Exercise and immune system as modulators of intestinal microbiome: implications for the gut-muscle axis hypothesis

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ABSTRACT

Exercise is a possible modulator of intestinal microbiome composition, since some investigations have shown that it is associated with increased biodiversity and representation of taxa with beneficial metabolic functions. Conversely, training to exhaustion can be associated with dysbiosis of the intestinal microbiome, promoting inflammation and negative metabolic consequences. Gut microbiota can, in turn, influence the pathophysiology of several distant organs, including the skeletal muscle. A gut-muscle axis may in fact regulate muscle protein deposition and muscle function. In older individuals, this axis may be involved in the pathogenesis of muscle wasting disorders through multiple mechanisms, involving transduction of pro-anabolic stimuli from dietary nutrients, modulation of inflammation and insulin sensitivity. The immune system plays a fundamental role in these processes, being influenced by microbiome composition and at the same time contributing to shape microbial communities. In this review, we summarize the most recent literature acquisitions in this field, disentangling the complex relationships between exercise, microbiome, immune system and skeletal muscle function and proposing an interpretative framework that will need verification in future studies.

Keywords: Gut microbiota; Sarcopenia; Inflammation; Exercise immunology; Sport

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1. Introduction

1.1 The physiology of human intestinal microbiome

The human intestinal microbiome is composed of a complex ecosystem of more than 10^{14} bacteria, viruses, fungi, Protozoa and Archea that live symbiotically with the host in the gut lumen (45,61,82). Although there is increasing interest in the role of Protozoa, fungi (the "mycome") and viruses (the "virome"), most of the existing research has been focused mainly on bacteria, thanks to the availability of high-throughput sequencing techniques of bacterial DNA (16S rRNA microbial profiling, shotgun metagenomics) and fecal metabolomics (52,77,93,94).

The concentration of bacteria generally increases from the small intestine to the colon, and different bacterial populations are harbored in different tracts of the gastrointestinal system (123). Although recent research has shown that the fecal microbiota composition does not completely overlap with the intestinal mucosa-associated microbiota (134), gut microbiota composition has been determined from fecal samples in most studies (45,61,82,123).

It is generally agreed that the intestinal microbiome composition is shaped during early childhood, influenced by genetic and environmental factors. These factors include geography, delivery mode, breastfeeding, weaning, and exposure to environmental bacteria (34,54,78). The maturation of the gut microbiota towards the adult-type is reached by the age of 3 years (128). Interestingly, this process seems to exert a great influence on the development of the immune system, promoting immunogenic tolerance towards symbionts and immune activation against pathogens (40,50,110). Conversely, alterations in this process may induce a shift towards unappropriated type 2 immune responses, favoring the pathogenesis of allergy or autoimmune diseases, according to some theories (71,74).

In adulthood, the human gut microbiota composition remains relatively stable over time, exhibiting resilience to disruptors, such as stress, acute diseases, or antibiotic administration (65). This means that, after a brief exposure to disruptors, the microbiome faces a substantial, but transient, perturbation, followed by partial or total recovery of previous taxonomic composition (96).

The healthy microbiome includes a limited number of highly represented taxa, such as *Bacteroides* and *Prevotella* spp., and a large number (up to 2000 identified to date) of minor players with low representation but high metabolic activity (42,65). In

healthy subjects, microbiota composition shows a certain degree of inter-individual differences, that can be again explained by genetic (13) and environmental factors (28), such as the place of living, cultural habits, diet, diseases, medications, and immune system function (99). Dietary factors, and particularly carbohydrate and protein intake, are generally considered as the main determinants of gut microbiota composition (5,76,132).

In the older age, species richness and diversity of microbiome decline, inter-individual variability increases, and resilience to perturbations is reduced (24,86). Thus, the microbiome composition shifts towards a higher representation of opportunistic pathogens, and is related to increasing prevalence of malnutrition, frailty, disability and multimorbidity (49,112,114).

1.2 The intestinal microbiome in human diseases

A large body of literature has demonstrated that the presence of acute and chronic diseases, not limited only to the gastrointestinal system, is associated with alterations of the gut microbiota composition (67,99). These alterations, globally referred to as "dysbiosis", include reduced biodiversity, loss of commensals with possible beneficial metabolic activities and overgrowth of opportunistic pathogens (55,60,81). Dysbiosis implies a disruption of the mutual equilibrium between gut bacteria and host physiology (16). As a consequence, intestinal permeability increases, allowing bacteria or bacterial toxins and metabolites to enter into the host circulation and promote subclinical inflammation (64). Dysbiosis also reduces bioavailability of nutrients, affecting the microbial metabolism of several beneficial substances (73).

As a result, gut microbiota may exert a great influence on the functionality and pathophysiology of several organs anatomically distant from the gastrointestinal tract. For example, gut microbiota dysbiosis may be involved in the pathophysiology of dementia (17,115), Parkinson's disease (108) ("gut-brain axis"), chronic kidney disease (95), nephrolithiasis (113) ("gut-kidney axis"), asthma (73) ("gut-lung axis") and even osteoporosis (84) ("gut-bone axis").

However, most of these associations linking dysbiosis with extra-intestinal diseases have been demonstrated in studies with a cross-sectional design. Longitudinal studies, demonstrating a causal relationship between microbiota composition and disease onset and course, are still lacking (99). Thus, the real clinical implications of gut microbiota dysbiosis and interventions targeted at modifying gut microbiota composition are still poorly understood.

2. Exercise and gut microbiota: the yin and yang

2.1 Beneficial effects of exercise on gut microbiota

Exercise is considered as one of the main environmental factors possibly influencing gut microbiota composition (99). The complex relationship between exercise and microbiome, and its possible implications for athletic performance have already been reviewed elsewhere (10,27,87). However, research in this field has made substantial improvements in the last few years, and some recent acquisitions deserve to be mentioned and discussed.

Exercise is generally considered a positive modulator of gut microbiota biodiversity. This concept has been supported by investigations performed in animals (8,18,57,75), and then confirmed in human studies.

In a case-control study, microbial diversity was much higher in a group of professional rugby players than in age-, sex- and body size-matched controls not performing sports (26). Recently, shotgun metagenomics analyses of the fecal samples from the same groups highlighted that athletes had a different microbiome composition also from a functional point of view, with increased microbial representation of genes involved in carbohydrate and amino acid metabolism, and short-chain fatty acid (SCFA) production (7). In another study, the average abundance of taxa involved in energy and carbohydrate metabolism, including *Prevotella* and *Methanobrevibacter smithii,* resulted significantly higher in professional than amateur cyclists, and was correlated with the frequency of training (89). However, these studies could not fully disentangle the contribution of exercise and diet in determining different microbiota compositions in different groups, since participants followed a wide range of dietary regimens.

The intensity of training is also important: light exercise programs induce only subtle modifications of gut microbiota composition in sedentary subjects (31). Therefore, the findings of studies performed in athletes should not automatically be transferred to all subjects undertaking non-competitive exercise.

According to three different studies (37,38,127), fecal microbiota biodiversity is correlated with cardiorespiratory fitness in adult subjects. However, in one of these studies, performed in 71 premenopausal Finnish women, this relationship was mediated by body composition (127). Another study, performed in 19 active and 21 sedentary women aged ≤40 years old, confirmed that the microbiome abundance of several bacterial taxa was significantly correlated with the body fat or lean mass percentage (15). Thus, the possible association between exercise and microbiota should be further investigated, carefully taking into account possible confounders, such as dietary habits, nutrient intake, and parameters of body composition.

The influence of body composition on microbiota was emphasized also by the findings of one intervention study, where two groups of sedentary subjects, one lean and one obese, underwent a 6-week structured exercise program, followed by a 6-week washout period (2). After exercise training, both lean and obese participants experienced a change in gut microbiota composition, but the overall representation of species with known anti-inflammatory properties and the microbiome capacity of producing SCFA was higher in lean subjects, highlighting a body mass index (BMI)-dependent response to training. However, all the changes reversed towards the baseline status after the washout period (2). Interestingly, in exercised healthy young males undergoing a period of forced inactivity, cessation of exercise was associated with changes in gastrointestinal physiology (i.e. reduction of bowel movements and increased consistency of feces) before alterations of gut microbiota composition and function could be detected (103,104,105). These circumstances suggest that the microbiome is resilient to acute changes in exercise habits, and that maintenance of exercise is needed to induce long-lasting modifications of intestinal microbial ecosystem.

The modifications of intestinal microbiome composition induced by exercise can exert beneficial effects on the whole organism, modulating pathological processes. For example, exercise-induced microbiota changes are able to attenuate the

clinical course and outcome of experimental models of myocardial infarction or chemically-induced colitis, especially by modulating the inflammatory response (1,63). The key mediators in these processes may be SCFA, and particularly butyrate, whose production by gut microbiota has been shown to increase after exercise in humans (2).

2.2 Negative effects of exercise on gut microbiota

Despite the findings of the studies discussed above, other investigations have questioned the concept that the exercise-induced changes in gut microbiota composition are always favorable for the host physiology. Endurance high-intensity exercise, especially if not proportioned to training level, may in fact represent a huge stressor for the organism. These conditions can induce ischemic events in the gut mucosa, associated with acute gastrointestinal symptoms including abdominal pain, nausea, and diarrhea (32). From a gut microbiota perspective, these phenomena may be associated with increased intestinal permeability allowing several bacteria and their toxic products to enter systemic circulation and activate systemic inflammation (51,97). A basic mediator in these processes is represented by microbiota-derived lipopolysaccharide (LPS) (97), exerting a wide range of pathological actions on the host (4).

Moreover, the high-intensity exercise-induced dysfunction of the intestinal mucosa may promote profound and rapid changes in microbiota. For example, in a group of soldiers, a 4-day military training program of Arctic cross-country ski-march resulted in deep changes in fecal microbiota composition and functionality. Namely, there was an expansion of a large number of taxa, including opportunistic pathogens, at the expense of dominant taxa, such as *Bacteroides,* and taxa with known production of anti-inflammatory mediators (51). In amateur athletes, the fecal microbiome functionality acutely changed after a half-marathon race, exhibiting a pro-inflammatory profile with a completely different fecal metabolome (131). Similar changes have also been demonstrated in animal models (129). Interestingly, the administration of probiotics or prebiotics seems to attenuate these unfavorable changes of gut microbiota after exercise to exhaustion (22,48,97), although the benefits are uncertain in case of lower intensity of exercise (118,119).

In summary, regular exercise training seems to be associated with higher biodiversity and beneficial functions of intestinal microbiome. The microbiota may thus represent a mediator of the exercise-induced health benefits, although diet and body composition may play a relevant role in this association. On the other side, there are also some studies supporting that exercise to exhaustion may be associated with detrimental consequences for the microbiome. The effects of exercise on the intestinal microbiome may thus depend on its intensity and timing, and future studies should help to disentangle this relationship.

3. Can the microbiota influence muscle pathophysiology? The gut-muscle axis hypothesis in age-related sarcopenia

Recently, several research groups have independently hypothesized that the gut microbiota composition may influence the emergence of sarcopenia, i.e. the loss of muscle mass and function occurring with aging (33,44,83,90,112,116). A study performed on rat models of sarcopenia has actually demonstrated that age-related muscle mass wasting is associated with a distinct fecal microbiota composition, with reduced representation of several taxa with purported anti-inflammatory and pro-anabolic actions on the host tissues, including *Clostridium* XIVa cluster, *Butyricicoccus, Sutterella, Coprococcus* and *Faecalibacterium* (102). Sarcopenic rats also exhibited a different fecal microbiota functionality, with rearrangements in the expression of bacterial genes involved in nutrient biosynthesis and catabolism (102).

To date, no studies have investigated the composition and functionality of fecal microbiota in older humans with sar-

Table 1: Overview of the possible pathophysiological mechanisms involved in the gut microbiota-mediated regulation of skeletal muscle function (gut-muscle axis) and of the corresponding effects in case of gut microbiota dysbiosis.

Hypothesized pathways involved in gut microbiota modulation of muscle function	Possible skeletal muscle effects in case of dysbiosis
Bioavailability of dietary proteins and specific amino acids (tryptophan) (36,62,101)	↓ protein synthesis ↓ IGF-1-mediated anabolic effect
Synthesis of vitamins, including folate, vitamin B12, riboflavin (59,111)	↓DNA synthesis, methylation and repair ↓oxidative stress neutralization capacity ↓amino acid biosynthesis ↓energy production
Biotransformation of nutrients, including polyphenols (resveratrol) and ellagitannins (97,106)	↓mitochondrial biogenesis ↓ exercise resistance capacity ↓ oxidative stress neutralization capacity
Permeability of intestinal mucosa (20,25)	↑ inflammation (stimulation of TLRs) ↑ muscle protein catabolism ↓ insulin sensitivity with anabolic resistance
Biotransformation of bile acids (53)	↓ activation of anabolic pathways regulated by farnesoid X receptor
Synthesis of short-chain fatty-acids (19,118,123)	↓ stimulation of anabolism ↓ insulin sensitivity ↑ systemic and local pro-inflammatory cytokine production

IGF-1 = Insulin-Like Growth-Factor-1; TLR = Toll-Like Receptors

copenia (116). However, there is some indirect evidence supporting the hypothesis of a gut-muscle axis, in which the intestinal microbiota composition can influence muscle mass anabolism and functionality.

The physio-pathological substrates of sarcopenia are represented by reduced muscle capillarity, reduced insulin sensitivity, and increased subclinical inflammation, resulting in altered mitochondrial biogenesis and function, and altered anabolic/catabolic balance of muscle protein synthesis (14,69,70). From a clinical point of view, muscle mass loss may also be favored by several conditions that are frequently found in geriatric patients, including malnutrition, low dietary protein intake, intestinal malabsorption, altered digestion and subclinical cognitive deficits (58,83).

In this context, the intestinal microbiota composition may influence the onset of sarcopenia at multiple levels. The presence of gut microbiota dysbiosis is in fact associated with several metabolic alterations, involving protein synthesis, release of pro-anabolic mediators, inflammation and insulin sensitivity. All these elements can modulate skeletal muscle physiology, as summarized in Table 1.

First, a dysbiotic intestinal microbiota can reduce the bioavailability of dietary proteins (102) and particularly of some amino acids, like tryptophan, involved in modulation of inflammation and promotion of muscle protein synthesis (21,36,62). Gut bacteria are also involved in the synthesis of many vitamins, including folate, vitamin B12 and riboflavin, exerting several beneficial and pro-anabolic effects in skeletal muscle cells, ranging from amino acid biosynthesis to oxidative stress neutralization during exercise (59).

Moreover, a healthy intestinal microbiota can effectively transform some dietary nutrients into metabolic mediators that, once absorbed into systemic circulation, can exert beneficial effects on inflammation, insulin sensitivity, anabolism, and antioxidant capacity. Conversely, a dysbiotic microbiota may lack these functions, with some negative consequences on muscle health. Polyphenols, including resveratrol, and ellagitannins contained in pomegranates and berries represent the most relevant examples of nutrients that, after microbial metabolism, enter systemic circulation and exert beneficial effect for the muscle (98,107). Interestingly, endurance training seems to enhance the bioavailability of dietary polyphenols, probably through its beneficial modulations of intestinal microbiota (88).

Moreover, the age-related alterations of gut microbiota composition (24), occurring independently from the level of exercise training, can promote gut mucosa dysfunction, with increased permeability. This phenomenon may result in the systemic absorption of microbial byproducts and toxins, including LPS (20). In skeletal muscle cells, circulating LPS can contribute to activate Toll-Like Receptors (TLR) 4 and 5, promoting NF-kB pathway activation, with reduced insulin sensitivity, enhanced protein catabolism and inflammatory cytokine production (72,106). In animal models, TLR4 activation determines muscle atrophy (35). In aging human beings, TLR4 activation is associated with metabolic endotoxinemia, decreased insulin sensitivity and reduced quadriceps muscle strength and volume (41).

But probably the most studied mechanism involved in gut microbiota modulation of muscle function is the bacterial production of metabolic mediators, including bile acids and SCFA (20,25). A healthy gut microbiota can produce secondary bile acids, that are well known activators of farnesoid X receptor stimulating myocyte anabolism (53). SCFA, and particularly butyrate, are generally synthetized by a large number of gut bacteria, including *Faecalibacterium, Butyricimonas,* and *Succinivibrio*, highly represented in healthy subjects but with reduced abundance in older individuals (19). These mediators have several beneficial metabolic activities, summarized in Table 2, ultimately influencing skeletal muscle protein deposition through modulation of the systemic anabolic/catabolic balance (6,19). The administration of butyrate and probiotics with similar functionality to animal models of muscle wasting resulted in massive improvements in muscle mass (120,125). Unfortunately, human studies on this topic are still lacking to date.

The metabolic action of gut microbiota was however confirmed in a study by Blanton and colleagues, where the transplantation of the dysbiotic fecal microbiota from malnourished African children to germ-free mice resulted in mouse failure-to-thrive (12).

Furthermore, the administration of rifaximin to mouse models of hepatic encephalopathy surprisingly resulted in improved skeletal muscle mass and function (56). Rifaximin is able to

Table 2: Summary of the main physiological functions of short-chain fatty acids (butyrate, acetate, propionate) produced by the intestinal microbiota (6,19,118,123). The most relevant functions possibly involved in the gut-muscle axis are shown in italics.

Substance	Function
Acetate	*Direct modulation of systemic inflammation*
	Antagonization of LPS-driven inflammation
	Improvement in insulin sensitivity
	Stimulation of skeletal muscle glucose uptake
	Peripheral modulation of satiety via GLP-1 and PYY
	Central appetite modulation
	Promotion of lipolysis
	Induction of adipose tissue differentiation
	Increased energy expenditure via thermogenesis
Propionate	*Increased Treg cell differentiation*
	Direct modulation of systemic inflammation
	Antagonization of LPS-driven inflammation
	Peripheral modulation of satiety via GLP-1 and PYY
	Promotion of lipolysis
	Induction of adipose tissue differentiation
	Increased energy expenditure via thermogenesis
Butyrate	*Direct modulation of systemic inflammation*
	Antagonization of LPS-driven inflammation
	Improvement in insulin sensitivity
	Stimulation of skeletal muscle glucose uptake
	Histone deacetylase inhibition
	Reduced intestinal permeability
	Peripheral modulation of satiety via GLP-1 and PYY
	Central appetite modulation
	Promotion of lipolysis
	Induction of adipose tissue differentiation
	Increased energy expenditure via thermogenesis

GLP-1 = Glucagon-Like Peptide-1; PYY = Peptide YY; LPS = Lipopolysaccharide

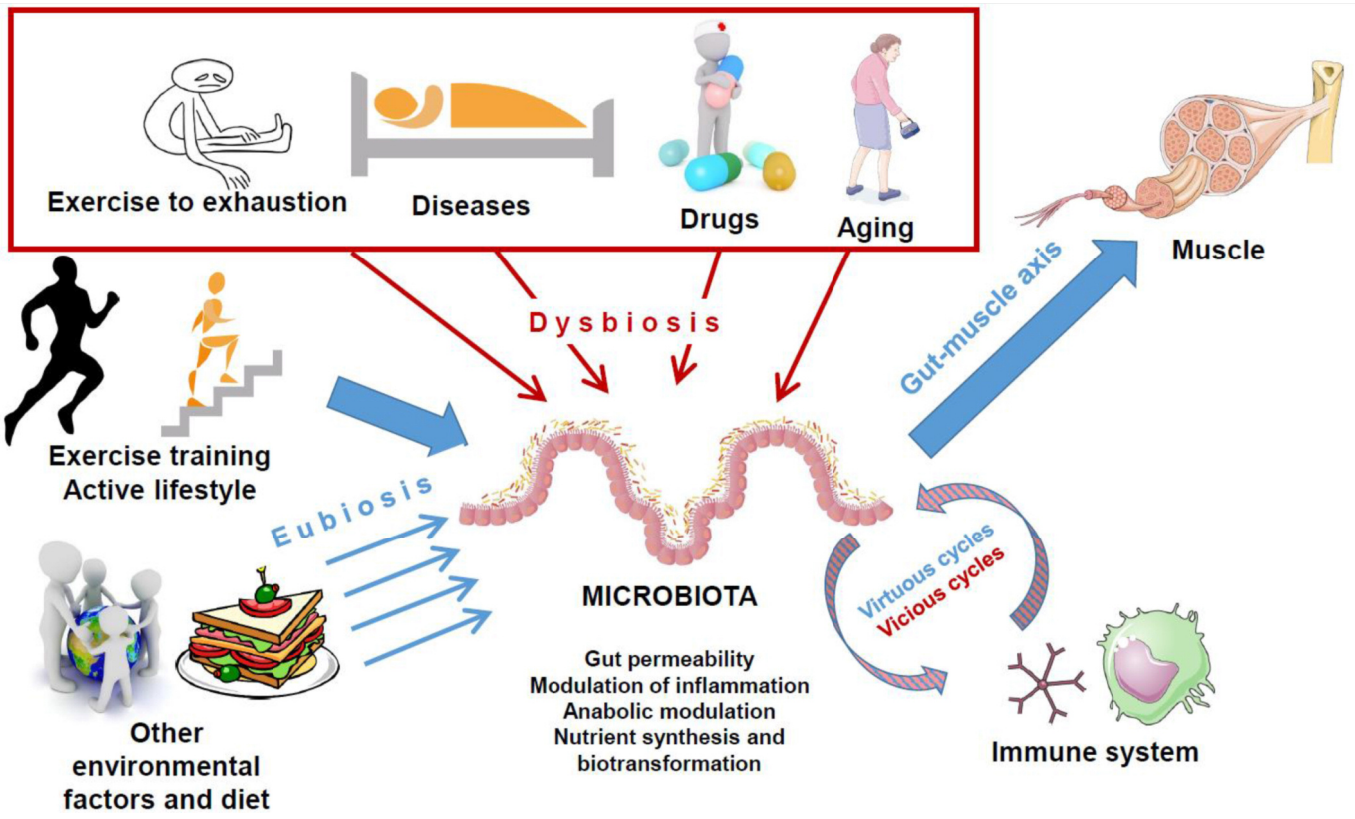

Figure 1: Representation of the hypothetical conceptual framework connecting exercise, intestinal microbiome, inflammation, immune system function and skeletal muscle pathophysiology at the current literature state of art.

selectively kill pathobionts, favoring the expansion of bacterial populations with purported beneficial activities, such as Bifidobacteria or Lactobacilli (91). The observed effects on mice may depend on its capacity to reduce the gut microbiota dysbiosis associated with hepatic encephalopathy.

In summary, the results of several pre-clinical studies support the hypothesis that gut microbiota dysbiosis may be associated with muscle wasting, especially in age-related sarcopenia. However, confirmation of this possible gut-muscle axis in human studies is still lacking, and the clinical relevance of these supposed mechanisms is still uncertain.

4. Exercise and the gut-muscle axis

Although studies on humans are lacking, several preclinical studies support the hypothesis that the intestinal microbiota can modulate skeletal muscle physiology not only in age-related sarcopenia, but in all ages and physiological states (20,25). In this context, exercise may represent a strong modulator of gut microbiota composition. Thus, the gut-muscle communication in human pathophysiology may be bidirectional (25), with gut microbiota representing a "cross-road" among environment, lifestyle, and skeletal muscle (112).

In this scenario, some authors have hypothesized that many of the well-known positive health effects of exercise may be mediated by its beneficial modifications on the gut microbiota (23,79,80). However, when there is an exercise overload, these possible beneficial effects are overweighed by increased intestinal permeability and oxidative stress, promoting inflammation and a catabolic state that negatively impacts the functionality of skeletal muscle (29). Moreover, the harmful effects of inactivity on the muscle and vascular system may be at least partly mediated by negative changes of the gut microbiota towards dysbiosis (23,109).

In healthy subjects who regularly perform physical activity, a homeostatic equilibrium between intestinal microbiota and skeletal muscle may be present, with exercise promoting healthy microbiota composition, and microbiota favoring muscle health. This equilibrium may be disrupted by sedentary lifestyle or excessive exercise, resulting in dysbiosis of the gut microbiota. Other factors promoting dysbiosis, such as drugs or acute illnesses, may also be associated with reduced muscle mass and function. In fact, dysbiosis influences gut permeability, systemic inflammation, anabolism and nutrient availability. All these mechanisms are involved in muscle physiology and represent the substrates of the gut-muscle axis, as depicted in Figure 1.

In summary, the gut-muscle axis may be two-way, with microbiota influencing the muscle, and exercise contributing to shape microbiota composition. The intensity and frequency of exercise may have great importance in determining which way of the axis is prevalent, and its physio-pathological consequences.

5. Immune system and the gut-muscle axis: virtuous and vicious cycles

A healthy gut microbiota has a fundamental role in shaping local and systemic immune response to gut bacteria through the whole lifespan, favoring the maintenance of tolerance towards antigens from commensals and activation against antigens from pathogens (40). On the other side, gut micro-

biota dysbiosis favors the loss of immunologic tolerance to commensals, the impairment of epithelial barrier function and an inbalance in the activation of anti-inflammatory T_{reg} lymphocytes and pro-inflammatory Th17 lymphocytes (43,60). These phenomena may contribute to the onset of several infectious, inflammatory and autoimmune diseases, including inflammatory bowel diseases, type 1 diabetes and multiple sclerosis, with gut microbiota playing an active pathogenic role (9).

However, besides this "outside-in" relationship, there is also an important "inside-out" control of immune system over gut microbiota (47,117). The immune system is in fact able to influence the gut microbiota composition at multiple levels. Both innate and adaptive immunity are involved. The possible mechanisms are synthetized in Table 3, and include production of antimicrobial peptides from intestinal cells, mucus secretion, immunoglobulin A (IgA) activation, toll-like receptor (TLR) activation, lymphocyte transfer and differentiation, presence of invariant natural killer T cells (iNKTC) (47,60,68,101,130). The presence of specific inbalances in each one of these pathways may be associated with the emergence of gut microbiota dysbiosis (47,68).

In fact, human beings infected with the Human Immunodeficiency Virus (HIV) exhibit deep changes in the structure and functionality of the intestinal microbiome, with increased biodiversity due to overgrowth of opportunistic pathogens and decreased representation of taxa with anti-inflammatory properties (121,122,133). Similar alterations of gut microbial community structure have been detected also in patients with IgA deficiency (39). These findings support the concept that the immune system functionality influences gut microbiota composition.

Conversely, the presence of specific functionalities in the gut microbiome, related to fatty acid metabolism, PPAR-signaling, lipid biosynthesis and kynurenine pathway of tryptophan metabolism, may enhance systemic immunity activation and promote control of HIV infection (124). Therefore, a complex interplay between microbiome and immunity exists, and the physio-pathological consequences depend on the type of equilibrium reached (117).

Some metabolic mediators, such as bile salts, may play a relevant role in this equilibrium. In the gut lumen, bile salts can in fact undergo metabolic transformations into compounds with immunoregulatory and anti-inflammatory properties, particularly on Kupffer cells and intrahepatic lymphocytes (100). Bile salts have also the capacity of selecting specific subpopulations of the gut microbiota that are able to metabolize them, contributing to shape the intestinal microenvironment (100).

Table 3: Overview of the mechanisms involved in immune system control of gut microbiota composition (47,60,68,100,128).

Immunity type	Mechanism
Intestinal innate immunity	Production of mucus (barrier function)
	Production of antimicrobial peptides by Paneth cells
	Production of α-defensins by epithelial cells
	Activation of NOD-like receptor and production of IL-18 by epithelial cells
	Release of non-specific immunoglobulins
	Invariant Natural Killer lymphocyte activation
	Expression of Resistin-like molecule β
	Leptin expression
Intestinal acquired immunity	IgA response to gut microbiota antigens
	Activation of CD4+ T cells in intestinal mucosa
	Activation of Foxp3+ T cells in intestinal mucosa
	Activation of Toll-Like Receptors with lymphocyte stimulation
	HLA class I and II loci expression
Systemic acquired immunity	IgG response to gut microbiota antigens penetrated in systemic circulation

Table 4: Summary of the main features of aging immune system involved in increased gut mucosa permeability and in age-related gut microbiota dysbiosis (11,66,124).

Immunity type	Age-related alterations
Innate	Reduced Paneth cell function
	Reduced glycosylation of mucins
	Reduction of M cell number and function
	Increased levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α
	Decreased levels of anti-inflammatory cytokines including IL-10
	Increased production of adipokines
	Impaired phagocytic function of macrophages
	Overgrowth of pro-inflammatory $CX3CR1^{Int}$ macrophages
	Reduced chemotactic capacity in neutrophils
Acquired	Reduced antigen presentation capacity by dendritic cells
	Reduced T cell priming by dendritic cells
	Reduced differentiation of CD4+ CD25+ T cells
	Reduced T cell secretion of TGF-β
	Increased T cell secretion of IFN-γ
	Overgrowth of T_{reg} lymphocytes
	Reduced homing of IgA-secreting B lymphocytes
	Reduced IgA secretion capacity
	Loss of naïve T and B cells

Whatever the mediators involved, the equilibrium between immune system and microbiome may be strongly influenced by environmental factors. Positive modulators of gut microbiota composition, including regular exercise, may induce a beneficial equilibrium with the immune system, resulting in a virtuous cycle helping to maintain health (27,85). Conversely, factors that disrupt gut microbiota composition, such as exercise to exhaustion, illness and aging, may cause a perturbation of the equilibrium between microbiome and immune system. As a result, systemic inflammation is chronically activated, sustaining further alterations of the microbiota towards dysbiosis promoted by the altered immune system regulation (30,46). So, a vicious circle arises.

These postulated mechanisms are highlighted in Figure 1.
A healthy gut microbiota, and a positive interaction with the immune system, may be crucial for the gut-muscle axis, and may influence the maintenance of muscle mass and functionality, especially in exercised subjects (29). Conversely, dysbiosis resulting from a negative interaction with the immune system may influence muscle wasting disorders, particularly during aging (112).
Age-related gut microbiota dysbiosis is associated with increased gut mucosa permeability in both animal models and humans (92,111). The reduced intestinal epithelial barrier function is accompanied by several alterations in immune system, involving both innate and acquired immunity (Table 4) (11,66,126). These alterations ultimately promote local and systemic inflammation, with overproduction of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (66,126). Inflammation negatively impacts the gut-muscle axis and is involved in the pathogenesis of several age-related conditions, including not only sarcopenia and frailty (126), but even cancer (11). Moreover, IL-1β further stimulates intestinal epithelial tight junction permeability and promotes local dysbiosis (3), in a vicious cycle supporting skeletal muscle wasting and loss of function.
In summary, the relationship between intestinal microbiome and immunity may be two-way, and the resulting equilibrium may exert important functions on the functionality of the gut-muscle axis and on muscle health. More research is however needed to disentangle these complex relationships, and to reveal their actual relevance from a clinical perspective.

6. Conclusions

The relationship between exercise, immune system, gut microbiota, and skeletal muscle pathophysiology is very complex and not completely elucidated at the current state of the art. In Figure 1, we present a possible interpretative framework, showing that the gut microbiota is at the cross-road between environmental stimuli and host physiology, undergoing a continuous interplay with the immune system and the skeletal muscle.
Future studies should clarify whether gut microbiota dysbiosis is pathophysiologically associated with muscle wasting disorders, and if exercise may positively influence this putative gut-muscle axis. Furthermore, the influence of the microbiome-immune system interplay on skeletal muscle mass and functionality should be investigated in both experimental models and human beings.

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