Gut microbes and muscle function: can probiotics make our muscles stronger?

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Abstract

Evidence suggests that gut microbiota composition and diversity can be a determinant of skeletal muscle metabolism and functionality. This is true in catabolic (sarcopenia and cachexia) or anabolic (exercise or in athletes) situations. As gut microbiota is known to be causal in the development and worsening of metabolic dysregulation phenotypes such as obesity or insulin resistance, it can regulate, at least partially, skeletal muscle mass and function. Skeletal muscles are physiologically far from the gut. Signals generated by the gut due to its interaction with the gut microbiome (microbial metabolites, gut peptides, lipopolysaccharides, and interleukins) constitute links between gut microbiota activity and skeletal muscle and regulate muscle functionality via modulation of systemic/tissue inflammation as well as insulin sensitivity. The probiotics able to limit sarcopenia and cachexia or promote health performances in rodents are mainly lactic acid bacteria and bifidobacteria. In humans, the same bacteria have been tested, but the scarcity of the studies, the variability of the populations, and the difficulty to measure accurately and with high reproducibility muscle mass and function have not allowed to highlight specific strains able to optimize muscle mass and function. Further studies are required on more defined population, in order to design personalized nutrition. For elderly, testing the efficiency of probiotics according to the degree of frailty, nutritional state, or degree of sarcopenia before supplementation is essential. For exercise, selection of probiotics capable to be efficient in recreational and/or elite athletes, resistance, and/or endurance exercise would also require further attention. Ultimately, a combination of strategies capable to optimize muscle functionality, including bacteria (new microbes, bacterial ecosystems, or mix, more prone to colonize a specific gut ecosystem) associated with prebiotics and other 'traditional' supplements known to stimulate muscle anabolism (e.g. proteins), could be the best way to preserve muscle functionality in healthy individuals at all ages or patients.

Keywords   Skeletal muscle; Probiotic; Ageing; Cachexia; Sarcopenia; Exercise; Athlete

Introduction

Skeletal muscles represent 40% of the total mass in human beings and are involved in various fundamental functions such as mobility (locomotion and posture) and the maintenance of thermoregulation and glucose/amino acid metabolism.[S1] These tissues, accounting for 50–75% of all body proteins, can also be considered as the protein reservoir that can be solicited in catabolic situations.[S1] Muscle protein status is the consequence of a tight regulation between protein synthesis and degradation leading to protein anabolism after meal intake that counterbalances the catabolism in the fasted state. The daily meal-related cycles of anabolism/catabolism can be modified on a longer-term basis by age, physiological state, and/or lifestyle and/or fitness, and either leads to muscle mass loss (sarcopenia and

cachexia) or gain (recovery after stress and resistance exercise). These factors are known to impact significantly on muscle mass within days, weeks, or months. Nutritional strategies have already been developed to improve muscle mass or limit loss.[1] This includes protein supplementation and micronutrients capable to increase insulin sensitivity or limit low-grade inflammation and oxidative stress.[1,S2] These strategies, implemented alone or in association, have been proven to stimulate muscle mass and function, but their efficiency is not optimal and depends greatly on the targeted population.[2,S2] In the case of the elderly population, muscle mass loss (sarcopenia) has been shown to be multifactorial[3] and potentiated by a loss of appetite and less physical activity. On the other hand, in the athletic population, muscles are highly solicited by effort, subjected to repeated (micro) damages and always along the ridgeline of the optimum anabolic condition necessary to recover from injuries.[S3]

In these diverse populations, there is an increasing interest to develop more specific or novel strategies capable to optimize muscle anabolism complementary to the ones currently used. Some are based on gut microbiota, which is suspected to be causal in the development of several metabolic disorders. Indeed, microbiota changes have been shown to be correlated to and sometimes related to the development of obesity, diabetes, and cardiovascular diseases, all these pathologies being generally associated with the establishment of an insulin-resistant state and low-grade inflammation.[S4] In parallel, it has been repeatedly demonstrated that muscle catabolism is frequently associated, in many physiological states, with inflammation and insulin resistance.

The coincidence between alteration of gut microbiota composition, physiological state impairment, and muscle catabolic states suggests that microbiota, directly or indirectly, could influence muscle mass status and regulation. The hypothesis of the 'gut–muscle axis' (i.e. the impact of gut microbiota and its interaction with the host gut on skeletal muscle metabolism and function) has therefore been proposed by several authors, particularly in sarcopenic older people.[4–6,S5] Among potential mechanisms, gut microbiota could intervene in the regulation of the sensitivity of skeletal muscle to anabolic stimuli.[4,7] This explains why recent research projects have developed nutritional strategies including probiotics to target muscle mass and function. Probiotics are living microorganisms that have positive effects on health when ingested in adequate quantities. For a long time, marketed probiotics were mainly lactic acid bacteria (LAB) and bifidobacteria strains from a human or food origin, but current strategies are focusing more and more on the use of bacteria already present in the gut. The mechanisms of probiotics action are complex and often poorly understood, but one of their aims is to modulate the composition of an altered gut microbiota.

The aim of the present review is first to report the recent data on the interaction and underlying mechanisms that have

been demonstrated or hypothesized explaining the link between microbiota and muscle. Then, we will focus on probiotic strategies that have been developed so far to limit muscle mass loss or favour muscle mass gain in populations in catabolic or anabolic states.

Shift in microbiota composition and alteration of muscle mass and function: what can be the link between microbiota, gut, and muscle?

Sarcopenia

Sarcopenia is defined as the progressive and generalized loss of skeletal muscle mass and strength[8] and has recently been classified as a disease by the World Health Organization. This disease is still often associated with old age and can also occur earlier in life (inflammatory diseases and organ failure with cachexia). Its diagnosis requires the presence of low levels for three parameters: muscle strength, muscle quantity/quality, and physical performance as an indicator of severity.[8] As the number of elderly people and life expectancy increase, the proportion of sarcopenic elderly, although difficult to determine, could reach 20% of people over 60 years old.[S6] Moreover, sarcopenia is recognized as a central factor in the pathophysiology of the frailty syndrome considered to be a major public health problem due to the numerous consequences it entails (e.g. risk of falls, hospitalizations, and dependency).[9] The frailty syndrome is multidimensional and characterized by a cumulative decline in several systems and functions of the body that include physical aspects (i.e. phenotype of frailty described by Fried *et al*.) and also cognitive and social aspects.[S7] Consequently, part of frailty phenotype includes some characteristics of sarcopenia (significant weight loss, low handgrip strength, and gait speed).[9]

Several groups have studied microbiota composition in sarcopenic, frail, or more frequently elderly population over the last decade (*Figure* 1). It is now well established that the gut microbiota composition evolves with age and is characterized by a reduction of microbiota diversity with an important inter-individual variation.[10,11,S8] The analysis of gut microbiota composition in cohorts of elderly people showed a shift in microbiota composition in function of their age with the health status playing an important part.[12,13] The gut microbiota composition of frail elderly people is more altered compared with people of the same age who are in a better health.[10,14,15] In accordance with this, individuals with high frailty scores (determined with Groningen Frailty Indicator or Rockwood Clinical Frailty Scale) showed a significant reduction in relative abundance of lactobacilli, *Bacteroides/*
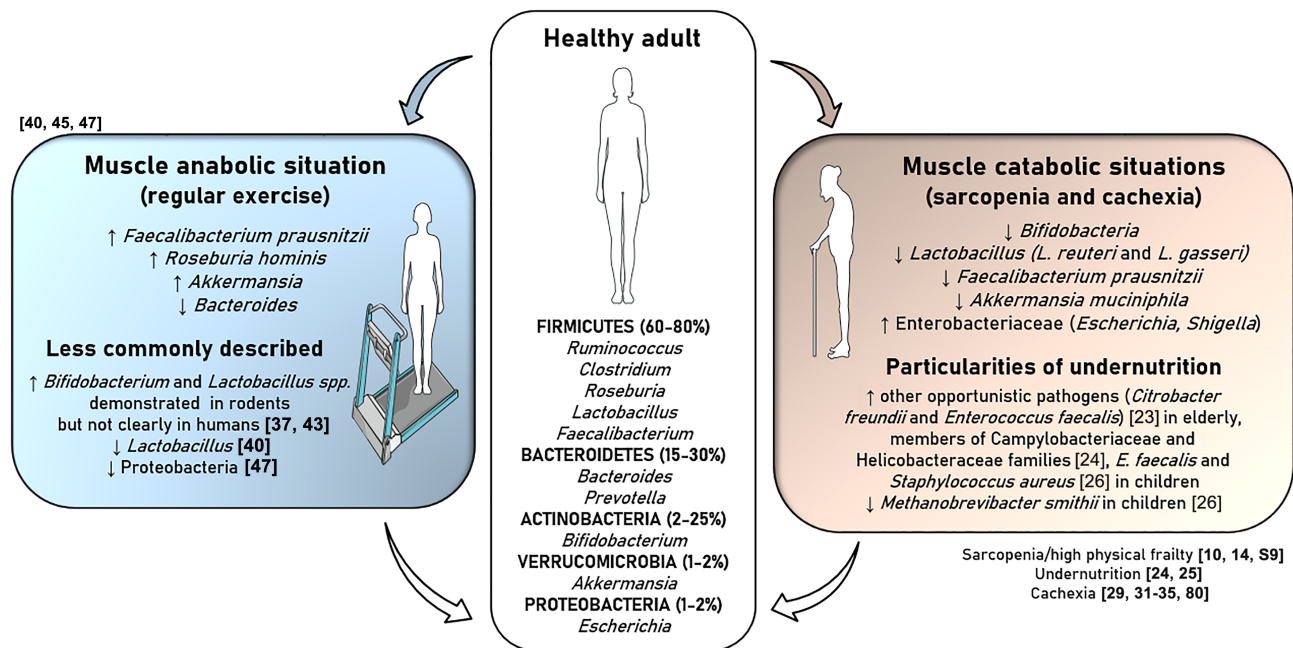
Figure 1 Major phyla and genera of the gut microbiota of healthy adult humans and impacts of physical exercise and sarcopenia/undernutrition and cachexia. The major phyla of the healthy intestinal microbiota are Firmicutes, Bacteroidetes, Actinobacteria, and to a lesser extent Verrucomicrobia and Proteobacteria. Exercise increases the proportion of *Faecalibacterium*, *Roseburia*, and *Akkermansia* genera. Studies in rodents have clearly shown an increase in *Bifidobacterium* and *Lactobacillus* spp. with exercise practice, a result more mitigated in humans. Frailty (and its major components: sarcopenia and undernutrition) and cachexia are associated with a decrease in these beneficial genera and an increase in opportunistic pathogens from Enterobacteriaceae family. Undernutrition (in both elderly and children) has the particularity to be associated with a strong increase in different pathogens.

Prevotella, and *Faecalibacterium prausnitzii* and increased Enterobacteriaceae compared with people with low frailty scores.[14,16,S9] Note that *F. prausnitzii* particularly is recognized as an indicator of good intestine health as metabolites produced by *F. prausnitzii* (e.g. butyrate) present anti-inflammatory properties.[S10] In parallel, studies using cohorts have shown that sarcopenia is associated with decreased short-chain fatty acid (SCFA—including acetate, propionate, and butyrate derive from fermentation of non-digestible food)-producing bacteria (*Roseburia*, *Eubacterium* for instance) relatively to non-sarcopenic controls.[13,15]

Experimental design/models such as germ-free mice have added valuable evidences on the potential causal role of microbiota on the control of muscle mass and function. Axenic and antibiotic-treated mice present a decreased muscle mass and strength compared with their conventional counterparts.[17–19] Interestingly, these alterations in muscle mass and function can be restored by the transplantation of a microbiota or after a natural reseeding.[17,18] Objectifying the causal role of the microbiota is complex in old individuals due to the numerous confounding factors that are known to modify microbiota composition: medication, lower appetite, and nutritional status or associated chronic pathologies.[20,S11] However, increasing evidences suggest that the gut microbiota may play among other factors, a causal role in the devel-

opment of muscle mass loss and function in elderly.[4,5,7] Fecal microbial transplantation (FMT) from elderly volunteers in either good (HF) or bad (LF) physical function to germ-free animals showed that HF mice presented greater muscle strength compared with LF group.[21] HF microbiota was enriched in *Prevotella* and *Barnesiella* compared with LF. These bacterial genera contain genes coding for enzymes that produce SCFAs (i.e. acetate, propionate, and butyrate), which is the opposite of what was described earlier in the old sarcopenic population. These promising results should be confirmed in the diverse elderly population.

Undernutrition

Partially connected with the issue of frailty described earlier, undernutrition (i.e. inadequate intake of dietary nutrients relatively to requirement) and its impact on microbiota have been investigated in two extreme populations in terms of age: children and elderly. In developed countries, the elderly population can suffer from undernutrition, particularly in frail and hospitalized people. The anorexia of ageing (i.e. decrease in appetite and/or food intake in old age) can worsen the age-related loss of muscle mass and precipitate the entry of elderly people into a phase of dependence.[22]

One study showed similarities between the microbiome of high frail nursing home residents (≥65 years) and malnourished population.[23] These two populations both showed an increase in *Ruminococcus gnavus*, a species recently associated with inflammatory joint disease,[S12] as well as a decrease in butyrate producers (*Roseburia intestinalis*) (*Figure* 1). A particularity of people at risk of malnutrition or malnourished is the significant increase of specific opportunistic pathogens, which can cause serious infections.[23] In severely malnourished children compared with healthy children from the same country and at the same age, the microbiota is enriched in Proteobacteria phylum (Enterobacteriaceae or Campylobacteriaceae and Helicobacteraceae families).[24–26]

This colonization can be responsible for the development of intestinal inflammation leading to an alteration of small intestine morphology (decreased surface area, increased permeability, and apoptosis of epithelial cells) and ultimately resulting in intestinal malabsorption.[24]

Smith *et al*. were the first to suggest a causal relationship between the gut microbiome in a state of extreme undernutrition, the kwashiorkor syndrome.[27,S13] FMT from Malawian twin patients with or without a kwashiorkor syndrome to germ-free mice was performed. The combination of Malawian diet (plant-based diet deficient in proteins and in micronutrients, and usual diet of the targeted population) and microbiome from the kwashiorkor twin led to a phenotype of the undernourished children in the recipient mice (i.e. weight loss). Same conclusions were obtained by Blanton *et al*.[28] Refeeding kwashiorkor microbiota-transferred animals with ready-to-use therapeutic food did not help to improve significantly the rodent's health,[27] but the growth of these mice was restored when co-housed with mice having healthy microbiota.[28] Two taxa (*R. gnavus* and *Clostridium symbiosis*) have been highlighted as potential key determinants in the regulation of weight gain and growth in such nutritional situations, by promoting protein synthesis (and lean mass growth) rather than altering protein oxidation.[28] The role of these specific bacteria on growth rate and muscle mass as well as translation to humans requires further investigation.[S14,S15]

Cachexia

A rapid muscle mass loss and a shift in gut microbiota composition also occur in cachectic population. Cachexia is a multifactorial syndrome characterized by a generalized fatigue, loss of body weight, skeletal muscle, and adipose mass, as well as reduced food intake; this term is being frequently used in context of cancer.[29,30,S16] This syndrome is also associated with a systemic inflammatory state, characterized by high rates of pro-inflammatory markers such as C-reactive protein (CRP).[30] An altered microbiota is observed in cancer cachexia (*Figure* 1), and several hypothesized mechanisms

(systemic inflammation or gut barrier dysfunction) suggest a causal role of microbiota in cachexia development.[29] In cancer patients (colorectal, breast, and lung cancers), a decreased abundance of *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium* genera is observed concomitantly with an increase of Enterobacteriaceae and *Enterococcus*.[31–33] Similar microbial colonic patterns are also found in other non-cancerous cachectic patients presenting chronic kidney, heart, or liver diseases.[29,34,35] The associated increase in circulating pro-inflammatory cytokines is then able to induce muscle atrophy by different mechanisms (insulin resistance, inflammation, and associated oxidative stress), which are detailed in the succeeding text.

Physical activity

Physical activity and exercise are known to be strong determinants of health status. Even if a lack of physical activity is correlated to a poor health status and imbalanced microbiota, the causal role of microbiota to promote health in such a context remains to be studied in detail. Few studies, particularly in humans, have investigated the effects of exercise on gut microbiota composition and/or functions (Supporting Information, *Table* S1). But, as nutritional status and physical activity are generally tightly intertwined, the exact role of exercise *per se* on health outcomes remains complex to evaluate.[36] In rodent studies where exercise and nutrition are more controlled (*Table* S2), regular physical activity is associated with an elevated bacterial diversity and richness in the gut/faecal microbiota.[37,38]

Generally, a decrease of the Firmicutes/Bacteroidetes ratio is observed,[37–40] and basically, microorganisms altered in catabolic situations are changed the other way round with exercise or in athlete population (*Figure* 1). Still, the nature and/or intensity of exercises may alter differentially microbiota composition (voluntary exercise vs. forced exercised[41,42]). For instance, in rodents subjected to voluntary access wheel exercise, the bacterial diversity and main phyla abundance were not strongly altered, but forced exercise (treadmill) led to increased proportion of harmful and pro-inflammatory bacteria vs. free-exercised animals and sedentary controls.[43,S17] A study demonstrated that forced treadmill exercise exacerbates inflammation and causes mortality while voluntary wheel training is protective in a mouse model of colitis.[44]

As said earlier, people exercising tend to have more 'healthy' diets, including a higher dietary fibre intake.[36] This can explain why some bacteria associated with good immunological and metabolic health such as *F. prausnitzii*, *Roseburia hominis* (i.e. butyrate producers) and *Akkermansia muciniphila* (i.e. acetate and propionate producer) tend to be more abundant with physical activity.[45–47] Likewise, exercise has been shown to be associated with the abundance of

other butyrate producers[40,45] or lactate producers,[37,43] and it is known that lactate is converted into butyrate by butyrate-producing bacteria in the gut.[S18] This suggests that the combination of exercise and microbiota-targeted nutrition should be more closely investigated.

In this sense, and similarly to what was already performed in catabolic situations to assess the causal role of microbiota on muscle mass and function, studies have used FMT from exercised mice to axenic rodents. In high-fat-fed mice, the impact of FMT from normal-fed or exercised animals on recipient's phenotype has been studied.[48] Even if diet is more powerful than exercise to shape the gut microbiota, FMT from exercised animals (fed with normal or high-fat diet) led to a reduced fat mass and inflammation (TNF-α and IL-1 expression in liver), to an increased glucose tolerance, and to a decreased serum low-density lipoprotein (LDL) compared with sedentary animals fed with the high-fat diet.[48] The anti-inflammatory properties of the microbiota from exercised mice have been further proven by Allen *et al*. Indeed, FMT from mice having free access to running wheel during 6 weeks in germ-free mice subjected to inflamed colon (colitis)[49] allowed a higher microbiota diversity, limited colitis, and improved body mass compared with same animals transplanted with microbiota from sedentary mice.[49]

Regular mechanisms of muscle function that could be recruited to explain microbiota–muscle axis

Many reviews summarize how microbiota can modulate health status in various physiological/pathological states. These covered metabolism regulation (e.g. energy expenditure, lipogenesis/lipolysis, and insulin secretion), appetite control, and cognitive function.[50,S10] Among these functions, some of them concern muscle (inflammation, energy/nutrients metabolism, insulin resistance, and oxidative stress). A recent review also details how molecules synthesized by the microbiota could be considered as links between microbiota and regulation of whole-body metabolism.[51] We will focus on major microbiota-related regulatory mechanisms involved in muscle mass and functionality: insulin sensitivity and inflammation, both mechanisms being tightly connected with the presence of an oxidative stress and alterations of nutrients metabolism at the muscle level.

Insulin sensitivity—role of short-chain fatty acids

Insulin is key in the regulation of skeletal muscle homeostasis as it stimulates the entry and use of glucose in muscle cells in the postprandial state and preserves muscle mass by inhibiting

protein breakdown.[S19] It is known for decades that elderly people develop insulin resistance associated with a loss of muscle mass and strength.[S20] The installation of a local/systemic inflammation and oxidative environment in a context of increased lipotoxicity are the most documented causes of decreased muscle insulin sensitivity in elderly or obese populations.[S19] On the contrary, the practice of moderate physical activity improves insulin sensitivity and increases glucose uptake, glycogen synthesis, and protein anabolism in muscle.[52] Because of this, exercise training can be therefore considered to be a therapeutic strategy against insulin resistance and sarcopenia.[S21] Many nutritional strategies have also been tested in that field such as micronutrients/anti-inflammatory/antioxidant substances (e.g. vitamins A and E, and polyphenols) and amino acid supplementation (leucine or leucine-rich proteins). A new paradigm arises from princeps studies from Bäckhed *et al*.[S22] and Turnbaugh *et al*.,[S23] in nutritionally induced insulin resistance rodent models that have shed light on the causal role of gut microbiota in tissue insulin sensitivity.[53,54] These data have been confirmed in humans with fecal microbial transplantation (FMT) from healthy patients to individuals with metabolic syndrome where peripheral insulin sensitivity was improved (using rate of glucose disappearance under hyperinsulinaemic–euglycaemic clamp).[55] However, the beneficial effect of FMT on insulin sensitivity parameters was not always observed.[56] The role of nutrition itself remains considered as the major driver of insulin sensitivity and entails the development of complementary studies on FMT implementation (donor effect).

Among the numerous metabolites produced by microbiota within the gut lumen and that could constitute links between microbiota, gut, and muscle insulin sensitivity, SCFAs have been extensively studied.[57,S24,S25] SCFA-producing bacteria have repeatedly been shown to be increased by exercise by contrast with individuals with sarcopenia and cachexia. Most of the SCFAs found in the gut lumen are terminal products of anaerobic fermentation of non-digestible dietary fibres by microbes and are mainly produced in the distal ileum and colon.[S24] Molar ratios in the colon for acetate, propionate, and butyrate are approximately 3:1:1, and they account for ≥95% of the total SCFAs.[S24] Then, SCFAs are absorbed by intestinal cells, and butyrate enters into the TCA cycle as acetyl-CoA to provide 60–70% of the colonocyte energy needs.[57] The remaining SCFAs are then found in the portal vein and reach the liver (~80% of propionates are taken up by the liver and ~40% for acetate), propionate being used as a substrate in gluconeogenesis pathways.[57,58,S24] Ultimately, a small proportion of SCFAs (mainly acetate) reach skeletal muscles[S24] (*Figure* 2).

Short-chain fatty acids are involved in glucose/lipid homeostasis, in the regulation of inflammation, and in the connection of gut with other distance tissues.[57,59,S24,S25] SCFA supplementation restored/improved muscle mass and/or strength, previously decreased in germ-free and
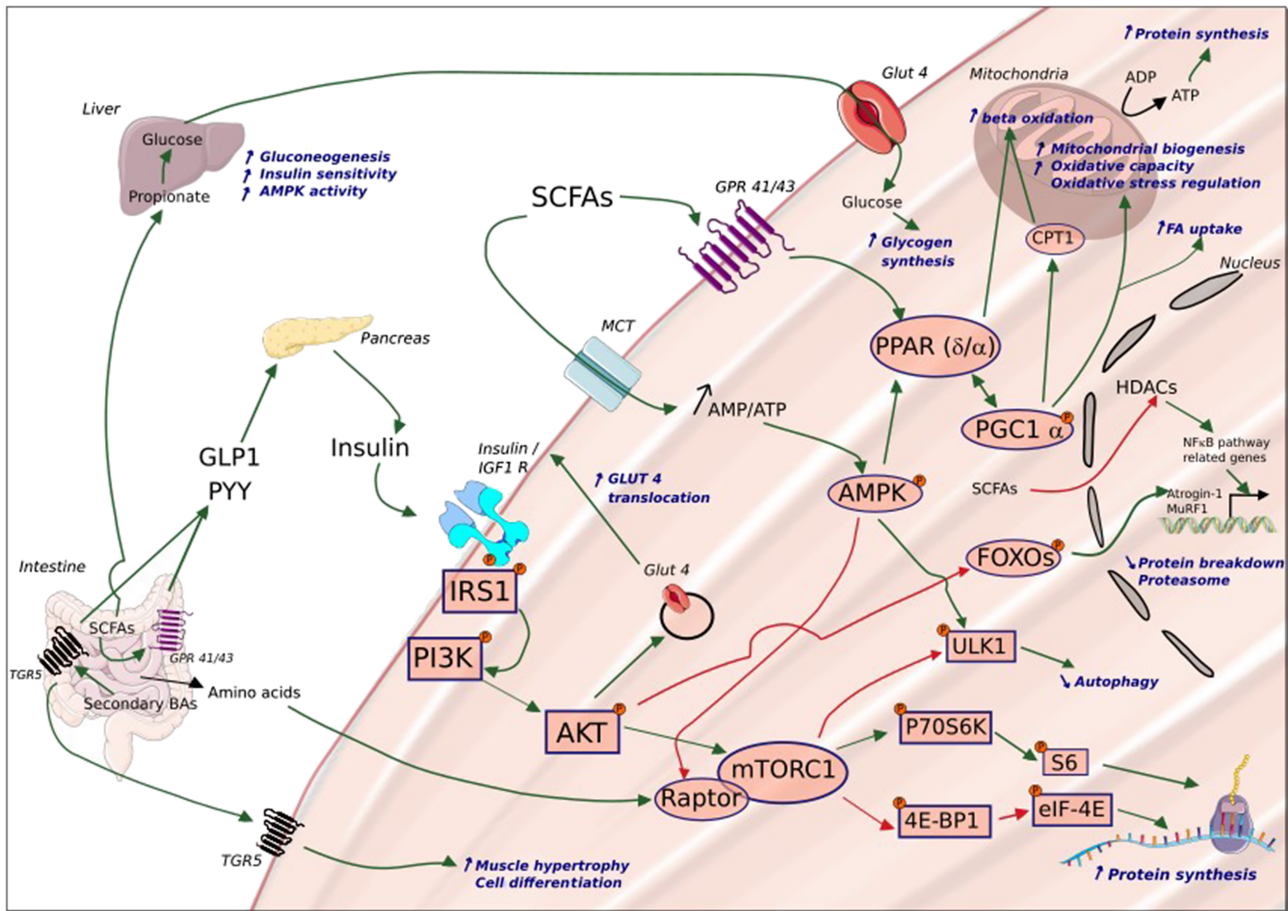
Figure 2 How probiotic can be involved in maintaining skeletal muscle health: regulatory signals and metabolites. Probiotics can promote the production of metabolites such as short-chain fatty acids (SCFAs), secondary bile acids (BAs), and some amino acids that can ultimately modulate muscle function. SCFAs, via their interaction with G protein-coupled receptor (GPR) 41/13 receptors in the ileum and colon, promote the secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) by intestinal L cells, which in turn stimulate insulin secretion by the pancreas and improve insulin sensitivity in peripheral organs. At the muscle level, actetate uptake and oxidation as well as its and interaction with GPR receptors leads to phophorylation of AMPK as well as activation of peroxisome proliferator-activated receptor δ and α. In parallel, insulin, IGF-1, and amino acids stimulate the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mTOR pathway, which increases glucose uptake [via glucose transporter type 4 (GLUT4)], promotes protein synthesis (via activation of S6K), and reduces muscle proteolysis via inhibition of forkhead box O (FOXO) and Unc-51 like autophagy activating kinase (ULK1) factors. SCFAs can also inhibit HDACs that participated in regulation of expression of various genes in the nucleus, including the expression of nuclear factor-κB (NF-κB) pathway-related genes. Secondary BAs also promote GLP-1 secretion via activation of G protein-coupled bile acid receptor, Gpbar1 (TGR5), and play a role in muscle trophicity by mechanisms that still require to be further investigated. 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; CPT1, carnitine palmitoyltransferase I; eIF-4E, eukaryotic translation initiation factor 4E; IGF1-R, insulin-like growth factor receptor; IRS1, insulin receptor substrate 1; MCT, monocarboxylate transporter; mTORC1, mechanistic target of rapamycin complex 1; P70S6K, p70S6 kinase; PGC-1α, Pparg coactivator 1 alpha; PPARα, peroxisome proliferator-activated receptor-α; Raptor, regulatory-associated protein of mTOR. Green arrow: activation; red arrow: inhibition.

antibiotic-treated rodents.[17–19,60] Supplementation with acetate (in the diet or subcutaneous injection) promotes glucose uptake and glycogen content in skeletal muscles of rats and shown to decrease lipid intramuscular accumulation through increased fatty acid uptake and oxidation in rabbits.[61] Similarly, oral butyrate supplementation in mice prevents oxidative stress and muscle mass loss by increasing mitochondrial function and biogenesis as well as the number of type I (oxidative) fibres in skeletal muscles.[60,62] These metabolic effects

of SCFAs could be direct on skeletal muscles, for acetate as it is present in peripheral blood, but probably not for butyrate and propionate as they only marginally reach the peripheral blood stream. However, these effects could be also indirect via a stimulation of glucagon-like peptide 1 (GLP-1) secretion, a gut hormone that is responsible for a stimulation of insulin secretion, glucose storage in the liver, and glucose uptake in skeletal muscles.[63] Other indirect effects of SCFAs on muscle include increased blood flow and anti-inflammatory

properties via epigenetic mechanisms.[64] Succinate is like propionate, a substrate for gluconeogenesis, and De Vadder *et al.* suggested that glucose produced from succinate by intestinal cells, when detected in the portal vein (portal signal), may increase satiety, energy expenditure, glucose tolerance, and insulin sensitivity.[65,66]

Inflammation

It has been postulated that an altered microbial ecosystem composition could be associated with an imbalance between intestinal anti-inflammatory and pro-inflammatory responses leading to a chronic systemic low-grade inflammation, also named 'inflammageing' in the elderly population.[67] One possible cause of inflammageing is the age-related deregulation of the immune system (immunosenescence) leading to a decrease in the ability to counteract intestinal colonization by pathogenic bacteria as well as by 'gut leaking', which generates a systemic inflammation[68] by enhancing circulating endotoxin levels (e.g. LPS). LPS is transported in the blood by the LPS binding protein and then recognized by the 'pattern recognition receptors' [including toll-like receptor 4 (TLR4)] localized on innate immune cells. The binding of LPS on TLR4 results in the recruitment of various intracellular moieties (e.g. MAPK) triggering cellular signalling cascades like the pro-inflammatory pathway NF-κB.[69] Of note, NF-κB pathway can be also activated by pro-inflammatory cytokines and ROS, molecules whose production is increased in cachexia, intense exercise training, or even sarcopenia. NF-κB has been shown to be involved in muscle atrophy by participating in muscle protein degradation (ubiquitin–proteasome pathway), inducing inflammation, and blocking muscle fibre regeneration[70] (*Figure* 3).

Ageing is associated with a progressive increase in inflammation markers including tumour necrosis factor-α (TNF-α), CRP, and interleukin 6 (IL-6).[S28] Plasma levels of TNF-α and IL-6 are also elevated in rodent models of cancer cachexia[71] or cancer cachectic patients.[72] The elevation of these cytokines is known to be involved in weight and muscle loss and poorer outcomes in patients with cancer.[71] Likewise, obesity and diabetes induced by high-fat diet are associated with increased inflammatory markers levels, leading to deleterious impact on skeletal muscles anabolism.[53,S29] By contrast, exercise can have either a positive or negative impact on the inflammatory status and points out that the balance between the different inflammatory markers has also to be taken into account.[73] Nevertheless, increased evidence suggests that regular physical exercise has an overall anti-inflammatory impact on sedentary and obese people.[74] However, when exercise is more intense, as for athletes, this could lead to an acute inflammatory response in skeletal muscle with release of pro-inflammatory cytokines.[73]

High circulating LPS is known to induce increased expression and production of pro-inflammatory cytokines by the immune cells and lead to muscle atrophy by regulating several metabolic pathways including protein homeostasis and mitochondrial function.[S30] In animal models of acute inflammation, a stimulation of muscle proteolysis (ubiquitin—proteasome pathway), a decreased protein synthesis (via mTOR-regulated initiation of translation), but also induction of cell apoptosis and, or inhibition the differentiation of satellite stem cells are observed.[75,S31] Such similar mechanisms may also occur even in a situation of low-grade inflammation as we have shown in rats presenting modest increased alpha-2 macroglobulin plasma levels (marker of systemic inflammation in rats) without any diagnosed or induced intestinal inflammation, an inhibition of muscle postprandial protein synthesis, whereas it is normally stimulated in adult or not inflamed old animals.[76] Interestingly, ibuprofen treatment of the same rats restored the blunted protein synthesis, suggesting that inflammatory status was the cause of the lack of response of protein synthesis to dietary anabolic stimuli.[76] Accordingly, in humans, IL-6 is also correlated to muscle atrophy when it is related to low-grade chronic inflammation as observed in elderly or certain cancerous patients with a down-regulation of protein synthesis.[4,77] Gut leaking and associated circulating LPS can also, during prolonged practice of intense exercise, promote an important ROS production responsible for oxidative stress that can generate insulin resistance within skeletal muscles with mitochondrial dysfunction, apoptosis, or autophagy.[53,S32] In this sense, it is extremely complex to separate inflammation from oxidative stress and consequences on muscle insulin sensitivity. For instance, IL-6 effect can impact on insulin signalling pathways in muscle that is associated with an increased risk of T2D in humans.[69] However, the role of IL-6 is paradoxical and depends on the dose and the manner of administration in rodents and the presence of other cytokines.[77] IL-6 could have an anti-inflammatory effect and play a key role in muscle growth (effect on satellite cells) as a myokine when it is secreted by the muscle itself during physical exercise.

How probiotics can modulate muscle mass and function in catabolic and anabolic states?

As we have developed earlier that microbiota activity and diversity could be a determinant in muscle mass and function in various physiological/pathological situations, strategies capable to target gut microbiota can then be considered as a lever of action to fight against muscle mass loss and function (sarcopenia and cachexia) or optimize muscle performance (athletes, sarcopenia, and recovery after a pathology). In such, probiotics could complement already existing strategies, and

Figure 3 Interaction between microbiota and skeletal muscle in a context on increased inflammation. An altered composition of the gut microbiota ecosystem can lead to gut leaking and entry of bacterial endotoxins such as lipopolysaccharide (LPS) in peripheral blood. This is also associated with a decreased production of beneficial metabolites [e.g. short-chain fatty acids (SCFAs)] in the gut lumen. LPS can trigger the production of inflammatory cytokines and reactive oxygen species (ROS) by macrophages via toll-like receptor 4 (TLR4) receptors. In skeletal muscle, tumour necrosis factor-α (TNF-α) activates the expression of nuclear factor-κB (NF-κB) pathway-related genes that decrease cell differentiation and proliferation (via inhibition of myogenin and myoD). Interleukin (IL)-6 and IκB kinase (IKK) can inhibit insulin receptor substrate 1 (IRS1), which, associated with an induction of insulin resistance, limits the activation of mechanistic target of rapamycin complex 1 (mTORC1) and thus protein synthesis. In addition, because protein kinase B (AKT) is no longer activated, it can no longer exert its inhibitory role on forkhead box O (FOXO), which leads to an increased expression of the ubiquitin E3 ligases Atrogin-1 and MuRF1 and promotes muscle proteolysis. Similarly, Unc-51 like autophagy activating kinase (ULK1) that is not inhibited by mTORC1 cannot inhibit autophagy. When these regulatory mechanisms become chronic, an imbalance between protein breakdown/synthesis occurs and causes muscle atrophy. 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; eIF-4E, eukaryotic translation initiation factor 4E; GLUT4, glucose transporter type 4; GPR, G protein-coupled receptor; IGF1-R, insulin-like growth factor receptor; IL-6 R, interleukin-6 receptor; JAK, Janus kinase; P70S6K, p70S6 kinase; PI3K, phosphoinositide 3-kinase; Raptor, regulatory-associated protein of mTOR; STAT, signal transducer and activator of transcription; TNF-α R, tumour necrosis factor-α receptor. Green arrow: activation; red arrow: inhibition.

they have been repeatedly demonstrating capability to limit the occurrence of insulin resistance, modulate metabolic pathways in various tissues, or limit inflammation and oxidative stress.[78,S33] Additionally, some probiotics seem to be capable to target specifically some of these metabolic functions or regulatory mechanisms even in non-catabolic or anabolic situations. As an example, two different types of probiotics (*Lacticaseibacillus casei* LC122 or *Bifidobacterium longum* BL986) have been tested in 10-month-old (i.e. adult, not old) mice for a 12 week period.[79] The two probiotic strains increased skeletal muscle mass and grip strength in these rats

compared with age-matched controls.[79] Interestingly, the two bacterial species seemed to act on different cellular pathways because *Lactobacillus casei* showed an antioxidant potential (increased SOD and GPx activities in muscle and intestines) while *B. longum* presented anti-inflammatory properties (decreased TNF-α, IL-6, and IL-1β levels in the muscle and colon).[79] The remaining question is to highlight whether or not these probiotics are capable to be also efficient on muscle when challenged, that is, in situations of muscle loss (sarcopenia and cachexia) or gain (following exercise) in animal models and humans.

Probiotics in catabolic states

Muscle catabolic conditions in rodents

Cancer Lactic acid bacteria have been tested in two experiments using rodents subjected to cancer-induced cachexia[80,81] (*Table* 1). A first experiment used leukaemic mice supplemented with *Limosilactobacillus reuteri* 100-23 and *Lactobacillus gasseri* 311476 for 2 weeks (in drinking water, 10^8 CFU/day).[80] The second experiment, in mice presenting intestinal polyposis, consisted in a daily supplementation in *L. reuteri* ATCC-PTA-6475 for 3 months at a lower dose (10^5 CFU) than the previous study. Both experiments showed that probiotics limited skeletal muscle mass loss,[80,81] which could be associated with an increased fibre size.[81] Here, an inhibition of proteolysis was observed (the ubiquitin–proteasome pathway with MuRF1 and Atrogin-1 decreased gene expression[80]). MuRF1 and Atrogin-1 were also down-regulated in muscle from mice presenting adenocarcinoma and supplemented with lyophilized kimchi containing *Leuconostoc mesenteroides* CJ LM119 and *Lactiplantibacillus plantarum* subsp. *plantarum* CJ LP133 vs. cancerous non-supplemented animals.[82] In all these experiments, inflammation associated with cancer implantation was down-regulated by probiotic supplementation.

Probiotics can also stimulate anabolism (protein synthesis) in mice with colon carcinoma as in An *et al*. (2019), where a positive impact on AKT and mTOR phosphorylation and a decreased AMPK phosphorylation were also observed. Lastly, Bindels *et al*. have also tested the effect of a symbiotic (*L. reuteri* 100-23 in association with oligofructose) for 2 weeks in leukaemic mice.[83] This led to a restoration of *Lactobacillus* population in the gut, maintenance of muscle mass, and expression of genes involved in permeability and immunity in the gut to the level of control animals. All these data confirm that some bacteria (mainly LAB)—associated or not with prebiotics—could preserve anabolism in skeletal muscle via both an inhibition cancer-induced inflammation leading to decreased proteolysis and a potential stimulation of protein synthesis.

Ageing In the field of sarcopenia (*Table* 1), attention should be given to the age of rodents. Indeed, in rats, for instance, ageing-related muscle loss generally occurs significantly from 18 months of age onwards.[84] Any adult rat that is non-growing in terms of fat free mass (i.e. 12 months) cannot be considered as the equivalent of an elderly human individual but as a middle-aged adult. With these considerations

Table 1 Studies assessing the effect of probiotics in cachectic and old rodent models

Reference	Model	Probiotic	Dosage	Results
(Bindels et al., 2012)	BALB/c BaF3 leukaemic and cachectic mice (6 weeks)	*Limosilactobacillus reuteri* 100-23 and *Lactobacillus gasseri* 311476	2 weeks (10^8 CFU/day for each strain)	↑ tibialis mass ↓ systemic inflammation (IL-6) ↓ MuRF-1 and Atrogin-1
(Varian et al., 2016)	C57BL/6 ApcMIN leukaemic and cachectic mice (8 weeks)	*Limosilactobacillus reuteri* ATCC-PTA-6475	3 months (10^5 CFU/day)	↑ muscle mass and fibre size
(Varian et al., 2016)	CD-1 mice (8 weeks to 10 months)	*Limosilactobacillus reuteri* ATCC-PTA-6475	10 months (10^5 CFU/day)	↑ muscle mass and fibre size ↑ growth hormone ↑ survival rate
(An et al., 2019)	Cachectic adenocarcinoma Balb/c mice (6 weeks)	Freeze-dried kimchi + *Leuconostoc mesenteroides* CJ LM119 and *Lactiplantibacillus plantarum* CJ LP133	3 weeks	↑ leg muscle mass ↓ Atrogin-1 and MuRF-1 ↓ ubiquitin–proteasome ligase ↓ p-AMPK and ↑ pAKT and p-mTOR ↑ PGC-1α ↓ NF-κB
(Chen et al., 2019)	SAMP8 mice (7 months)	*Lacticaseibacillus paracasei* PS23	12 weeks (10^9 CFU/day)	↑ lean mass ↑ grip strength ↑ protein digestibility ↓ oxidative stress (protein carbonyl) ↑ SOD and GPx (muscles)
(Hor et al., 2019)	Sprague–Dawley rats + D-galactose	*Limosilactobacillus fermentum* DR9 or *Lacticaseibacillus paracasei* OFS 0291	12 weeks (10^{10} CFU/day)	↑ running time, speed, work, and power (treadmill exhaustion test) ↑ p53 expression in gastrocnemius muscle
(Ni et al., 2019)	C57BL/6 mice (10 months)	*Lacticaseibacillus casei* LC122 or *Bifidobacterium longum* BL986	12 weeks (10^9 CFU/day)	↑ quadriceps and gastrocnemius mass ↑ grip strength ↑ intestinal barrier (mucus production, claudin-1, and ZO-1) ↓ muscle inflammation (TNF-α, IL-6, and IL-1β) *Bifidobacterium* ↑ muscle antioxidant capacities (SOD and GPx) *Lactobacillus*

kept in mind, several studies have investigated the impact of probiotic supplementation of variable duration (from several weeks to entire life) in rodents. Varian *et al*. have tested the effect of a supplementation of the strain *L. reuteri* ATCC-PTA-6475 on the muscle characteristics and life expectancy in normal mice. Muscle weight, fibre size, and animals' survival were increased following probiotic treatment when it was ingested on a long time basis.[81] Of note, mice strain used in this study was CD1 that is characterized by a low life expectancy or an accelerated ageing process compared with other strains. Indeed, all CD1 animals were dead between 500 and 600 days of life. However, this effect has been found in other less specific strains (C57/BL6 mice) with even shorter duration of probiotic supplementation.

Models of accelerated ageing have also been used to demonstrate the impact of probiotic supplementations. This is the case for senescence-accelerated mouse prone-8 (SAMP8) and D-galactose-induced ageing rodents. A 12 week supplementation of *Lacticaseibacillus paracasei* PS23 was performed in 26-week-old SAMP8 mice[85] and led to an increased lean mass and restored muscle strength (grip force) to the same level as 16 week SAMP8 mice. This was associated with an improved oxygen consumption rate in the muscle and mitochondrial biogenesis. In parallel, muscle protein carbonyl content, a marker of oxidative stress, was lower, and the expression of the antioxidant enzymes SOD and GPx in muscles increased in SAMP8 animals vs. controls. Inflammation state was also improved via a decreased pro-inflammatory/anti-inflammatory balance in both plasma and muscle (increased IL-10 levels and expression and decreased pro-inflammatory cytokines). In a model of D-galactose-induced premature ageing Sprague–Dawley rats, Hor *et al*. have shown that treatment with different LAB strains (*Limosilactobacillus fermentum* DR9, *L. paracasei* OFS 0291, or *Lactobacillus helveticus* OFS 1515) for 12 weeks led to an increased exercise performance following a treadmill exhaustion test compared with untreated rats.[86] Strength and physical endurance of the older rodents supplemented with each probiotic was increased at a level relatively similar to that of young rats without D-galactose treatment. This was explained by a decrease in senescence markers (p53 involved in apoptosis induction), increase of anabolic factors (IGF-1 mRNA) in muscle and bone,[86] and improved inflammatory status. Interestingly, all strains tested did not target the same muscle pathways: oxidation status, AMPKa2, and myogenic factors (e.g. Myf5/MyoD), suggesting that in anabolic situations, probiotic strains effects could target different signalling or metabolic pathways and tissues.

Taken together, data obtained after supplementation of probiotics in animal models subjected to catabolic states show that the effect is null or anabolic on muscle mass and function. An anti-inflammatory effect is generally observed, probably due to gut leaking, increased inflammation, and up-regulation of the NF-κB pathway at the gut then whole-body and muscle levels.[87] The muscle targets are numerous and could differ between strains: stimulation of anabolic factors such as IGF-1, stimulation of myogenic factors or mTOR pathway, regulation of energy status in the muscle (via AMPK), and inhibition of proteolysis and oxidative status.

Muscle catabolic conditions in humans

As a prelude to this part, it should be said that studies investigating the impact of probiotics on muscle mass and function in cancer patients or the elderly are scarce if non-existent. Yet probiotics are beginning to be considered (alone or in association with other strategies) as very promising new tools to maintain muscle mass and function in both the elderly population[88] and cancer patients.[89]

Cancer In cancer patients, probiotic strategies most used are lactobacilli genera and more specifically *Lacticaseibacillus rhamnosus* CG. Even if a majority of studies are related to colorectal cancer, the supplementation in probiotics was also studied in many varieties of cancers.[89] The most documented impact of probiotics on gut health is the limitation of occurrence of diarrhoea and improved global gut health and microbial composition that is compromised either by anticancer treatment or by the cancer itself (for colorectal for instance).[90] In colorectal cancer, probiotics have been shown to limit colonization of pathogenic bacteria, improve gut barrier function, and reduce inflammation and oxidative stress in humans.[91,S34] A positive effect on muscle mass and function has only been hypothesized but not effectively measured.

A protocol using probiotic supplementation (*L. plantarum* 299v) in cancer patients receiving enteral nutrition has recently been published, but results are not yet available.[92] Such protocols may add some valuable information on nutritional status and life quality, indexes that can be linked to muscle health status. Still, it should be kept in mind that in cancer and immunosuppressed patients, care has to be taken on the risk/benefit ratio you can expect from a supplementation of bacteria in these patients, even if probiotics.

Ageing The vast majority of studies using probiotic supplementations in the elderly have targeted digestive health and immunomodulation and concerned mainly bifidobacteria and lactobacilli genera or a mix of different strains (VSL#3 mix).[93] Overall, the probiotic supplementation in the elderly population led, in some cases, to beneficial modifications of gut microbiota with a decrease in pathogens but with relatively minor effects on health status of the host.[94,S35] The main effects were a slight increase in gut comfort and reduction in constipation,[95] but not always.[S36] A few studies reported slight positive effects on increased innate immunity (increased cytotoxicity of NK cells[96] and improvement cytokine profile[97]). Taken together, the overall small effects observed or the inconsistency of the results observed in health parameters measured in the elderly population can lie on the large heterogeneity of this population in terms of health status, dependency (frail concept[9]), and life habits (community

dwellers vs. residential care), all these 'basal' parameters affecting the response of the host to the probiotic supplementation and finally the validity of parameters used to evaluate immune or health state. To our knowledge, as for cancer patients, no study has directly measured muscle mass and function in the elderly population supplemented with probiotics. Still, the observed slight improvement of immune status and microbiota composition are in line with a potential beneficial effect on muscle via a reduction of inflammation. Additionally, probiotic (fructooligosaccharides and inulin) supplementation (that also targets gut microbiota) in frail old adults resulted in a significant improvement in their state of exhaustion and grip strength as well as a significant reduction in frailty index after 13 weeks of daily supplementation.[98,S37] These data suggest that a better assessment of the real impact of probiotics on muscle functionality in the old population is required.

Probiotics during exercise or in athletes

Rodent models

The supplementation in probiotics has been tested in healthy animals (in interaction or not with exercise) (*Table* 2). The aim of these studies was to determine if probiotics were capable to optimize muscle function and physical performance in

models that could mimic metabolic/physiological responses of young athletes.

The probiotics used were generally, again, lactobacilli and bifidobacteria.[99–102] In all studies, muscle function and/or physical performances were improved. *L. plantarum* TWK10 supplementation for 6 weeks increased muscle mass (gastrocnemius muscle) and improved forelimb grip strength and swimming endurance vs. control mice. *Ligilactobacillus salivarius* subsp. *salicinius* SA-03 and *Bifidobacterium* strain (*B. longum* OLP-01) treatment for 3–4 weeks also improved muscle mass and swimming endurance in young mice without any training intervention.[100,102] Lastly, heat-killed *Bifidobacterium breve* B-3 supplemented for 4 weeks improved muscle mass and grip strength in mice, the latter effect being already present after 2 weeks of treatment.[101] Two other probiotics: *Saccharomyces boulardii*[103] and *Veillonella atypica*,[104] have also been tested in rodents and improved VO$_2$max/aerobic performance and exhaustive treadmill running time, respectively.

Metabolically, at the muscle level, the increased performance can be explained by an increased number of oxidative type I fibres,[99] increased glycogen content,[100,102] and decreased fatigue-associated variables (plasma lactate, ammonia, creatine kinase, and blood urea).[99,100,102] The observed decreased plasma lactate levels and increased blood glucose after exercise[100,102] may be explained by an improved hepatic

Table 2 Studies investigating the effects of probiotics on muscle mass and function in young and healthy rodent models

Reference	Model	Probiotic	Dosage	Results
(Chen et al., 2016)	ICR mice (6 weeks)	*Lactiplantibacillus plantarum* TWK10	6 weeks (10^8 or 10^9 CFU/day)	↑ gastrocnemius mass ↑ type I fibres (gastrocnemius) ↑ grip strength ↑ swimming endurance ↓ markers of fatigue (plasmatic lactate, ammonia, and creatine kinase after exercise)
(Lee et al., 2019)	ICR mice (8 weeks)	*Bifidobacterium longum* OLP-01	3 weeks (10^8 or 10^9 CFU/day)	↑ grip strength ↑ swimming endurance ↑ glycogen in muscle ↓ markers of fatigue (plasmatic NH$_3$, creatine kinase, and blood urea nitrogen after exercise)
(Lee et al., 2020)	ICR mice (6 weeks)	*Ligilactobacillus salivarius* subsp. *salicinius* SA-03	4 weeks (10^9 or 10^{10} CFU/day) of gavage	↑ grip strength ↑ swimming endurance ↑ glycogen in muscle ↓ markers of fatigue (plasmatic lactate, ammonia, creatine kinase, and blood urea nitrogen after exercise)
(Toda et al., 2020)	Sprague–Dawley rats and C57BL/6J mice (8 weeks)	Heat-killed *Bifidobacterium breve* B-3	4 weeks (10^9 CFU/day)	↑ soleus mass ↑ grip strength (mice) ↑ mTOR pathway (pAKT and pS6K) ↑ p-AMPK and PGC-1α (rats)
(Soares et al., 2019)	Wistar rats (11 weeks)	*Saccharomyces boulardii*	10 days (10^8 CFU/day)	↑ aerobic performance, ↑ time to fatigue, ↑ maximum speed attained, and ↑ external work
(Scheiman et al., 2019)	C57BL/6 mice (12 weeks)	*Veillonella atypica*	2 weeks (10^9 CFU/day)	↑ exhaustive treadmill runtime ↑ lactate utilization and Cori cycle efficiency ↑ circulating SCFAs

Table 3 Studies investigating the effects of probiotics on physical performance and muscle recovery after training in humans

		(A) Physical performance		
Reference	Model	Probiotic	Dosage	Results
(Shing et al., 2014)	Trained male runners (27 ± 2 years) N = 10	Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, L. casei, Lactiplantibacillus plantarum, Limosilactobacillus fermentum, Bifidobacterium lactis, B. breve, B. bifidum, and Streptococcus thermophilus	4 weeks (10^9 CFU/day)	↑ runtime to fatigue in the heat
(Marshall et al., 2017)	Male and female endurance runners (23–53 years) N = 32	L. acidophilus CUL-60, L. acidophilus CUL-21, B. bifidum CUL-20, B. animalis subsp. lactis CUL-34, and fructooligosaccharides + glutamine	12 weeks (10^9 CFU/day for each strain)	No increase of exercise performance during an extreme marathon in the heat
(Huang et al., 2019a)	Amateur runners N = 16	Lactiplantibacillus plantarum TWK10	6 weeks (10^{10} CFU/day)	↑ muscle mass ↑ endurance performance
(Toohey et al., 2020)	Collegiate female athletes (20 ± 1 years) N = 23	Bacillus subtilis DE111	10 weeks (10^9 CFU/day)	No increase of exercise performance (muscle strength and power, and agility) ↓ body fat mass
		(B) Muscle recovery		
Reference	Model	Probiotic	Dosage	Results
(Gepner et al., 2017)	Elite male soldiers (20 ± 0.7 years) N = 17	Bacillus coagulans GBI-30, 6,086 + hydroxymethylbutyrate calcium (CaHMB) 3 mg/day	40 days (10^9 CFU/day)	↑ muscle integrity [↓ apparent diffusion coefficient (ADC) for the rectus femoris (RF)] with the combination of probiotic and CaHMB compared with CaHMB alone
(Komano et al., 2018)	Male athletes (19–21 years) N = 51	Heat-killed Lactococcus lactis JCM 5805	13 days (10^{11} CFU/day)	↑ physical condition ↓ cumulative days of fatigue ↓ cumulative illness days (common upper respiratory tract infection) ↓ articular pain No decrease of markers of muscle damage
		(C) Muscle recovery + physical performance		
Reference	Model	Probiotic	Dosage	Results
(Lu et al., 2006)	Untrained male and female students (19–23 years) N = 16	Spirulina platensis	3 weeks— 15 g soy or spirulina/day	↑ time to exhaustion ↑ antioxidant capacities (blood glutathione peroxidase, SOD, and malondialdehyde lactate dehydrogenase) ↓ fatigue markers (serum lactic acid)
(Cox et al., 2010)	Elite male distance runners (27 ± 6 years) N = 20	Limosilactobacillus fermentum VRI-033 (PCC)	4 months (10^{10} CFU/day)	No increase of exercise performance (VO$_2$max and running time) Tendency to ↓ systemic inflammation (↓ IFN-γ) ↓ number of total illness days (common upper respiratory tract infection)
(West et al., 2011)	Competitive male and female cyclists (35 ± 9 years) N = 99	Limosilactobacillus fermentum VRI-033 (PCC)	11 weeks (10^{10} CFU/day)	No increase of exercise performance (VO$_2$max and training performance) ↓ systemic inflammation (↓ IFN-γ, TNF-α, IL-6, GM-CSF, and IL-1ra)
(Jäger et al., 2016)	Recreationally trained men (21 ± 3 years) N = 29	Bacillus coagulans GBI-30, 6086 + casein	2 weeks (10^9 CFU/day)	Tendency to ↑ anaerobic power (vintage peak power) Tendency to ↓ muscle soreness (72 h post-exercise) ↑ perceived recovery (24 h post-exercise) Tendency to ↓ muscle damage markers (creatine kinase) With the combination of probiotic and casein compared with casein alone
(Carbuhn et al., 2018)	Collegiate female swimmers (age?) N = 16	Bifidobacterium longum 35624	6 weeks (10^9 CFU/day)	No increase of exercise performance (swim time, force, and aerobic and anaerobic performances)

(Continues)

Table 3 (continued)

(C) Muscle recovery + physical performance				
Reference	Model	Probiotic	Dosage	Results
(Ibrahim *et al.*, 2018)	Healthy young men with a sedentary lifestyle (19–26 years) N = 21	*Lactobacillus acidophilus* BCMC 12130, *Lacticaseibacillus casei* BCMC 12313, *Lactobacillus delbrueckii* subsp. *lactis* BCMC 12451, *Bifidobacterium bifidum* BCMC 02290, *B. infantis* BCMC 02129, and *B. longum* BCMC 02120	12 weeks (10^{10} CFU/day)	No modulation of inflammation and immunity No increase of muscle performance (strength and power) Tendency to ↓ systemic inflammation (↑ IL-10)
(Townsend *et al.*, 2018)	Male baseball athletes (20 ± 1 years) N = 25	*Bacillus subtilis* DE111	12 weeks (10^{9} CFU/day)	No increase of exercise performance (muscle strength and power, agility, and anaerobic fitness) and body composition ↓ systemic inflammation (↓ TNF-α)
(Hoffman *et al.*, 2019)	Elite male soldiers (19–21 years) N = 16	*Bacillus coagulans*	2 weeks (10^{9} CFU/day)	↑ mean jump power ↑ simulated casualty drag ↓ systemic inflammation (↓ TNF-α and IFN-γ and ↑ IL-10)
(Huang *et al.*, 2019b)	Triathletes N = 16	*Lactiplantibacillus plantarum* PS128	3–4 weeks (10^{10} CFU/day)	↑ peak anaerobic power and mean power ↑ VO₂ max endurance ↓ systemic inflammation (↓ TNF-α and IL-6 and ↑ IL-10) ↓ fatigue index (bike) ↓ fatigue markers (creatine kinase index and kidney injury after exercise)

gluconeogenesis from lactate (Cori cycle) as well as an increased lactate catabolism in oxidative muscle fibres (larger number of type I fibres). In addition, Lee *et al*. showed that animals' physical performances and metabolic parameters responded to the probiotic supplementation in a dose-dependent manner and such a concept should require further investigation.[100,102]

These metabolic adaptations are explained by a stimulation of the AKT and AMPK phosphorylation and increased expression of PGC-1α, as shown following 4 weeks of supplementation with heat-killed *B. breve*.[101] As an increased VO₂max as well as muscle oxidative capacity and glycogen content following exercise[99,100,102,103] are observed, an increased insulin sensitivity can be hypothesized in the probiotic-supplemented animals that explain improved performances.[105] Other mechanisms of action of probiotics are their anti-inflammatory role.[99,103] However, such mechanisms of action remain to be demonstrated in normal or exercised animals. Indeed, in the field of muscle performance, if studies exist on the impact of probiotics on muscle mass and function/performance (i.e. the primary goal), the mechanisms of action are less investigated.

Humans

The improvement of muscle mass and function in athletes or soldiers via probiotic supplementation, in addition/combination to already existing strategies, is more documented (*Table* 3). Initially, the studies were mainly focused on the immunomodulatory and anti-inflammatory properties of the probiotics to manage or prevent infections and digestive discomfort in athlete populations.[106] Recently, the purposes of probiotic supplementations have drifted on improvement of physical performance.[107]

Probiotic supplementation in athletes or soldiers was studied to address two distinct issues: (i) improvement of muscle performance (run speed and time to exhaustion, and muscle strength and power) and/or (ii) limitation of exercise-induced muscle damage after the practice of intense training. Supplementation of several probiotic strains (*Spirulina platensis* or *L. plantarum* or *Bacillus coagulans* or a multi-strain probiotic) a couple of weeks improved exhaustive endurance compared with placebo group.[108–111,S38] In parallel, physical performance of elite soldiers determined by several tests including the mean jump power (evaluation the strength and velocity of each jump) and the simulated casualty drag (consists of walking fast 50 m by dragging a bag of 48 kg) was improved with *B. coagulans* supplementation for 2 weeks.[110] However, the probiotic supplementation did not improve performance following the other proposed exercises (60 s pull-ups and 100 m shuttle run). Other authors investigated the effects of *L. fermentum*, *Bacillus subtilis*, and *B. longum* supplementations, but they did not mention any significant effects on physical performance.[112–118] This does not allow us to conclude on a clear beneficial effect of probiotic supplementations in human muscle performance. This apparent discrepancy in the results of the experiments can be explained by various reasons. It is clear that the studies vary greatly in terms of type of athletes (from amateur runners to

elite), gender, and sport practised (cycling, running, triathlon, and soldiers) and also the strain of probiotic used, duration of treatment (from 2 weeks to 4 months), and number of participants selected in the study.[108,117] Indeed, as said previously, the impact of exercise on microbiota could vary greatly depending on the intensity of exercise. Consequently, the beneficial effect of probiotics may also vary depending on the population targeted and the intensity of the exercise they practice.[111,113,115–118]

In the same studies discussed earlier, the report is clearer concerning the anti-inflammatory properties of probiotics.[115,117] Supplementation with *L. fermentum*, *L. plantarum*, *B. coagulans*, and *B. subtilis* reduces the production of circulating pro-inflammatory cytokines (e.g. TNF-α, IL-6, and IFN-γ) and/or increases the production of anti-inflammatory cytokines such as IL-10.[109,110,112,113] Finally, some probiotic strains (*Spirulina platensis*, *B. coagulans*, and *L. plantarum*) have been shown to decrease muscle fatigue markers (e.g. creatine kinase and serum acid lactic) and thus could promote muscle recovery after exercise.[109,119,S38] Hence, a combination of targeted markers of muscle performance and fatigue with muscle performance tests should be systematically considered to better evaluate the potential beneficial impact of probiotics in these populations.

Conclusions—perspectives

To date, probiotic strains (and mix of strains) have been proven efficient to improve muscle mass and function in rodents in both anabolic and catabolic situations. When translated to humans, the picture is less clear because of the scarcity of studies, high variability of targeted populations, and/or difficulties to measure accurately and repeatedly muscle mass and function. A decreased efficiency of response of humans to probiotics tested in animal models cannot be excluded. So far, many studies have used LAB and bifidobacteria, but currently, it is possible, through a reverse engineering approach, to isolate bacterial strains from a particular human microbiota (e.g. athletes) so that supplementation with these

probiotic strains reproduces some phenotypic characteristics of the donor. Similarly, the use of strict anaerobic bacteria such as *Faecalibacterium* or *Roseburia* strains, which are under-represented in catabolic situations, could represent an interesting alternative strategy in this context. A mixture of probiotics or even FMT (transfer of a 'complete' microbiota) should be also investigated in the near future as they could settle more efficiently and durably the host microbial ecosystem. Finally, to improve muscle and host microbiota functions, probiotic strains could be combined with other nutritional factors that target microbiota (e.g. prebiotics and polyphenols) and the muscle (e.g. proteins and energy) to optimize the effects. The gut microbiota–muscle axis thus offers a wide range of research opportunities.

Conflict of interest

None declared.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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