lead to the loss of self-tolerance, inducing deviant immune responses (imbalance of T-cells) and lymphocytic infiltrate, typical of T1D [117]. It is indeed possible that intestinal microbiota changes suppress the function of T-cells, provoking dysregulated immune responses and thus dysrupting immunological tolerance [39]. As mentioned, butyrate may play a critical role in the regulation of gut permeability and the assembly of tight junctions [118]. In animal models of autoimmune diabetes, not only gut permeability preceded diabetes onset, but also the environmental factors modulating permeability affected diabetes incidence [119].

The characterization of intestinal bacterial populations may be of great importance, not only in defining a specific gut microbiota profile associated to T1D, but also realizing how to reshape the microbiota composition in order to improve the disease-associated symptomatology. Indeed, understanding which class of bacteria is directly involved in the pathophysiology of T1D may allow a targeted intervention (e.g. probiotic supplementation, fecal transplantation) able to maximize benefits and reduce potential side effects. It would be of paramount interest to explore how and when the alterations induced in bacterial community (e.g. *Bacteroidetes*) of experimental models will be responsible for the T1D pathogenetic events.

The nonobese diabetic (NOD) mouse is the elective model to study spontaneous autoimmune T1D development [120]. Gut bacteria are in fact crucial modulators for T1D development in genetically susceptible NOD mice [121]. It has been shown that diabetes incidence is increased in the germ-free NOD mice, consistently with higher rates of T1D in countries with stringent hygiene practices [122]. We previously underwent the NOD model to moderate-intensity exercise in order to evaluate the immunological and inflammatory modulations in the T1D-course [123]. NOD trained mice benefited from glucose-lowering effects in the late states of diabetes, whereas control untrained NOD mice showed larger infiltrates at the end of the 12-week study. These findings suggested that exercise might exert a beneficial immune-modulation of systemic functions to both T1D and inflammation. Few exercise-studies have been conducted in NOD mice, possibly because of the extra stress that exercise could generate in a mouse strain *per se* very delicate, and prone to develop T1D and inflammation for numerous reasons.

In *db/db* mice (a model for T2D), low-intensity treadmill running determined a greater abundance of *Firmicutes* species and lower amount of

Bacteroides/Prevotella spp. in both normal and diabetic trained mice compared to sedentary littermates. Moreover, an increase in *Bifidobacterium spp.* level was only observed in exercised non-diabetic mice, suggesting that the presence of diabetes abolished the beneficial effect of exercise [124]. Also in humans with T2D, Qin et al. observed a reduction in universal butyrate-secreting bacteria along with a raise in opportunistic pathogens, indicating a moderate degree of gut microbial dysbiosis [125].

In adults with T1D, we have conducted several studies showing the multi-faceted positive effects of exercise on immunometabolism and autoimmunity [8,74,126,127].

Still, it is currently unclear whether the imbalance in the gut microbiota is a cause or a consequence of T1D. An observational study showed that gut microbiota of long-standing T1D patients with good glycemic control and high physical fitness is similar to matched-people without diabetes [128]: bacterial profiles of ten male young adults with T1D were comparable with those of the ten paired-controls. Importantly, this effect persisted even when the analysis accounted for the HbA_{1c} stratification.

Past interventions using probiotics and/or prebiotics were successfully employed to alleviate a cluster of morbid conditions, suggesting that gut microbiota could be an adjustable therapeutic tool. In this perspective, a plethora of studies tried to identify the factors determining the composition of the core healthy gut microbiota. Nevertheless, to date, physical exercise has been hardly considered as a therapeutic regulator of gut microbiota composition on the roadmap toward T1D-healing.

The role of intestinal microbiota in T1D has been already firmly established, therefore an action capable to modify the microbiome peculiarly is sought

in order to intervene successfully in the pathogenesis of T1D. Whether this challenge can be faced by physical exercise requires further research.

6. Conclusions

Although a growing body of evidence is pointing at the health-promoting effect of physical exercise in the modulation of gut microbiota, this intriguing pattern is far to be clearly elucidated. Few controlled studies on humans have been conducted in the attempt to confirm the findings of studies on animals, which have been carried out in greater numbers. Modality, intensity and duration of exercise are still fascinatingly unexplored as to providing the most beneficial treatment for several chronic- and immune-based diseases, through manipulation of gut microbiota.

Contribution statement

All authors were responsible for drafting the manuscript and revising it critically for valuable intellectual content. All authors approved the version to be published.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Captions

Figure 1. Flow-chart of microbiota fuctions, interactions, and imbalances

Gut microbiota functions are indicated in light blue, interactions by vertical lines, whereas disruptions are depicted in grey as a result of gut dysbiosis (dashed lines).

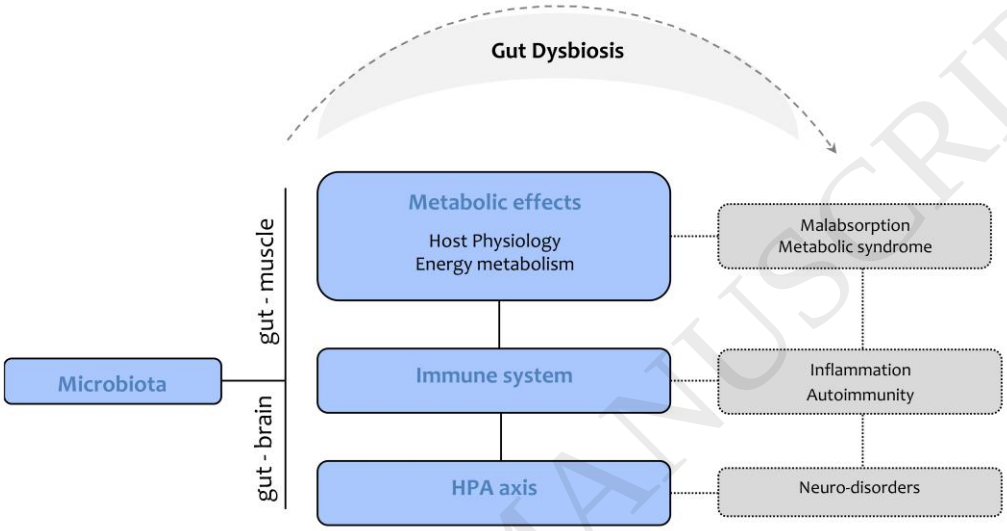

Figure 2. Microbiomal environment interacts with immune system

Within the large intestine commensal bacteria produce SCFAs (butyrate, acetate, propionate), which are implicated in multiple signals. Microbiota stimulates directly and indirectly the immune system (TLRs, T-, B-, NK-cells, cytokines) and, via SCFAs, promotes the release of serotonine (5-HT), neurotransmitter involved in the gut-brain axis. Exercise may enhance TLRs sensitivity. TLRs may be activated by LPS coming from the membrane of bacteria (muscle-gut axis).

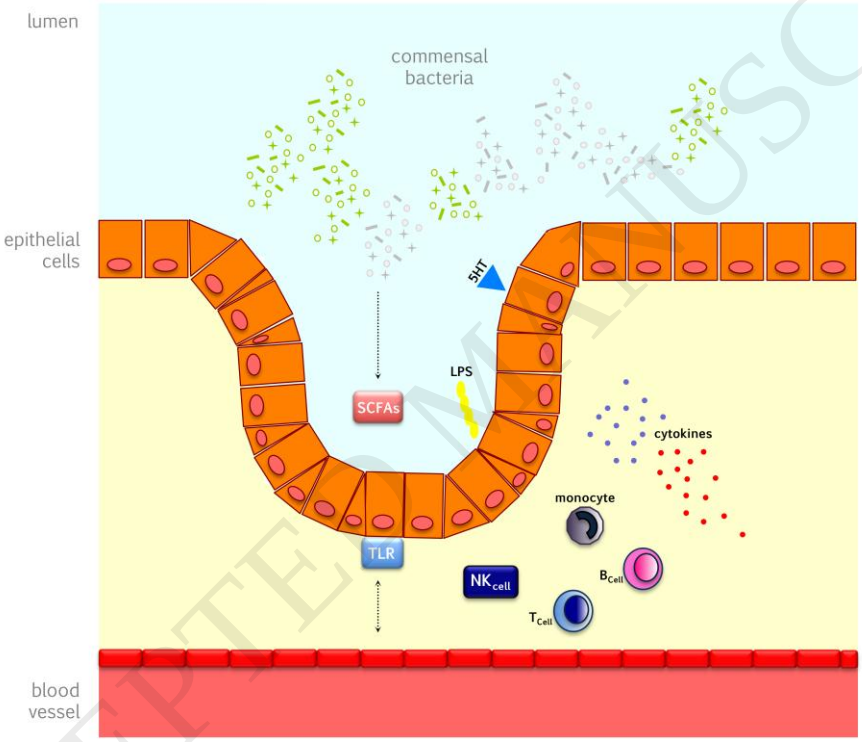

Figure 3.

The brain-gut-muscle axis in reshaping healthy microbiota through exercise

There is an intensive cross-talk orchestrated by physical exercise involving neuro-endocrine responses (HPA-axis), which continuously affect microbiota in a loop-feedback. As a stress, hopefully "a positive one" (depending on the intensity), exercise can be considered a *primum movens* in this circuit. However, the extent by which physical exercise contributes to microbial health, as well as the interactions among biological covariables and the microbiota, remain to be addressed.

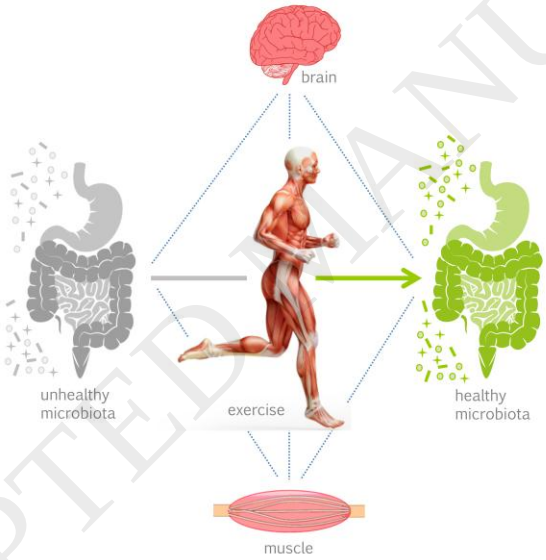

Table 1. Studies examining the effects of exercise on gut microbiota

Authors	Model	Exercise	Outcomes
Hsu et al.[48]	GF-, SPF-, BF-mice	Swimming-to-exhaustion test	↓glutathione, ↓catalase, ↓SCFAs, in GF and BF vs SPF
Matsumoto et al. [75]	Wistar rats	Voluntary wheel for 5 wks	↑butyrate; ↑butyrate-producing bacteria
Campbell et al. [49]	Wild-type, DIO mice	Voluntary wheel for 12 wks	↓Cox-2 in DIO trained mice; ↑*Faecalibacterium prausnitzii* in trained mice
Evans et al.[78]	Wild-type mice	Voluntary wheel for 12 wks	↓*Firmicutes:Bacteroidetes* ratio; ↑*Bacteroidetes*, ↓*Firmicutes*; ↓*Actinobacteria*; exercise prevented DIO
Cook et al. [82]	Wild-type mice	Treadmill (8-12 m/min, 40 min, 6 weeks, 5x/week), or voluntary wheel (30 d access to wheels)	↑diarrhea, ↑IL-6, ↑IL-1β, IL-17 colon gene expression, ↑mortality in treadmill group; alleviated ↓colitis symptoms, ↓ inflammatory gene in wheel group
Kang et al.[83]	Wild-type mice, DIO mice	Motorized wheel 1h/d, 5d/wk for 16 wks @ 7m/min	↓*Bacteroidetes*; ↑*Firmicutes*; ↑cognitive abilities despite HFD
Denou al. [84]	Wild-type mice	HIIT for 6 wks	↓*Firmicutes:Bacteroidetes* ratio
Queipo-Ortuño et al. [80]	Sprague-Dawley rats	Voluntary wheel for 6 d	↑*Bacteroidetes*, ↓*Firmicutes*; ↑diversity; ↓*Bacteroides;* ↑*Lactobacillus*
Liu et al. [85]	Ovariectomized rats	Voluntary wheel for 11 wks	↔*Bacteroidetes*; ↔*Firmicutes:Bacteroidetes* ratio; ↓*Firmicutes* in low-capacity running rats; ↑*Firmicutes* in high-capacity running rats
Allen et al. [129]	Wild-type mice	Voluntary wheel vs treadmill for 6 wks	↓Bacterial richness in voluntary wheel running mice; ↔*Bacteroidetes*, ↔*Firmicutes* in both groups

Lambert et al. [124]	*db/db* (T2D model) and *db/*$^{+}$ mice	Low-intensity treadmill, 5d/wk for 6 wks	↑*Firmicutes*, ↓*Bacteroidetes:Prevotella spp.* in both trained and untrained groups; ↑*Bifidobacterium spp.* in trained non-diabetic mice
Lamoureux et al. [130]	Wild-type mice	Voluntary wheel, treadmill	↔ abundance in gut microbes, ↔ host inflammatory response in both trained and controls
Petriz et al. [81]	Obese (Zucker), hypertensive (SHR) and Wistar rats	Treadmill, 30min/d, 5x/wk for 4wks	↑*Firmicutes*; ↓*Proteobacteria*; ↑*Lactobacillus*
Mika et al. [46]	F344 rats	Voluntary wheel for 6 wks in juvenile vs adult groups	↑*Bacteroidetes*, ↓*Firmicutes*, ↑bacterial genera in juvenile rats vs adults
Clarke et al. [95]	Humans, cross-sectional	Elite rugby players	↑diversity in athletes vs controls; ↑*Akkermansia* in low BMI athletes and controls; ↑protein intake, ↑CPK in athletes
Estaki et al. [98]	Humans, healthy subjects, cross-sectional	Observing levels of cardiorespiratory fitness	↑diversity, ↑butyrate-producing taxa in subjects with higher fitness
Yang al. [99]	Humans, premenopausal women, cross-sectional	Observing levels of cardiorespiratory fitness	gut microbiota compostion ∝ cardiorespiratory fitness level
Bressa al. [97]	Humans, healthy women, cross-sectional	Active women performing WHO-recommended low dose of exercise	gut microbiota compostion ∝ body fat/muscular mass; ↑*Faecalibacterium prausnitzii*, ↑*Akkermansia muciniphila* in active women
Stewart et al. [128]	Humans, subjects with T1D, and healthy controls, cross-sectional	Observing levels of physical fitness, glycemic control	Gut microbiota comparable between T1D-subjects in good glycemic control + high physical fitness vs healthy controls
Barton et al. [131]	Humans, cross-sectional	Professional rugby players	↑SCFAs,↑muscle turnover (fitness) in athletes vs control group
Paulsen et al. [100]	Humans, breast cancer survivors (BCS), cross-sectional	Observing levels of cardiorespiratory fitness, anxiety, fatigue	gut microbiota compostion ∝ changes in cardiorespiratory fitness level and anxiety in BCS

Abbreviations: ↑ = significant increase; ↓ = significant decrease; ↔ = unchanged; ∝ = correlates; BCS = breast cancer survivors; BF = *Bacteroides fragilis*-colonized; Cox-2 = cyclo-oxygenase 2; CPK = creatine phosphokinase; d = day; DIO = diet-induced obesity; GF = germ free; HFD, high fat diet; HIIT, high intensity interval training; m = meters; min = minutes; NOD = non-obese diabetic; SCFAs = short chain fatty acids; SPF = specific pathogen free; wk = week.