Oxidative Medicine and Cellular Longevity

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Lead Guest Editor: Ferdinando Franzoni
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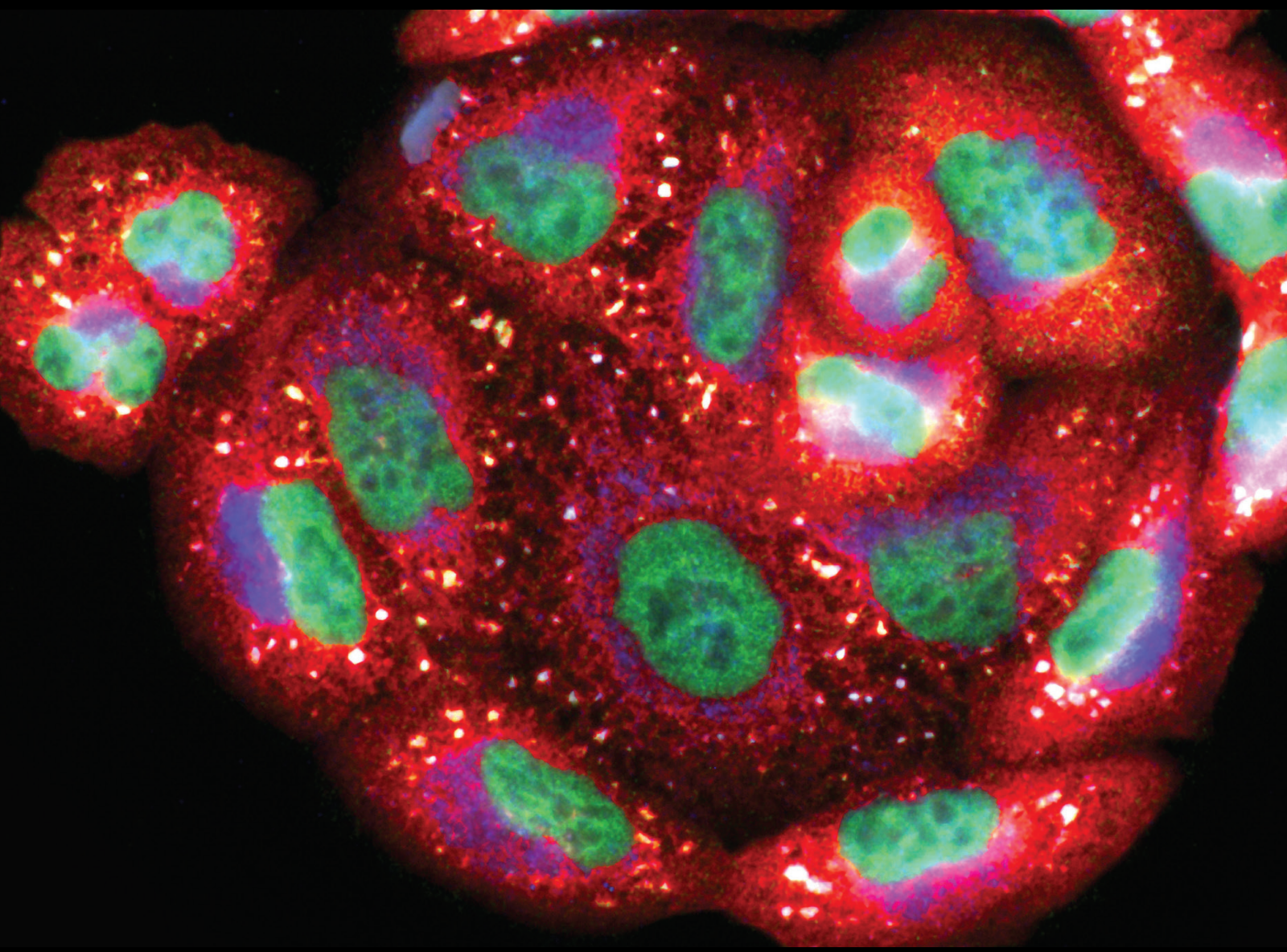

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*Review Article*

# A Minireview Exploring the Interplay of the Muscle-Gut-Brain (MGB) Axis to Improve Knowledge on Mental Disorders: Implications for Clinical Neuroscience Research and Therapeutics

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What benefit might emerge from connecting clinical neuroscience with microbiology and exercise science? What about the influence of the muscle-gut-brain (MGB) axis on mental health? The gut microbiota colonizes the intestinal tract and plays a pivotal role in digestion, production of vitamins and immune system development, but it is also able to exert a particular effect on psychological well-being and appears to play a critical role in regulating several muscle metabolic pathways. Endogenous and exogenous factors may cause dysbiosis, with relevant consequences on the composition and function of the gut microbiota that may also modulate muscle responses to exercise. The capacity of specific psychobiotics in ameliorating mental health as complementary strategies has been recently suggested as a novel treatment for some neuropsychiatric diseases. Moreover, physical exercise can modify qualitative and quantitative composition of the gut microbiota and alleviate certain psychopathological symptoms. In this minireview, we documented evidence about the impact of the MGB axis on mental health, which currently appears to be a possible target in the context of a multidimensional intervention mainly including pharmacological and psychotherapeutic treatments, especially for depressive mood.

## 1. Introduction

From a historical point of view, the pivotal role of the gut microbiota on an individual's health was first conceived by the Russian biologist E. Metchnikoff, who described some health benefits in a population of poor Bulgarians connected to the consumption of lactic acid bacteria in fermented milk [1].

On one side, *microbiota* refers to a specific population of organisms (i.e., bacteria, yeasts, and parasites) colonizing the skin, the respiratory, the uro-genital, and the gastrointestinal tract, where the majority of the population lives. The human gut is a complex, dynamic, and heterogeneous system which exert a marked influence on the host during homeostasis and disease. It contains $10^{13}$-$10^{14}$ microorganisms, and its weight is about one kilogram in the adult, with the majority of bacteria residing in the colon [2]. Through physiological functions, the microbiota can offer specific benefits to the host, such as strengthening gut integrity or shaping the intestinal epithelium, harvesting energy, protecting against pathogens, and regulating immunity [3]. In healthy adults, two bacterial phyla, *Bacteroidetes* and *Firmicutes*, dominate the gut bacterial composition, with smaller amounts of *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [2].

Alterations that affect the commensal flora impair microbial homeostasis and generate a condition called "*dysbiosis*"; particularly, gut *dysbiosis* is characterized by a significant decrease of *Bacteroidetes* and *Lactobacilli* [4]. In a similar way, *Lactobacillus* abundance is predominant in other body districts, including vagina and endometrium [5], and even in the latter, eubiosis exists if the percentage of endometrial *Lactobacilli* is greater than 90% [6].

On the other side, the *microbiome* consists of the genes that microbial cells harbor [7]. It comprises all the genetic material within a microbiota, the whole collection of microorganisms in a definite *situs*, in such a case, the human gut. This has been defined by some researchers as the "*metagenome of the microbiota*", too [8].

Evidence from literature documented that the alteration of the native microbial intestinal florae is being invoked in nutrition, human metabolism, direct host defense, immunological development, physiological and pathological aging, and even psychiatric disorders [9]. Starting from this assumption, microbiota manipulation may represent a promising tool as adjunct therapy for treating specific mental illnesses and their associated symptoms [10].

Moreover, the impact of the gut microbiota on skeletal muscle function and quality in terms of energy, neuromuscular connectivity, mitochondrial function, and endocrine and insulin resistance, has recently been the focus of some research attempts [11]. The gut microbiota may represent a challenging new therapeutic opportunity and advances in the field of exercise science may enrich the heritage of clinical neuroscience applied to psychiatric disorders. Studies reporting experiments on the gut microbiota intervention documented that specific probiotics have the potential to interact with the brain and exert a positive bacteria-mental functioning relationship [12]. Altered gut microbial profiles have been described in several psychiatric disorders and psychobiotics are currently employed as adjunct treatment to pharmacological and psychotherapeutic interventions. Many of these effects appear to be specific, suggesting a potential role of certain probiotic strains. Further, physical exercise inducing microbial changes with release of neuroendocrine factors may lower inflammatory and oxidative stress of the brain [13].

This mini-review briefly summarizes the progress of research on the muscle-gut-brain (MGB) axis highlighting the role of psychobiotics and physical activity in modulating the response of the microbiota and its effects on mental health, and discusses implications for clinical neuroscience research and therapeutics.

## 2. The MGB Axis: Communication Links and Role of Physical Activity in the Mutual Relationship between the Gut and the Skeletal Muscles

As well as regulating brain functions, the gut microbiota affects the skeletal muscle functioning. The graphical representation (Figure 1) depicts gut eubiosis and dysbiosis. In particular, intestinal *eubiosis*, conceived as the balance of the intestinal microbial ecosystem, favors the integrity of the gut barrier and prevents the translocation of liposaccharides (LPS) and other harmful products in the bloodstream, with positive effects on systemic inflammation which could alter muscle metabolism [14–16]. On the other hand, intestinal *dysbiosis*, an ecosystem where "*good*" and "*bad*" bacteria do not live in mutual harmony, [1] is also responsible for a decreased activation of AMPK (i.e., AMP-activated protein kinase) and PGC-1$\alpha$ (i.e., proliferator-activated receptor coactivator-1) signaling pathways, which are at the basis of autophagy mechanisms. Autophagy, in fact, is fundamental for the skeletal muscles to remove older organelles and myocytes and to preserve muscle functions [17]. Moreover, an impaired autophagy stimulates inflammation and oxidative stress that negatively affects muscle vitality [18].

An altered gut microbiota also affects insulin-like growth factor-1 (i.e., IGF-1) release. IGF-1 usually activates phosphatidylinositol 3-kinase (i.e., PI3K-AKT) signaling pathway that inhibits mRNA transcription and muscle protein synthesis [19]. In murine models, the lack of a gut microbiota decreases levels of IGF-1 reducing the transcription of genes fundamental for efficient mitochondrial functions within the skeletal muscles [20]. Therefore, intestinal dysbiosis promotes inflammation, oxidative stress, and alters muscle anabolism and mitochondria impairing muscle vitality [11].

In recent years, the interaction between the gut microbiota and the muscles has been receiving considerable attention from the scientific community [21]. It is now well established that the integrity of the muscular system correlates with regular physical activity. On the basis of such evidence, an attempt has been made to establish how the intestinal microbiota may influence the muscular system, or whether physical activity may lead to intestinal eubiosis or dysbiosis.

The positive interaction between physical activity and the gut microbiota is highlighted by the studies of Santacroce et al. [22] and Manders et al. [23], in which it is observed that a low amount of physical activity can induce a reduction in the risk of colon cancer, diverticulosis, and irritable bowel syndrome (IBS). These results are confirmed in the study of Monda et al. [24] documenting how regular physical activity reduces inflammation in the intestine. In their studies, Petersen et al. [25] and Scheiman et al. [26] showed that athletes have a greater biodiversity of the fecal microbiota and also a presence of mycobacterium correlated with the health status. Physical exercise modulates not only the expression of the gut microbiota in terms of microorganisms, but also the production of immunoglobulin A (i.e., IgA) and the reduction of B-cells and T-CD4 in murine models. Such modifications suggest that the gut microbiota also has immunomodulatory functions [27]. However, prolonged and strenuous exercise increases intestinal permeability. Such a mechanism causes a passage of the bacteria from the colon with the consequent risk of gastrointestinal problems [28]. When analyzing the scientific literature, it is always difficult to understand which type of physical activity (e.g., endurance exercise, resistance training exercise, acute or chronic exercise sessions, etc.) induces better changes [29]. Endurance exercise, that is a kind of cardiovascular exercise performed over a prolonged

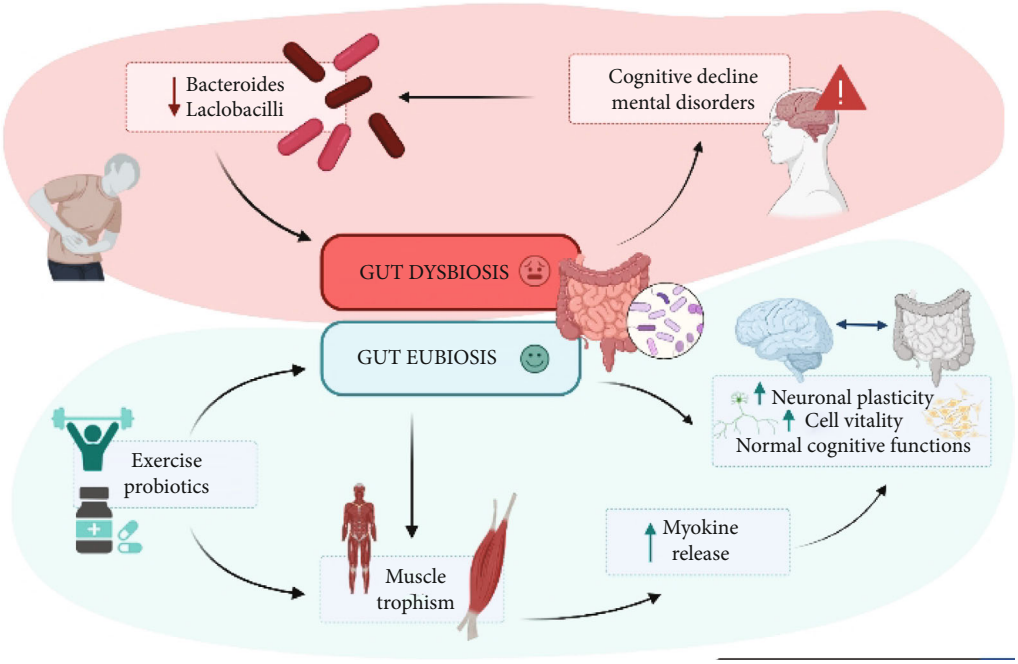


Figure 1: A representation of the gut eubiosis/dysbiosis effects on brain and muscle activities.

period of time [30], induces a number of major adaptations such as capillary neogenesis, mitochondrial biogenesis, and increased cardiofitness. In addition, endurance training increases *Lactobacillus*, *Bifidobacterium*, and *Blautia coccoides-Eubacterium rectale* species, while a decrease of *Clostridium* and *Enterococcus* has been found in a rat model [31].

Clarke et al. [32] showed that athletes (i.e., rugby players) had greater variability of the gut microbiota than sedentary individuals. The greater variability is the basis for an improved overall health. *Firmicutes* and *Lactobacillales* are two classes of microbes that seem to be affected by positive changes induced by endurance exercise (i.e., ability to last) [33]. Few studies pointing out the relationship between resistance training (i.e., all exercises in which a force is required to overcome a resistance) and the composition of the gut microbiota are present in literature [34]. In a recent study by Castro et al. [35], it was observed that 12 weeks of resistance training promoted the diversity and the composition of the gut microbiota in rats. In the trained group, an abundance of *Pseudomonas* and, in contrast, a decrease in *Serratia* and *Comamonas* were observed. Subsequently, in a study conducted in a human model by Moore et al. [36], it was observed that 6 weeks of resistance training can improve the integrity of the intestinal barrier in a group of elderly subjects by modulating the population of intestinal microbes. In conclusion, it should be noted that the relationship between physical activity and microbiota is inverse. In fact, some studies have shown that a correct composition of the intestinal microbiota (or *eubiosis*) improves athletic performance [37–39]. Indeed, it was observed that sport performance (i.e. endurance swimming) was better in specific pathogens (SPF) and *Bacteroides fragilis* mice than in germ-free mice. This result suggests that the composition of the gut microbiota may be crucial for athletic performance. Moreover, the study also showed a possible improvement of antioxidant systems in SPF mice, linked to an increased plasmatic levels of glutathione peroxidase and catalase [40]. In this regard, it has to be considered that intestinal microbiota exerts beneficial effects on the oxidative stress status; several microorganisms have antioxidant properties since they are able to improve the expression of antioxidant enzymes as well as controlling the release of proinflammatory cytokines [41]. The abundance of *Lactobacillus* species enhances the activity of superoxide dismutase (i.e., SOD), the levels of glutathione and the scavenging activity against hydroxyl radicals [42]. In contrast, *Escherichia coli* and *Enterococcus* abundance make organisms susceptible to oxidative stress damages [43]. Considering the above, in addition to a proper balanced diet, a moderate and regular exercise can modulate microbial species within the gastrointestinal tract, that, in turn, regulate inflammation and oxidative stress, with positive implications both on muscle performance [44] and brain health [43]. Indeed, muscle trophism is fundamental to ensure, in response to exercise, the release of hormone-like molecules called myokines, such as cathepsin B, FNDC5/irisin, and interleukin-6, which are able to regulate mental abilities [45].

With the aim of completing the MBG axis decription, it has to be noted that the gut-brain axis includes the vagus nerve (VN), a mixed nerve composed of 80% afferent and 20% efferent fibers with anti-inflammatory properties and the circumventricular organs (CO), the gut hormone signaling, the immune system, the serotonin, and the tryptophan metabolism and microbial metabolites such as short-chain fatty acids (SCFAs) [46]. The neuroactive compounds released by bacteria, such as the $\gamma$-aminobutyric acid (GABA), the serotonin, the dopamine, and the acetylcholine locally acting within the

enteric nervous system also reaches the brain by blood [47]. Other bacterial metabolites exerting neuroactive functions include long and SCFAs [2] such as acetate, propionate, and butyrate that are important metabolites in intestinal homeostasis maintenance. The existence of a gut-brain axis has been demonstrated in Alzheimer's disease (AD). In a murine model, gut inflammation, enteric dysmotility, and intestinal AD-related protein deposition were found in early stages of the disease [48]. Similarly, Palmitoylethanolamide (PEA), a lipid mediator, has proven to counteract intestinal dysmotility associated to AD. Specifically, PEA is able to prevent glial hyperactivation and the enteric deposition of AD-related proteins, with a decreased inflammatory status [49].

## 3. Psychobiotics and Physical Exercise in Mental Disorders

With regard to psychological well-being, some gastrointestinal diseases have been recognized as triggered by biopsychosocial factors, such as the IBS, often accompanied by depression and anxiety [50], and the inflammatory bowel disease (IBD). These syndromes are influenced by an individual's stress response because of the stimulation of the sympathetic nervous system and the inhibition of the vagus [2]. Stress, anxiety, and depressed mood may be manipulated by the gut microbiome [51]. Accordingly, a double-blind randomized controlled trial (RCT) on volunteers receiving a probiotic (i.e., Probio-Stick) containing *Lactobacillus acidophilus* and *Bifidobacterium longum* during a 3-week period significantly reduced stress-induced gastrointestinal symptoms (i.e., abdominal pain and nausea/vomiting). Another RCT documented multiple benefits of *Lactobacillus plantarum* assumed $1 \times 10^9$ cfu/day for 12 weeks in terms of reduced stress and anxiety [52]. The use of 24 billion cfu *Lactobacillus casei* strain Shirota (LcS) for 2 months was also shown to reduce anxiety symptoms in patients with chronic fatigue syndrome [53].

Altered gut microbial profiles have been found in some medical conditions, including psychiatric disorders [9]. Differently from healthy subjects, an increased bacterial diversity in feces of autistic children consisting of *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Firmicutes* has been found [54]. A recent systematic review concluded that major depressive disorder, bipolar disorder, and schizophrenia were not characterized by differences in the number or distribution (i.e., $\alpha$-diversity) of gut bacteria but display compositional differences compared to controls (i.e., $\beta$-diversity) [55]. Further, dysbiotic alterations of the gut microbiota may lead to local inflammation and increased permeability of the gastrointestinal wall leading to an augment of liposaccharides (LPS) circulation. They activate the production of systemic inflammation mediators (i.e., IL-1$\beta$, IL-6, IL-8 e TNF-$\alpha$) that have been found to be higher in psychiatric patients, such as those suffering from schizophrenia [56]. High levels of IL-6 and TNF-$\alpha$ were also found in patients with bipolar disorder during both mood alterations and euthymic phases [56]. The phenomenon known as "*leaky gut*" has been proposed to shed light on major depressive disorder (MDD), too, as a proinflammatory response induced by external and internal stressors and by an increased translocation of the LPS from gram-negative bacteria [57].

Psychobiotics include a range of substances that may affect the gut-brain axis signaling, including probiotics (i.e., living microorganisms contained in food products or supplements), prebiotics (i.e., the substrate used by the host organism conferring health benefits), synbiotics (i.e., a combination of probiotics and prebiotics), and postbiotics (i.e., metabolites of bacterial fermentation and bioactive compounds) [58]. Specifically, probiotics have some effects in ameliorating certain psychopathological symptoms by improving intestinal homeostasis. Their supplementation may serve in adaptation to exercise as aiding muscle recovery and supporting skeletal muscle [59]. Akkasheh et al. [60] found a decreased Beck Depression Inventory (BDI) total score after complementary treatment with probiotic administration (i.e., *Lactobacillus acidophilu, Lactobacillus casei,* and *Bifidobacterium bifidum*, $2 \times 10^9$ cfu/g) for 8 weeks in patients with MDD. Similar results on the same psychodiagnostic scale were reached by Kazemi et al. [61] by using a formula containing freeze-dried *Lactobacillus helveticus* and *Bifidobacterium longum* at a dosage of ten billion colony-forming units (i.e., $\geq 10 \times 10^9$ CFU) per 5 g. sachet on an 8-week treatment. Further, a change in the 17-item Hamilton Depression Rating Scale score and BDI score from baseline to week 8 were found after an adjunctive therapy of *Clostridium butyricum* MIYAIRI 588 in patients with treatment-resistant MDD [62]. Finally, substantial shifts to the microbial community in response to dietary patterns may cause important health implications, as reported in attention deficit hyperactivity disorder [63].

Beyond probiotics assumption, physical exercise has been shown to be a significant factor causing changes in qualitative and quantitative composition of the gut microbiome [64]. Specifically, studies reported that exercise may have positive effects on gut microbiota increasing butyrate-producing bacteria (i.e., *Roseburia hominis*, *Faecalibacterium pausnitzii*, and *Ruminococcaceae*), for diversity and balance between beneficial and pathogenic bacterial communities, and colon health [65, 66]. Moderate intensity physical exercise (i.e., <70% VO2max) provide beneficial effects to the human body, thanks to physiological and metabolic adaptations, with changes in skeletal muscle including mitochondrial biogenesis, concentration of the substrate transporting proteins, activity of the enzymes involved in metabolic pathways, and glycogen storage in the muscle [67] whereas intensive physical exercise (i.e., >70% VO2max) may disturb the homeostasis of the gut microbiota [13] by increasing gastrointestinal wall permeability and by diminishing the gut mucus thickness, potentially favoring pathogens to enter the bloodstream, thus increasing inflammation levels [29]. A parallelism can be drawn with regard to physical activity and mood, because moderate exercise has been shown to be useful in supporting affective state while intense exercise may lead to its deterioration [68]. An adequate level of physical activity increases the synaptic transmission of monoamines, releases endorphins, and improves positive emotions experienced after the exercise [68]. A recent systematic review has shown that combined resistance and aerobic training or aerobic training alone may

have positive effect on the microbiota, incrementing some bacteria phyla (i.e., *Bacteroidetes, Firmicutes, and Proteobacteria*) although further research with higher methodological rigor is needed to better understand such a relationship [9]. Studies on physical activity in clinical samples pointed out that it can normalize reduced levels of brain-derived neurotrophic factor (i.e., BDFN), with neuroprotective effects on the brain while other investigations have documented anxiolytic effects of aerobic exercise for induced-panic symptoms [69]. In addition, the aforementioned effects of physical activity on the gut microbiota suggest that the better the composition of the microbiota, the greater the capacity for nutrient degradation. Greater nutrient degradation results in both greater macronutrient availability and glycemic control [70]. All these effects have an impact on the neuronal activity. For example, it has been demonstrated that athletes present an enriched profile of SCFAs (especially, acetate, propionate, and butyrate), due to the specific activity of the microbacteria modulated by physical activity [66]. Subsequently, the produced SCFAs act as a nutritional substrate to support microglia function and this leads to an improvement in mental abilities [71].

## 4. Conclusion and Implications for Clinical Neuroscience Research and Therapeutics

The exact composition of the gut microbiota is different for each individual, and it is still unclear what may constitute a healthy profile. Determining a healthy microbiota should be a prerequisite for evaluating clinical deviations and proceeds towards tailored interventions. Such a kind of observation can be taken into consideration by clinicians to study in-depth the modification of the microbiota, also in the case of psychotropic medication orally taken [72, 73]. Alterations of the gut microbiota composition have been found in some psychiatric disorders but heterogeneity in terms of ethnicity, age, comorbidities, medication, unhealthy nutrition, antibiotics use, aging, and environmental factors, complicates a definite description [74, 75]. All these factors should be considered when planning a study on the microbiota and interpreting results. The probiotics could be useful when ingested in a definite quantity through the interaction with commensal gut bacteria and their benefits are mediated by several mechanisms referred to the hypothalamic-pituitary-adrenal (i.e., HPA) axis, the immune response and inflammation, and the production of neurohormones and neurotransmitters [76]. The rebalancing of a dysbiotic flora through the use of psychobiotics represents a therapeutic goal as a complementary intervention to standard care, especially for depressive symptoms [77, 78], even if additional RCTs in clinical populations are warranted to better evaluate their efficacy. Further, the stimulation of the vagus nerve is also recognized as an effective neurophysiological treatment in depression [79] because of the possibility to alter the cerebrospinal fluid concentration of neurotransmitter or their metabolites (e.g., GABA, and 5h1AA), and influence the functionality of certain brain regions that are dysregulated in mood disorders (i.e., orbitofrontal cortex, insula, thalamus and hypothalamus, and cingulate and hippocampus) [80]. Food hygiene and probiotics supplementations should be carefully taken into account as an integrative aspect of a multidimensional intervention on psychiatric disorders, due to the fact that many pathologies report unbalanced diet (e.g., consumption of highly saturated fats and sugar, low fiber intake, etc.) or difficulties in weight management, potentially impacting microbiota profile [81]. To this end, psychoeducational interventions focused on balanced diet adherence for a healthy lifestyle may improve quality of life of psychiatric patients, and nutritional psychiatry should be called into question with the final aim of improving clinical outcomes of standard treatments.

Evidence of positive effects of physical activity in mental disorders are limited to date. Nevertheless, outdoor activities are associated with greater feelings of revitalization, increased energy and positive engagement with tension, confusion, and anger decrease [82] and should be considered in structured psychotherapeutic protocols for depression, such as cognitive-behavioral ones implementing motor activation [83, 84]. Physical exercise further improves behavioral outcomes in psychiatric disorders by psychological mechanisms of body scheme reinforcement, changes in health attitudes, greater awareness in proprioception, and counteracts inactivity as a typical feature of patients with depression [85]. However, physical exercise as a psychosocial additional intervention for psychiatric disorders needs to be better investigated by rigorous RCTs [86] because of paucity and methodological limitations of the existing studies.

In the opinion of the authors, evidence on probiotics supplementation and physical activity in depressed mood treatment as adjunctive strategy in the context of a multidimensional intervention including pharmacology and psychotherapy is somewhat interesting. However, advances on MGB axis research have to be carefully integrated with clinical data derived from blood tests, neuropsychological and psychodiagnostic measures, and functional status examination, to better depict the relationship among the microbiota, the brain, and the musculoskeletal system.

## Conflicts of Interest

The authors declare no conflict of interest.

## Authors' Contributions

D.M.C. was responsible for the conception of the work. D.M.C. J.F. and G.S. wrote the manuscript. F.F. S.D. and G.C. revised it critically.

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*Research Article*

# Clinical Effect of Abdominal Massage Therapy on Blood Glucose and Intestinal Microbiota in Patients with Type 2 Diabetes

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The aim of the study was to investigate the clinical effects of abdominal massage on patients with type 2 diabetes mellitus (T2DM) and its influence on the intestinal microflora. We conducted a randomized, controlled clinical trial. A total of 60 patients with T2DM, who met the inclusion criteria, were randomly allocated to the control group, the routine massage group, and the abdominal massage group. The control group received health education and maintained their hypoglycemic drug treatment plan. The routine massage group and the abdominal massage group received different massage interventions. In addition to glucose and lipid metabolism indicators, we quantitatively analyzed the gut microbiota to assess the effects of massage on the intestinal microflora of patients with T2DM. Compared with the control group, the abdominal massage improved levels of glycated hemoglobin, total cholesterol, Enterobacter, and Bifidobacteria with significant differences ($P = 0.02$, $P = 0.03$, $P = 0.03$, and $P = 0.03$). The comparison within group showed that the levels of the four bacterial genera in the abdominal massage group revealed significant differences before and after treatment ($P = 0.006$, $P < 0.001$, $P < 0.001$, and $P = 0.002$). The comparison between the routine massage group and the abdominal massage group was not significantly different in all levels of test indices. The abdominal massage group regulated levels of Enterobacter and Lactobacilli to a greater extent than the routine massage group. Additionally, abdominal massage decreased Enterococcus levels. The results of this study showed that abdominal massage has clinical advantages over routine massage. Specifically, this intervention may correct microflora disturbances to a certain extent.

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic endocrine disease characterized by insulin resistance and insufficient insulin secretion. As a global public health concern, approximately 537 million adults had DM in 2021. The prevalence of DM in obese people continues to increase [1]. However, once patients are diagnosed with T2DM, they can only be treated with long-term maintenance hypoglycemic drugs. In view of the side effects of hypoglycemic drugs, massage therapy, which is safe, effective, and less toxic with few or no side effects, represents a valuable treatment option [2].

Conventional massage consists of regular and rhythmic movements of the therapist's hands on body tissues, including nerves and muscles, to achieve certain goals. Several studies [3–5] have shown that massage improves glucose and lipid metabolism disorders by regulating muscle, inflammatory factors, and pancreatic islet function. Compared with routine massage, abdominal massage is not only easy to perform, but can also improve gastrointestinal function and lipid metabolism [6–9].

Intestinal microflora disorders and abnormal glucose and lipid metabolism are important etiological factors in T2DM [10]. Recent studies show that inflammatory markers are correlated with the diabetic control indices, i.e., glycated

hemoglobin (HbA1c) levels in the diabetic population [11]. Not only DM but also its complications such as diabetic nephropathy, frailty, and proteinuria are associated with inflammation [12–14]. On the other hand, the intestinal microbiota has a close relationship with inflammation [15]. Once the intestinal microbiota is imbalanced, it will generate systemic inflammation, which is the characteristic of DM and its complications.

Abdominal massage affects gastrointestinal responses by stimulating parasympathetic nerves [16], but its effectiveness and specific mechanism in the treatment of DM are not clear. It is possible that abdominal massage may modulate the composition of the gut microbiome, thereby affecting metabolism. The objective of this study was to assess the safety and therapeutic effects of abdominal massage in T2DM patients through clinical observation and its effects on the intestinal microflora. Our study findings will provide a basis for understanding the mechanism of abdominal massage in metabolic diseases and the relationship between skeletal muscle movement and the intestinal microflora.

## 2. Materials and Methods

*2.1. Design.* We performed the randomized clinical study following the principles of the Declaration of Helsinki. The study took place between September 2020 and February 2022 after registering it in the Chinese Clinical Trial Registry and obtaining approval from the Ethics Committee of the affiliated hospital of Nanjing University of Traditional Chinese Medicine (2019-210-KY). The trial registration number was ChiCTR2000031688. The subjects were informed about the objectives of the study and provided signed informed consent.

*2.2. Participants.* We recruited 60 patients with T2DM from the massage department of the affiliated hospital of Nanjing University of Traditional Chinese Medicine and the outpatient department of Nanjing Jiqingmen Hospital. We randomly allocated the patients to one of three groups (a control group, a routine massage group, or an abdominal massage group) in a 1 : 1 : 1 ratio. Four patients were lost to follow-up, and two patients were excluded from the study. Therefore, a total of 54 subjects completed the eight-week treatment and follow-up. There were no significant differences in age, course of disease, gender, body weight, or BMI among the three groups ($P > 0.05$; Table 1). Due to the particularity of using conventional hypoglycemic drugs in the control group, it was impossible to blind the subjects and therapists. However, throughout the trial, the data managers, analysts, and evaluators remained blinded to groups to minimize possible confusion of the trial results.

*2.2.1. Inclusion Criteria.* Patients with all of the following conditions were included in the study: those who meet the diagnostic criteria for patients with T2DM based on the World Health Organization (WHO) diagnostic criteria, the patient is between 35 to 80 years of age, those who agree to the interventions and provide signed informed consent, the patient had not received massage therapy for T2DM during the last 2 weeks, the patient did not participate in other ongoing clinical studies, and the drug regimen for T2DM is metformin alone. Medication for other underlying diseases that are not excluded from the exclusion criteria, assessed by the attending physician and in the patient's own opinion, is acceptable without change during the 2-month trial.

*2.2.2. Exclusion Criteria.* Patients with one of the following conditions were excluded from the study: type 1 diabetes, late-onset autoimmune diabetes in adults, gestational diabetes, and other secondary diabetes; diseases affecting the cardiovascular, nervous, digestive, or hematopoietic system; or severe metabolic and organic complications of diabetes, such as ketoacidosis and diabetic nephropathy. Additionally, pregnant or lactating women, patients with alcohol or psychotropic substance abuse, and patients with mental illness were excluded. Similarly, patients who had modified the amount and type of drugs consumed within two months before the trial, those who had participated in other clinical trials in the previous two years, those who had consumed antibiotics or micro-ecological drinks within one month before the trial, and those with a weight change of >5% in the first two months of the trial were excluded.

*2.2.3. Dropout and Elimination Criteria.* The following patients were eliminated from the study: patients who violated the research protocol, who were lost to follow-up, who had additional circumstances that affected the judgment of curative effect, or patients who had poor compliance or withdrew from the study.

*2.3. Interventions*

*2.3.1. Control Group.* Patients were provided with health education, guidance on healthy eating habits, and exercise. The routine hypoglycemic drug treatment plan was maintained. The intervention continued for eight weeks.

*2.3.2. Routine Massage Group.* The treatment was based on "Tuina Therapeutics" by Professor Fan Binghua [17]. Firstly, the patient was in a prone position, and the therapist performed chiropractic sessions five times. The therapist pressed and kneaded the bladder meridian, focusing on BL13, BL20, BL21, BL22, and BL23 for three minutes each. Subsequently, they rubbed BL23 and BL31-34 to diathermy, pinched and pushed from both lower limbs to the Achilles tendon three times, and pressed and rubbed KI1 to diathermy. Secondly, the patient was in a supine position. A one-finger Zen push method was applied to the Ren meridian, focusing on RN15, RN13, RN12, RN6, and RN4. This session was performed three times. The holding method from the front of both lower limbs to the ankle joints was performed twice. The therapist tapped GB34, ST36, and SP6 for one minute each. Lastly, the patient was in a seated position. The therapist kneaded GB20, DU16, and DU20 by thumb for one minute each. Subsequently, they pinched the neck and GB21 for one minute each and tapped the shoulder and back once. The whole procedure lasted

Table 1: Characteristics of participants ($\chi \pm s$).

| Group | Number (male/female) | Age (y) | Duration (y) | Weight (kg) | BMI ($kg/m^2$) |
|---|---|---|---|---|---|
| Control | 17 (7/10) | 64.24 ± 9.24 | 6.88 ± 6.26 | 66.91 ± 8.82 | 25.66 ± 1.12 |
| Routine massage | 18 (9/9) | 67.50 ± 6.72 | 8.11 ± 7.88 | 67.57 ± 6.93 | 25.20 ± 1.25 |
| Abdominal massage | 19 (9/10) | 63.11 ± 6.83 | 6.16 ± 5.59 | 70.27 ± 7.81 | 26.00 ± 2.18 |
| *P* value | 0.87 | 0.21 | 0.67 | 0.40 | 0.33 |

approximately 30 minutes, three times a week, one time every other day, for eight weeks, with rest on Sundays.

*2.4. Abdominal Massage Group.* The selection and manipulation of acupoints in the abdominal massage group were derived from clinical experience. First, the patient laid on their back. The therapist pressed and rubbed RN13, RN12, and RN10 for five minutes each. Second, at 30 r/min, the therapist rubbed the abdomen in a clockwise manner for 15 minutes. Third, the therapist used the one-way holding method from ST21 to ST29 on both sides. Four, the therapist rubbed under the flank to induce diathermy. The whole procedure lasted approximately 30 minutes, three times a week, once every other day, for eight weeks, with rest on Sundays.

*2.5. Test Indices.* The patients fasted after 21 : 00 in the evening. We collected venous blood on an empty stomach at 7 : 00 the next morning once before and after treatment. Fasting blood glucose (FPG) and postprandial blood glucose (PBG) were measured using the glucose oxidase method. High performance liquid chromatography was used to detect glycated hemoglobin (HbA1c). Total cholesterol (TG), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using an automatic biochemical analyzer (HITACHI 3100, China). Fluorescence quantitative PCR was used to detect Enterococcus, Enterobacter, Bifidobacterium, and Lactobacillus. Before and after treatment, fresh fecal specimens from the middle and back sections of all test patients were collected in the morning and quickly stored at −80 °C. DNA extraction was completed within 24 h after sampling. The sample library construction, sequencing, and analysis services were completed by China Shanghai Meiji Pharmaceutical Biotechnology Co, Ltd.

*2.6. Statistical Analysis.* We performed statistical analysis using SPSS22.0 software. The Shapiro-Wilk test was used in the normality analysis of the study. We expressed normally distributed data with homogeneity of variance as mean ± standard deviation (SD). Using the Chi-square test, we analyzed the count data. When the measurement data met the requirements of normal distribution and homogeneity of variance, the paired *t*-test was used for the comparison of the two samples within the group before and after treatment, the independent *t*-test was used for the comparison between the groups, one-way analysis of variance was used for comparison of multiple groups, and the LSD method was used for pairwise comparison between groups. A rank-sum test was used for those who did not meet the requirements, and a nonparametric test was used for comparison of multiple groups of sample data. We set statistical significance at $P < 0.05$.

Table 2: Number of dropouts or eliminated participants.

| Group | Number | Dropouts or eliminated | *P* value |
|---|---|---|---|
| Control | 17 (85.0%) | 3 (15.0%) | 0.57 |
| Routine massage | 18 (90.0%) | 2 (10.0%) | |
| Abdominal massage | 19 (95.0%) | 1 (5.0%) | |

## 3. Results

We randomly allocated 60 patients with T2DM to one of three groups: control, routine massage, or abdominal massage group. Four patients dropped out, and two patients were excluded from the study. Therefore, a total of 54 patients completed the trial. The number of cases in the control, routine massage, and abdominal massage groups was 17, 18, and 19, respectively. The dropout and elimination rates from the three groups were analyzed using the Chi-square test. The rates of dropout and elimination were comparable ($P = 0.57$; Table 2).

*3.1. Baseline Characteristics.* There were no differences in age ($P = 0.87$), disease duration ($P = 0.67$), gender ($P = 0.87$), body weight ($P = 0.40$), and BMI ($P = 0.33$) among the three groups (Table 1).

*3.2. Data Analysis of Efficacy Indicators before and after Treatment*

*3.2.1. Comparison of FBG, PBG, and HbAlc Indexes.* When comparing before and after treatment within a group, we observed that the levels of FBG, PBG, and HbAlc had no statistical differences in the control group and were significantly different in the abdominal massage and routine massage groups (control: $P = 0.07$, $P = 0.09$, and $P = 0.31$; abdominal massage: $P < 0.001$, $P < 0.001$, and $P < 0.001$; and routine massage: $P < 0.001$, $P < 0.001$, and $P < 0.001$). Pairwise comparison between groups showed that there were significant differences between the control group and

Table 3: Comparison of FBG, PBG, and HbAlc values before and after treatment ($\chi \pm s$).

| Groups | FBG (mmol/L) | | PBG (mmol/L) | | HbAlc (%) | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| Control | 8.02 ± 0.96 | 7.70 ± 0.88 | 11.28 ± 1.82 | 10.59 ± 1.86 | 7.20 ± 0.59 | 7.13 ± 0.60 |
| Routine massage | 8.63 ± 1.44 | 7.21 ± 1.08** | 13.02 ± 2.60 | 9.74 ± 1.86** | 7.53 ± 0.98 | 6.68 ± 0.83** |
| Abdominal massage | 8.76 ± 1.63 | 7.08 ± 0.92** | 13.19 ± 3.16 | 9.72 ± 1.96** | 7.49 ± 1.17 | 6.45 ± 0.97**# |
| $F$ value | 1.40 | 2.03 | 2.88 | 1.21 | 0.63 | 3.13 |
| $P$ value | 0.26 | 0.14 | 0.07 | 0.31 | 0.54 | 0.05 |

Comparison within group, $^{*}P < 0.05$ and $^{**}P < 0.01$. Comparison with control group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$.

Table 4: TC and TG levels before and after treatment ($\chi \pm s$).

| Group | TC (mmol/L) | | TG (mmol/L) | |
|---|---|---|---|---|
| | Before | After | Before | After |
| Control | 4.43 ± 1.09 | 3.86 ± 0.71* | 2.12 ± 2.72 | 1.68 ± 1.64 |
| Routine massage | 5.01 ± 0.93 | 4.50 ± 0.70# | 2.13 ± 1.04 | 1.93 ± 1.02 |
| Abdominal massage | 5.52 ± 0.65 | 4.59 ± 0.61**# | 2.24 ± 2.44 | 1.55 ± 1.00 |
| $F$ value | 3.37 | 3.36 | 0.01 | 0.22 |
| $P$ value | 0.05 | 0.05 | 0.99 | 0.80 |

Comparison within group, $^{*}P < 0.05$ and $^{**}P < 0.01$. Comparison with control group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$.

Table 5: HDL and LDL levels before and after treatment ($\chi \pm s$).

| Group | HDL (mmol/L) | | LDL (mmol/L) | |
|---|---|---|---|---|
| | Before | After | Before | After |
| Control | 1.19 ± 0.26 | 1.04 ± 0.22** | 2.73 ± 0.84 | 2.23 ± 0.50* |
| Routine massage | 1.21 ± 0.10 | 1.18 ± 0.12 | 3.03 ± 0.81 | 2.59 ± 0.47* |
| Abdominal massage | 1.31 ± 0.34 | 1.27 ± 0.35# | 3.24 ± 0.55 | 2.62 ± 0.52* |
| $F$ value | 0.59 | 2.22 | 1.08 | 1.92 |
| $P$ value | 0.56 | 0.13 | 0.35 | 0.17 |

Comparison within group, $^{*}P < 0.05$ and $^{**}P < 0.01$. Comparison with control group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$.

the abdominal massage group in the level of HbAlc ($P = 0.02$ ) and there were no significant differences between the control group and the routine massage group and between the routine massage group and the abdominal massage group in the levels of FBG, PBG, and HbAlc ($P = 0.14$, $P = 0.19$, $P = 0.11$; $P = 0.68$, $P = 0.97$, and $P = 0.40$) (Table 3).

FBG, PBG, and HbAlc values in the routine massage and abdominal massage groups were lower after the intervention. The clinical efficacy of the abdominal massage in reducing the level of HbAlc was higher than no massage (control).

*3.2.2. Comparison of TC and TG Indexes.* When comparing before and after treatment within a group, we observed that the level of TC had no statistical difference in the routine massage group and was significantly different in the control and abdominal massage groups ($P = 0.07$, $P = 0.01$, and $P = 0.005$). The level of TG had no statistical differences in all groups (control: $P = 0.27$; abdominal massage: $P = 0.27$; and routine massage: $P = 0.60$). Pairwise comparison between groups showed that, when compared with the control group, the abdominal massage and routine massage groups were significantly different in the level of TC ($P = 0.03$ and $P = 0.046$) and had no statistical differences in the level of TG ($P = 0.83$ and $P = 0.66$). Compared with the routine massage group, the abdominal massage group had no statistical difference in the levels of TC and TG ($P = 0.76$ and $P = 0.52$) (Table 4).

Therefore, the level of TC in the control and abdominal massage groups decreased after treatment. The clinical efficacy of the abdominal massage in reducing the level of TC was higher than no massage.

*3.3. Comparison of HDL and LDL Indexes.* When comparing before and after treatment within a group, we observed that the level of HDL had no statistical difference in the abdominal massage and routine massage groups and was significantly different in the control group ($P = 0.43$, $P = 0.20$, and $P = 0.002$). The level of LDL was significantly different in all groups after treatment (control: $P = 0.01$; routine massage: $P = 0.049$; and abdominal massage: $P = 0.02$). Pairwise comparison between groups showed that, when compared with the control group, the abdominal massage group was significantly different in the levels of HDL ($P = 0.046$) and

Table 6: Enterococcus and Enterobacter levels before and after treatment ($\chi \pm s$).

| Group | Enterococcus (LogN/g) | | Enterobacter (LogN/g) | |
|---|---|---|---|---|
| | Before | After | Before | After |
| Control | 8.53 ± 0.85 | 8.48 ± 0.56 | 9.38 ± 0.67 | 9.38 ± 0.61 |
| Routine massage | 8.48 ± 0.98 | 8.39 ± 0.78 | 9.34 ± 1.07 | 9.16 ± 0.89* |
| Abdominal massage | 8.53 ± 1.10 | 8.22 ± 0.97** | 9.19 ± 0.85 | 8.81 ± 0.74**# |
| $F$ value | 0.01 | 0.53 | 0.24 | 2.56 |
| $P$ value | 0.99 | 0.59 | 0.79 | 0.09 |

Comparison within group, $^{*}P < 0.05$ and $^{**}P < 0.01$. Comparison with control group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$.

Table 7: Bifidobacterium and Lactobacillus levels before and after treatment ($\chi \pm s$).

| Group | Bifidobacteria (Log N/g) | | Lactobacilli (Log N/g) | |
|---|---|---|---|---|
| | Before | After | Before | After |
| Control | 8.13 ± 0.59 | 8.05 ± 0.58 | 6.84 ± 0.91 | 6.79 ± 0.92 |
| Routine massage | 8.10 ± 0.73 | 8.51 ± 0.77** | 6.32 ± 1.19 | 6.63 ± 1.16* |
| Abdominal massage | 8.07 ± 0.65 | 8.56 ± 0.74**# | 6.64 ± 1.29 | 6.99 ± 1.09** |
| $F$ value | 0.04 | 2.84 | 0.91 | 0.52 |
| $P$ value | 0.96 | 0.07 | 0.41 | 0.60 |

Comparison within group, $^{*}P < 0.05$ and $^{**}P < 0.01$. Comparison with control group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$.

the abdominal massage and routine massage groups had no statistical differences in their levels of LDL (P = 0.10, P =0.11). Compared with the routine massage group, the abdominal massage group had no statistical difference in the levels of HDL and LDL ($P = 0.39$ and $P = 0.90$) (Table 5).

Therefore, the level of LDL in all groups decreased after treatment, and the level of HDL decreased only after no massage treatment. The clinical efficacy of the abdominal massage in reducing the level of LDL had no difference with routine massage and control groups.

### 3.4. Observation Index Data Analysis before and after Treatment

*3.4.1. Comparison of Enterococcus and Enterobacter Indexes.* When comparing before and after treatment within a group, we observed that the level of Enterococcus had no statistical differences in the control and routine massage groups ($P = 0.65$ and $P = 0.38$), and the level of Enterobacter had no statistical difference in the control group and was significantly different in the routine group ($P = 0.93$ and $P = 0.03$). The levels of both bacteria were significantly different in the abdominal massage group (P =0.006, P <0.001). Pairwise comparison between groups showed that, when compared with the control group, the abdominal massage and routine massage groups had no statistical differences in their levels of Enterococcus ($P = 0.32$ and $P = 0.75$). The abdominal massage group was significantly different, and the routine massage group had no statistical difference in the level of Enterobacter ($P = 0.03$ and $P = 0.41$). Compared with the routine massage group, the abdominal massage group had no statistical difference in the levels of both bacteria ($P = 0.50$ and $P = 0.17$) (Table 6).

Therefore, the level of Enterobacter in the routine massage and abdominal massage groups decreased after treatment, and the level of Enterococcus decreased only after abdominal massage treatment. The clinical efficacy of the abdominal massage in reducing the level of Enterobacter was higher than no massage.

*3.4.2. Comparison of Bifidobacteria and Lactobacilli Indexes.* When comparing before and after treatment within a group, we observed that the levels of Bifidobacteria and Lactobacilli had no statistical difference in the control group and were significantly different in the routine massage and abdominal massage groups (control: $P = 0.23$ and $P = 0.56$; routine massage: $P < 0.001$ and $P = 0.04$; and abdominal massage: $P < 0.001$ and $P = 0.002$). Pairwise comparison between groups showed that, when compared with the control group, the abdominal massage group was significantly different and the routine massage group had no statistical difference in the level of Bifidobacteria ($P = 0.03$ and $P = 0.06$). The abdominal massage and routine massage groups had no statistical differences in their levels of Lactobacilli ($P = 0.59$ and $P = 0.66$). Compared with the routine massage group, the abdominal massage group had no statistical difference in the levels of both bacteria ($P = 0.82$ and $P = 0.31$) (Table 7).

Therefore, the levels of Bifidobacteria and Lactobacilli in the abdominal massage and routine massage groups increased after treatment. The clinical efficacy of the abdominal massage in reducing the level of Bifidobacteria was higher than no massage.

## 4. Discussion

T2DM presently has no known treatment. Injections of insulin plus the usage of oral hypoglycemic medications make up the standard treatment for diabetes mellitus (DM). The drawbacks and side effects of these treatments,

however, include gastrointestinal issues, anemia, liver and kidney damage, and lactic acid toxicity [18].

Massage is one of the most popular and effective methods for relaxation and therapy [19]. Manual massage is commonly used in the treatment of pathological conditions affecting the skeletal muscles [20–22]. Recently, mechanisms by which massage is beneficial for the musculoskeletal system have been investigated. According to the findings, massage has an immunomodulatory effect on skeletal muscles, affecting protein and ribosome synthesis and degradation, anabolic signaling, and satellite cell abundance [23]. The effects of manual massage have been classified as reflexive, mechanical, and psychological, resulting in vasodilation and improved circulation [24]. Interestingly, massage induces skin microcirculation, expands blood vessels and improves blood flow, promotes insulin secretion, improves the nervous system and the function of the vegetative nervous system, enhances the immune function and metabolism of the body, and promotes the utilization of glucose by muscle tissue, thereby reducing blood sugar levels [25, 26]. As a complementary treatment strategy, it is crucial to explore the most effective massage method in the treatment of T2DM.

Abdominal massage, also named visceral massage in China, involves mechanical and manual manipulations that are used in the treatment of prediabetes, overweight, and obesity [27]. Numerous studies have shown that abdominal massage reduces thigh, infraumbilical, arm, and postpartum abdominal circumference; decreases flank subcutaneous fat deposits and serum lipid lipids; and improves skin laxity [28–30]. Additionally, the effects on gastrointestinal function have been extensively studied [31]. The function of abdominal massage in lipid metabolism and gastrointestinal function suggests that it could be beneficial in the treatment of T2DM.

Our study findings revealed that both abdominal and routine massage reduce the levels of FBG, PBG, and HbAlc. Besides, abdominal massage improves the levels of TC and LDL. It proved the regulative effects of massage on glucose and lipid metabolism disorders in patients with T2DM. In addition, these interventions can increase the number of beneficial bacteria (e.g., Bifidobacteria and Lactobacillus) and reduce the number of pathogenic bacteria (e.g., Enterococcus and Enterobacter). Dysbiosis is a causative factor in T2DM because it affects energy metabolism and storage and promotes chronic inflammation. In terms of energy [32], the intestinal microflora improves glucose and lipid metabolism by producing short-chain fatty acids, encouraging fat synthesis and storage, and so on. In contrast, intestinal dysbiosis generates the production of endotoxins, leading to systemic inflammation, destruction and apoptosis of pancreatic $\beta$ cells, and insulin resistance [33]. In this study, abdominal massage modulated the intestinal microbiota in T2DM patients, although more research is required to determine the precise mechanisms. We measured three laboratory indices to determine the impact of abdominal massage on glucose metabolism (FBG, PBG, and HbAlc). Future research should evaluate the pancreatic islet function and insulin resistance indexes.

The ability to regulate intestinal microflora suggests that there are interactions between the skeletal muscle and the gut microbiota during abdominal massage. Manual abdominal massage includes different techniques directed to the abdominal soft tissues, including kneading, friction, rubbing, and pinching. This intervention involves the passive movement of abdominal skeletal muscles and of the rectus abdominis, internal oblique, external oblique, and transverse abdominis. According to the evidence, muscular disorders may arise from microbial factors that trigger innate immunity and low-grade systemic inflammation [34]. On the other hand, the metabolic and inflammatory states, muscle function, and gut microbiota are all interconnected [35]. Active skeletal muscle exercise, such as that used in fitness, has been shown to affect the composition and activity of the microbiota in studies using human and animal models [36, 37]. We verify that improving the gut microbiota's equilibrium can be accomplished by passive abdominal skeletal muscle activity. Thus, abdominal massage therapy is a complementary form of treatment for T2DM.

## 5. Conclusion

In patients with T2DM, abdominal massage significantly reduced abnormalities in the intestinal microbiota and glucose metabolism. Abdominal massage was simpler to do than regular massage because it did not require changing the patient's body posture. Abdominal massage is quite convenient for both doctors and patients, especially given the increased prevalence of DM in obese persons. This study will advocate abdominal massage as a trustworthy treatment option for T2DM patients.

## Data Availability

The study data are available upon request. We welcome specific proposals for future collaboration.

## Conflicts of Interest

The authors declare no conflicts of interest.

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*Review Article*

# Mechanisms Involved in Gut Microbiota Regulation of Skeletal Muscle

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Skeletal muscle is one of the largest organs in the body and is essential for maintaining quality of life. Loss of skeletal muscle mass and function can lead to a range of adverse consequences. The gut microbiota can interact with skeletal muscle by regulating a variety of processes that affect host physiology, including inflammatory immunity, protein anabolism, energy, lipids, neuromuscular connectivity, oxidative stress, mitochondrial function, and endocrine and insulin resistance. It is proposed that the gut microbiota plays a role in the direction of skeletal muscle mass and work. Even though the notion of the gut microbiota–muscle axis (gut–muscle axis) has been postulated, its causal link is still unknown. The impact of the gut microbiota on skeletal muscle function and quality is described in detail in this review.

## 1. Introduction

Skeletal muscle is one of the largest organs, accounting for roughly half of the total body weight. Skeletal muscle produces heat, regulates blood sugar, storing amino acids, and alters the physiological characteristics of the body [1]. Skeletal muscle mass and function decline have been reported to affect 8%–13% of older adults [2], with clinical effects including frailty, loss of mobility, falls, fractures, disability, and increased mortality [3]. Numerous factors contribute to the loss of skeletal muscle mass and function, such as inflammatory states [4], age-related changes in the hormonal environment [5], insulin resistance [6], gut physiology [7], DNA damage, and mitochondrial dysfunction [8]. These mechanisms are enhanced in the presence of insufficient protein energy [9].

The physiological characteristics of skeletal muscle have been extensively studied in the past few decades, providing unique insights into the interconnection among organs [10]. As with the products secreted by skeletal muscle, external factors that may act on skeletal muscle can also play an important role in peripheral tissues. The gut microbiota has the potential to influence muscle function and quality [11]. The gut microbiota is increasingly being seen as a key factor in human wellbeing and disease, especially in older adults [12]. Although the gut microbiota is known for its role in nutrient absorption, it is closely associated with many other physiological processes [13]. Therefore, the interaction between the gut microbiota and human organs has become the focus of recent research [14].

Recent studies have demonstrated the existence of a gut microbiota-muscle axis, i.e., that muscle function and metabolism are largely dependent on the quantity and composition of the gut microbiota, and that the gut microbiota is expected to be a potential biological target for the prevention and treatment of muscle-related diseases such as sarcopenia and muscular dystrophy [15]. Furthermore, it is critical to clarify how the gut microbiota affects exercise load, modulates muscle function, and improves host fitness. The gut microbiota has a profound effect on skeletal muscle function and mass, and intervening in this axis may reverse the decline in skeletal muscle function and mass [13, 15–19].

This article reviews the progress of research on the effects of gut microbiota on the biological function of skeletal muscle and its mechanisms.

## 2. Gut Microbiota and Intestinal Barrier

*2.1. Gut Microbiota.* The human body consists of approximately 30 trillion cells that coexist with various microbial communities [20]. The human gut microbiome consists of 10–100 trillion microbes that are highly diverse, complex, constantly evolving, and colonize the digestive tract [21]. For host physiology, body homeostasis, and long-term health, functional interactions between gut microorganisms and hosts are critical. Although several studies have revealed how the gut microbiota impacts the liver and intestinal metabolism [22], there are few reports on how the gut microbiota regulates skeletal muscle, which is also one of the key metabolic organs [23]. The composition of the gut microbiome is influenced by a variety of factors, including genetics, age, diet, and exercise [24]. The human gut microbiota is dynamic throughout the life cycle, with the composition of gut microbes tending toward a steady state during the early years, but new research has found that the gut microbiota changes significantly in older adults (≥65 years) [25]. Antibiotics are known to cause changes in the microbiota composition, and older people are more inclined to use antibiotics more frequently [26], which may be one of the reasons for the changes in their gut microbiota composition.

To date, more than 9.9 million microbial genes have been found in human feces, with Bacteroides and Firmicutes accounting for the majority [27]. Probiotics are beneficial bacteria (e.g., *Lactobacillus*, *Bifidobacterium*, *Clostridium butyricum*, and *Bacillus subtilis*) [28]. Prebiotics are largely found in our gastrointestinal tract. Prebiotics are organic substances that the host cannot digest or absorb but which benefit the host's health. They feed beneficial bacteria and promote the growth and reproduction of beneficial bacteria [29]. The aging gut microbiota is highly characterized by a decrease in microbial diversity and beneficial bacteria, as well as a rearrangement of Bacteroides and Firmicutes, especially in older people, where individual differences in microorganisms can be greater [30, 31].

*2.2. Intestinal Barrier.* The intestinal tract of the organism has a relatively complete functional barrier, and intestinal barrier function refers to the function of the intestinal epithelium that can separate the intestinal lumen from the internal environment of the organism and prevent the invasion of pathogenic antigens. The normal intestinal barrier consists of mechanical barrier, chemical barrier, immune barrier, and biological barrier together [32].

The mechanical barrier is an intact intestinal mucosal epithelial structure closely connected to each other, which consists of a mucosal layer, intestinal epithelial cells, intercellular tight junctions, and submucosal lamina propria, and the intact intestinal mucosal epithelial cells and tight junctions between epithelial cells are the structural basis of the mechanical barrier [33]. Gastric acid, bile, various digestive enzymes, lysozyme, digestive juices, and antibacterial substances produced by parasitic bacteria in the intestinal lumen constitute the chemical barrier of the intestinal tract [34]. Stomach acid can destroy bacteria entering the gastrointestinal tract and inhibit bacterial adhesion and colonization of the gastrointestinal epithelium; lysozyme can destroy the cell wall of bacteria and cause bacterial lysis; digestive juices secreted by the intestine can dilute toxins and flush the intestinal lumen, making it difficult for potentially pathogenic bacteria to adhere to the intestinal epithelium [35, 36]. The immune barrier of the gut consists of immune cells, immune factors, and gut-associated lymphoid tissue. Immune cells initiate immune responses and form the intestinal mucosal immune system to protect the gut from external stimuli [36]. Immune factors enhance gut barrier function through immune rejection and bacterial clearance, in which immunoglobulin IgA plays an important role in regulating gut microbiota and maintaining immune homeostasis [37]. Gut-associated lymphoid tissue neutralizes antigenic substances by triggering local immune responses and can also secrete immunoglobulins to block the binding of bacteria to intestinal epithelial receptors, thereby effectively blocking the adhesion of harmful substances to the intestinal mucosa [38]. The normal parasitic flora in the intestine forms the biological barrier of the intestinal mucosa, and the metabolism of the gut microbiota can also regulate the mechanical, chemical, and immune barriers of the intestinal tract [39]. The biological barrier of the gut maintains the stability of the gut microbiota, and dysregulation of gut microbial homeostasis can lead to a decrease in beneficial microbes and an increase in harmful microbes, thereby compromising the health of the host [40].

Since birth, the microbiota has colonized the gastrointestinal tract and participates in many physiological processes in the host. Intestinal immune and endocrine function, energy homeostasis, and health are all influenced by the complex microbiota [41], which regulates inflammatory gene expression, innate immune effector cells (monocytes and macrophages), glucose tolerance, and gut hormone release, among other metabolic pathways [42, 43]. The gut microbiota and the gut barrier interact with each other. Intestinal cells regulate the composition of the gut microbiota by secreting antimicrobial peptides, and conversely, the gut microbiota can also affect the growth process of intestinal epithelial cells [34]. In mice, depletion of the gut microbiota compromises the intestinal epithelium, leading to altered patterns of microvillus formation and reduced cell renewal [44]. Probiotics form a biofilm to cover the intestinal mucosa, preventing the invasion of foreign bacteria, and they also produce acidic metabolites that lower the pH of the intestinal tract, thereby inhibiting the growth of harmful bacteria [45]. In addition, the accumulation of anaerobic bacteria and the invasion of exogenous pathogenic bacteria can lead to dysbiosis of the gut microbiota, damage the intestinal epithelial cells, and destroy the gut microbiota barrier [46].

*2.3. Gut Microbiota Affects Skeletal Muscle Mass and Function.* According to emerging evidence, the gut microbiota appears to play a role in regulating several muscle

metabolic pathways [47]. Individual differences in gut microbiota relative abundance are linked to muscle mass and body weakness [48, 49], and higher gut microbiota diversity is linked to increased muscle mass [50]. In young women, the diversity of the gut microbiota is also related to skeletal muscle mass [51]. Increased numbers of *Oscillospira* and *Ruminococcus* and decreased numbers of Barnesellacae and Christensenellacea taxa are found in people with muscle wasting and physical weakness [48]. When compared to older people with low functional muscular strength, those with higher levels of *Prevotella*, *Barnesiella*, and *Barnesiella intestinihominis* have greater muscle strength [52]. *Barnesiella* and *Prevotella* have genes that produce short-chain fatty acids (SCFAs) [53].

Several studies from rodents have suggested that gut microbes may be related to the function and quality of skeletal muscle. The effects of gut microbiota shortage on skeletal muscle were studied in two animal investigations, which revealed that a lack of gut bacteria causes muscle mass loss [54, 55].The abundant Rikenellaceae group found in the gut microbiota of older mice is linked to a dose-dependent rise in muscular frailty index [56]. Higher *Sutterella* to *Barnesiella* ratio, altered inflammation and immune function, and decreased gastrocnemius and triceps size in rats with muscle atrophy were compared with healthy adult rats [47]. Comparison of germ-free (GF) mice lacking gut microbiota and pathogen-free (PF) mice with gut microbiota revealed skeletal muscle atrophy and decreased muscle mass in GF mice [23]. Ghrelin-deficient mice develop microbial dysbiosis at a young age and then lose muscle mass and function as they get older [57]. A decrease in gut bacteria can directly lead to muscle atrophy, according to two new studies [23, 54].

Antibiotics change the microbiota, and metronidazole has been shown to upregulate the expression of neurogenic atrophy-related proteins in skeletal muscle in earlier studies, as well as histone deacetylase 4, myostatin (MyoG), and FOXO1/FXOX3-mediated protein degradation, leading to skeletal muscle atrophy, thereby reducing muscle mass in the hind limb and muscle fiber volume in the tibialis anterior muscle of mice [58]. Similarly, antibiotic-treated mice resulted in muscle atrophy, reduced muscle mass, decreased running endurance, and increased *ex vivo* muscle fatigue [26, 59]. However, after inoculation with natural microbes in antibiotic-treated mice, the mice had increased muscle mass and a muscle mass/body weight ratio [59].

*In vitro* studies have also shown that gut microbial products can directly affect muscle mass [60]. The levels of two intestinal microbial metabolites (indoxyl sulfate and p-cresol sulfate) increase with age and play a vital part in muscle function [61]. Indoxyl sulfate, a biomarker of uremic sarcopenia, accelerates muscle atrophy by increasing inflammation levels, oxidative stress, and myasthenic gene expression and is negatively correlated with muscle strength and physical exercise [62]. Similarly, the gut microbiota that produces p-cresol sulfate, through insulin resistance and increasing muscle lipid content, ultimately contributes to poor muscle status [63]. Conversely, SCFAs are the end product of colonic protein fermentation and have many important physiological functions.

## 3. The Gut Microbiome Regulates Skeletal Muscle through a Variety of Mechanisms

*3.1. Inflammation, Immunity, and Autophagy.* One of the major mechanisms contributing to the loss of skeletal muscle mass and function is systemic chronic inflammation. As research has progressed, the importance of the gut microbiota in skeletal muscle metabolism and immunological function has become recognized. The gut microbiota promotes metabolic homeostasis and immune function by strengthening the intestinal barrier [64]. Gut microbial disorders and loss of variety, in contrast, compromise the integrity of the intestinal barrier, allowing hazardous microbial products such as lipopolysaccharide (LPS) to enter the bloodstream, and these harmful substances trigger systemic inflammation and lead to metabolic disorders and decreased muscle function and mass [15]. Elevated LPS levels activate Toll-like receptor (TLR) 4 signaling, which leads to metabolic endotoxemia [65]. Activation of the TLR4 signaling pathway causes a significant increase in nuclear factor- (NF-) $\kappa$B protein levels (p50 and p65) and c-Jun N-terminal kinase phosphorylation, resulting in a decrease in human immune function [65]. Specifically, the TLR4 signaling pathway induces upregulation of proinflammatory cytokines (interleukin-6 and tumor necrosis factor-$\alpha$) through a cascade response, thereby inducing a systemic inflammatory response [66] (Figure 1).

In recent years, autophagy has received a lot of attention as a fundamental element in skeletal muscle mass and function regulation. Autophagy ensures skeletal muscle quality and function by removing dysfunctional organelles from senescent cells [67]. The AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-coactivator- (PGC-) 1 signaling pathways are known to regulate cellular metabolism and play essential roles in autophagy, inflammation, insulin resistance, and skeletal muscle. In addition, AMPK and PGC-1$\alpha$ signaling pathways are associated with the gut microbiota–muscle axis [68]. The activation of AMPK and PGC-1 decreases with age [69], and inhibition of AMPK and PGC-1$\alpha$ signaling pathways decreases autophagic activity, leading to a decrease in skeletal muscle mass and function [70]. Decreased autophagic activity exacerbates the inflammatory response, which in turn inhibits activation of the AMPK signaling pathway [71]. The reduced autophagic activity also clusters dysfunctional organelles in senescent cells, thereby increasing the production of reactive oxygen species (ROS). The level of the inflammasomes, including Nod-like receptor 3 (NLRP3), is stimulated by ROS [72]. The NF-$\kappa$B signaling mentioned above also stimulates the production of NLRP3 inflammasomes [73]. Thus, dysregulated autophagic activity and inflammatory responses play a pivotal part in the loss of skeletal muscle mass and function, and AMPK and PGC-1$\alpha$ signaling pathways are closely associated with the gut microbiota–muscle axis [68]. Further research into the relationships between the AMPK and PGC-1 signaling pathways, autophagy, inflammatory responses, and the gut microbiome could aid in the treatment of disorders characterized by skeletal muscle mass and function loss (Figure 1).

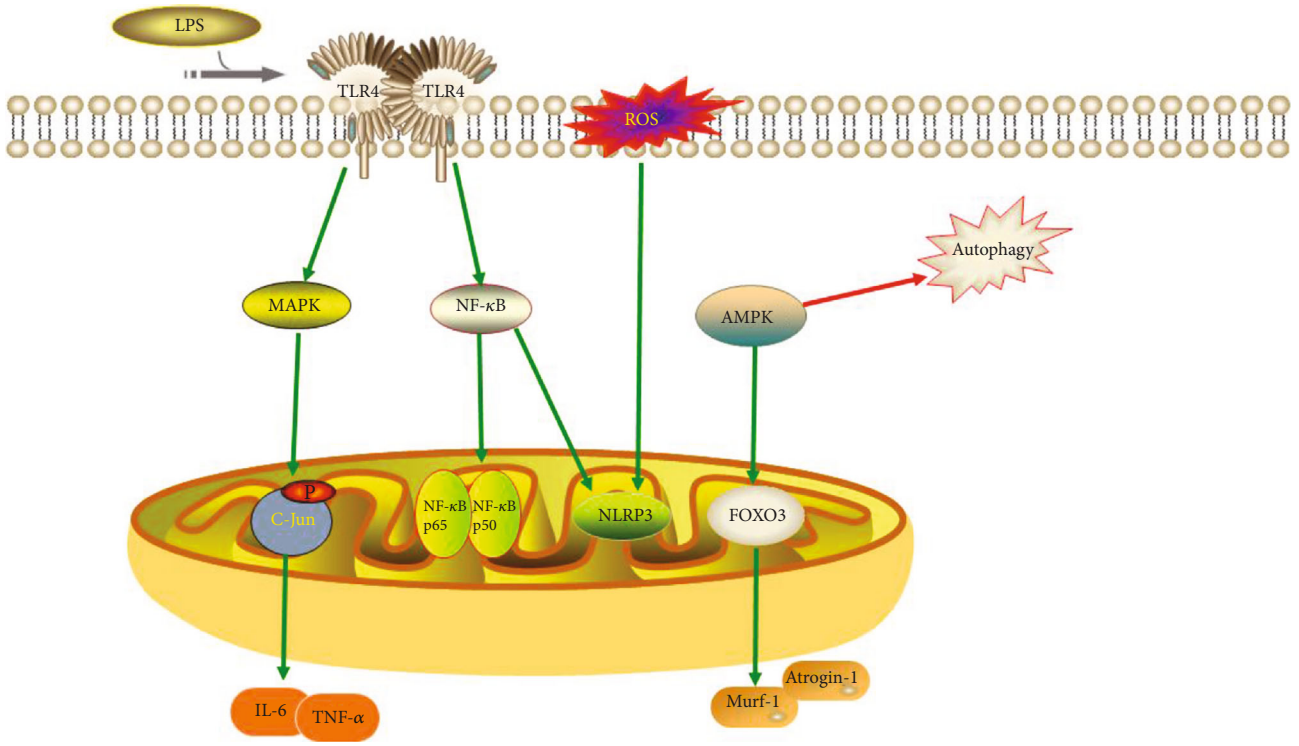


Figure 1: TLR4 signaling and the production of ROS induce inflammatory responses. AMPK signaling regulates autophagic activity and produces muscle atrophy factors.

Increased expression of atrophy marker genes, particularly Murf-1 and Atrogin-1, which play a critical role in muscle atrophy, is linked to the role of microbiota in the reduction of muscle mass and function [74]. FOXO transcription factors influence the production of Murf-1 and Atrogin-1 [74]. By activating the FOXO3-mediated protein breakdown pathway, AMPK modulates muscle fiber size [75]. Decreased muscle mass and strength in GF mice are associated with increased expression of FOXO, Murf-1, and Atrogin-1. The MyoG and FOXO3 pathways and their downstream target genes are regulated by the gut microbiota and their derived metabolites during protein synthesis and degradation [76]. The activation of AMPK signaling in GF mouse muscle suggests that the AMPK/FOXO3/Atrogin-1/Murf-1 signaling pathway may be implicated in the gut microbiota–muscle axis [23] (Figure 1).

*3.2. Endocrine System.* The endocrine system has an important role in muscle mass regulation, with insulin, insulin-like growth factor- (IGF-) 1, and growth hormone influencing muscle growth and development [77]. In general, insulin acts on skeletal muscle to promote glucose uptake and upregulates anabolic signaling, which influences the rate of muscle protein synthesis [78]. Dysregulation of the gut microbiota and disruption of epithelial regeneration can be founded in intestinal epithelial IGF-1 gene-deficient mice compared with normal mice [79]. Mechanistically, IGF-1 regulates muscle growth through the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and inhibits the mRNA transcription and translation process of muscle protein synthesis (MPS) [80]. The PI3K/AKT signaling pathway is a well-known insulin-resistance pathway [81], and it is disrupted in diabetic patients. Insulin production and beta-cell activity may be diminished once this route is blocked, worsening insulin resistance even more [82]. Insulin resistance causes muscle cells to be unable to utilize glucose and instead rely on glycogen or fat, which can lead to a loss of muscle mass and function [83] (Figure 2).

Glucocorticoids can induce skeletal muscle atrophy under pathological conditions [84]. One of the target genes for glucocorticoid receptor activation is Kruppel-like factor (KLF) 15, which is implicated in metabolic activities in skeletal muscle such as overexpression of branched-chain aminotransferase2, which leads to degradation of branched-chain amino acids (BCAAs) [85]. Loss of gut microbiota also leads to the degradation of BCAAs in muscle. Increased catabolism of BCAAs in GF mice is a key factor in muscle atrophy, and increased expression of genes involved in BCAA metabolism leads to reductions in muscle mass, hindlimb grip strength, and spontaneous activity in mice [23]. Catabolism of BCAAs is linked to skeletal muscle proteolysis and has the ability to modulate protein synthesis [86] (Figure 3).

*3.3. Protein Anabolism.* A balance between protein synthesis and breakdown keeps skeletal muscle mass in check. A state of negative muscle protein balance occurs when the rate of muscle protein breakdown (MPB) exceeds the rate of MPS over time, resulting in a reduction in skeletal muscle function and mass [87]. It is widely believed that the decrease in muscle

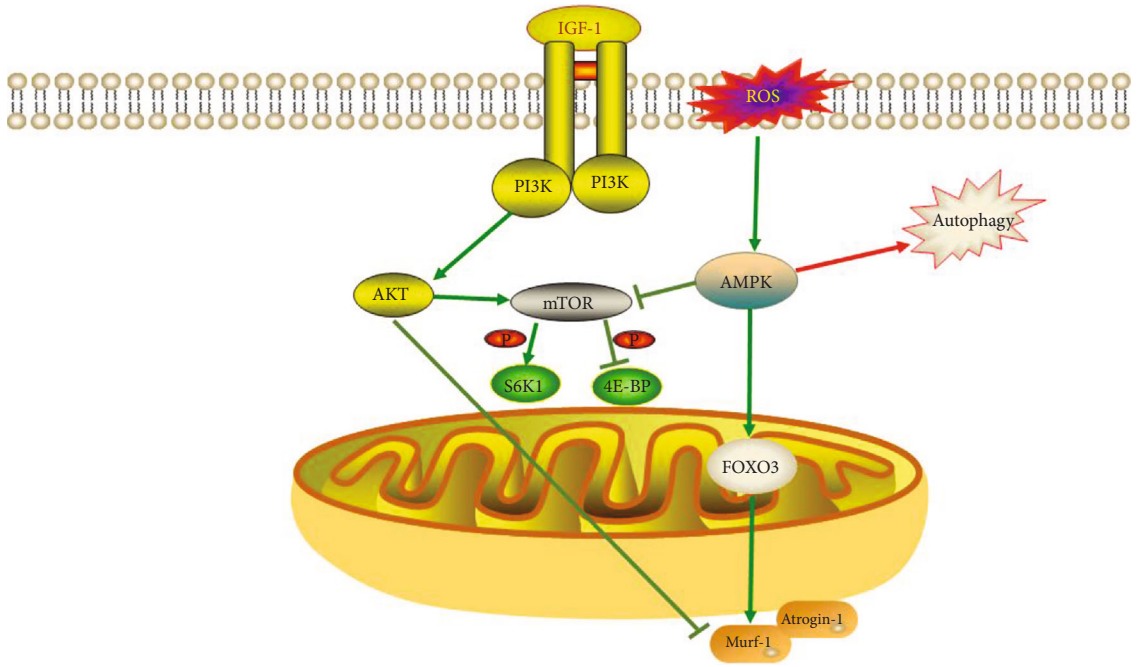


Figure 2: IGF-1 activates mTOR through PI3K/AKT signaling to stimulate protein synthesis. The PI3K/AKT signaling pathway inhibits the expression of myasthenic markers (Murf-1 and Atrogin-1). ROS inhibits mTOR activity by activating the AMPK signaling pathway.


Figure 3: Glucocorticoids inhibit protein synthesis by activating KLF15, which leads to the degradation of BCAAs.

function and mass is caused by diminished ability to stimulate MPS rather than by acceleration of MPB [88]; a metabolic phenomenon known as muscle anabolic resistance.

Mammalian target of rapamycin (mTOR) is a downstream target of PI3K/Akt. mTOR stimulates protein synthesis in two ways: phosphorylation and inactivation of eukaryotic initiation factor 4E-binding protein1 and phosphorylation and activation of ribosomal S6 kinase1 [89]. Many studies have demonstrated that mTOR signaling regulates MPS, and that inhibition of mTOR signaling results in decreased muscle function and muscle loss [90]. IGF-1 can activate mTOR activity by activating the PI3K/Akt signaling pathway, thereby stimulating protein synthesis [91]. Production of myasthenic markers (Murf-1 and Atrogin-1) is downregulated by the PI3K/Akt pathway [92]. However, phosphorylation and activation of AMPK can inhibit mTOR activity [93]. Decreased insulin sensitivity and inflammatory responses also reduce mTOR signaling. Reduced insulin sensitivity inhibits mTOR activity by reducing IGF-1 levels, and overproduction of inflammatory factors as well as ROS can inhibit the mTOR pathway by activating the AMPK pathway [9] (Figure 2).

An increasing number of studies have shown that the gut microbiota can produce a large number of bacterial metabolites to activate diverse receptors in host cells, thus maintaining homeostasis in the host. Bile acids (BAs) are metabolites produced by the microbiota [94]. BAs bind to cellular BA receptors, one of which is the nuclear farnesoid X receptor (FXR), to modulate host glucose and lipid metabolic signaling [95]. FXR is activated in the ileum and produces fibroblast growth factor (FGF) 19, which is called FGF15 in rodents. In previous research, BAs, BA receptors, and the FXR-FGF15/19 signaling pathway have all been linked to skeletal muscle mass and function [96]. The expression of FGF15/19 activates the protein kinase (ERK) signaling pathway and phosphorylation of ERK downstream targets p90 ribosomal S6 kinase and ribosomal protein S6 to catalyze protein synthesis [97]. In short, gut microbiota disorders inhibit the BA/FXR/FGF15/19/ERK signaling pathway, resulting in restricted protein synthesis and thus skeletal muscle atrophy [98] (Figure 4).

*3.4. Peroxisome Proliferator-Activated Receptors.* Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor family of transcription factors that are activated by fatty acids and their derivatives. After activation by ligand binding, PPAR heterodimerizes with retinoid X receptors, forming a heterodimer that binds to a PPAR response element upstream of the target gene promoter, ultimately regulating the transcription of the target gene [99]. There are three subtypes of PPAR: PPAR$\alpha$, $\beta/\delta$, and $\gamma$. PPAR$\alpha$ is highly expressed not only in the liver, heart, brown adipose tissue, and kidney but also in skeletal muscle [100]. It plays an important role in fatty acid catabolism by

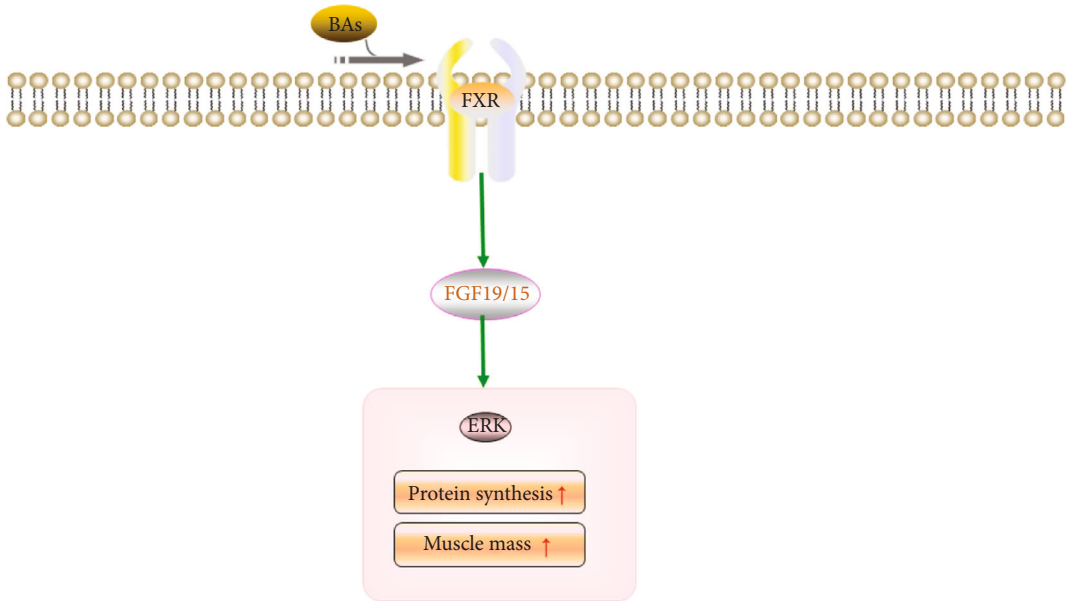


Figure 4: BAs promote protein synthesis and strengthen muscle mass through the FXR/FGF15/19 signaling pathway.

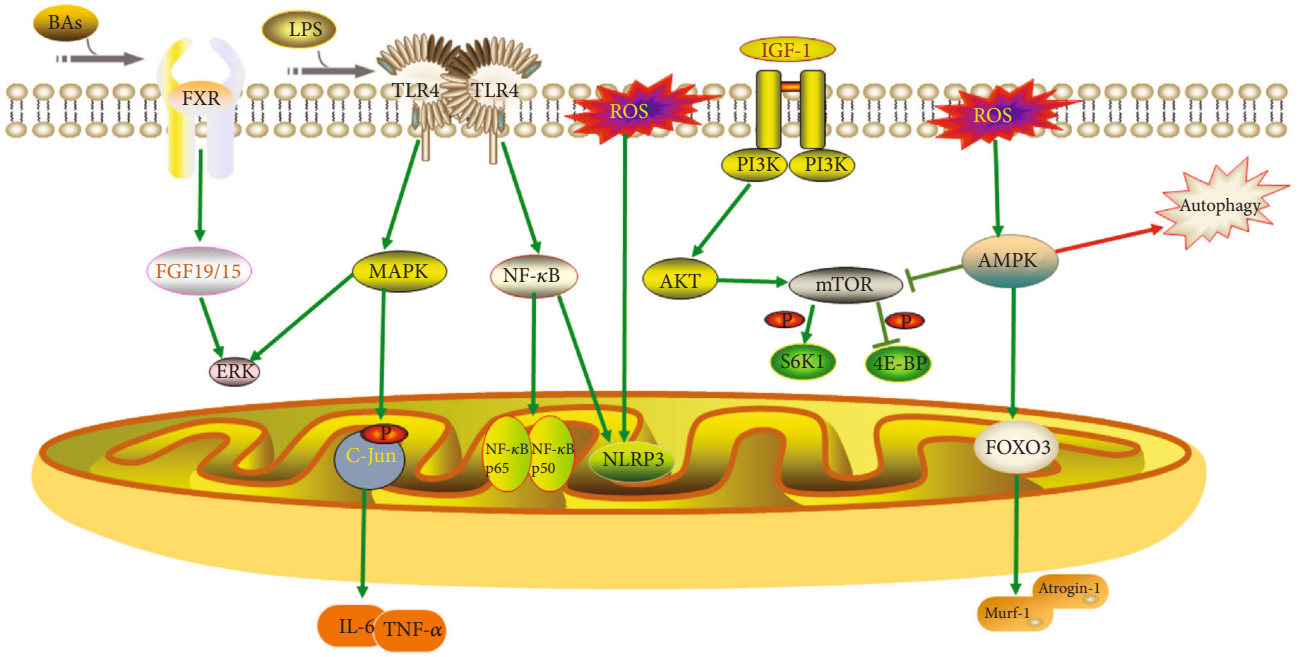


Figure 5: Mechanisms involved in the gut microbiota–skeletal muscle axis.

regulating peroxisomal and mitochondrial $\beta$-oxidation and microsomal $\omega$-oxidation of fatty acids; it is also involved in glucose metabolism and is key in controlling energy expenditure and suppressing inflammatory responses [101]. The expression of PPAR$\beta/\delta$ is more widespread in skeletal muscle, and it plays an important role in glucose and lipid metabolism, inflammatory response, energy expenditure, and muscle fiber type switching [102]. PPAR$\gamma$ is highly expressed in adipocytes and is associated with lipid deposition in muscle and other organs, affecting adipogenesis as well as triglyceride storage [103].

It has been shown that mice lacking PPAR$\beta/\delta$ have a reduced number of muscle satellite cells with decreased regenerative capacity, ultimately leading to muscle atrophy and decreased muscle mass and body weight, suggesting that PPAR$\beta/\delta$ regulates postnatal myogenesis and regeneration in mice [104]. Some mice with specific active PPAR$\beta/\delta$ have shown greater resistance to fatigue [105]. Abnormal energy metabolism and reduced muscle fibers have been observed in mice with PPAR$\beta/\delta$ knockout in muscle and adipocyte hypertrophy, and glucose intolerance with insulin resistance has also been observed [106]. PGC-1$\alpha$ has been shown to be

Table 1: The effects of gut microbiota on skeletal muscle.

| References | Objects | Methods | Results | Remarks |
|---|---|---|---|---|
| Chen et al. [123] | Mice | Supplementation of LP10 | Forelimb grip strength and endurance swimming time were increased | LP10 reduces the inflammatory response, improves glucose utilization, and increases the number of type I muscle fibers in the gastrocnemius muscle |
| Storelli et al. [124] | Drosophila | Supplementation of *Lactobacillus plantarum* | Increased protein synthesis and enhanced muscle anabolism | Upregulation of mTOR pathway and enhancement of MPS |
| Chen et al. [125] | Mice | Supplementation of NCE | Forelimb grip strength and endurance swimming time were increased | NCE alters gut microbiota composition and increases tissue glycogen content |
| Okamoto et al. [126] | LMC diet mice | Inulin supplementation combined with microbial transplantation | Endurance was improved | Muscle mass improvement was not found, and it may be difficult to promote muscle growth with a single supplement of inulin |
| Katsuki et al. [127] | Mice | Supplementation of *Lactobacillus curvatus* CP2998 | The myotubular diameter was restored | CP2998 prevents dexamethasone-induced muscle atrophy by inhibiting glucocorticoid receptor activation |
| Hsu et al. [128] | Mice | Supplementation of kefir | Significant improvement in forelimb grip strength score, endurance swim time, and muscle mass | Altered gut microbiota composition and increased tissue glycogen content |
| Ni et al. [129] | Mice | Supplementation of *Lactobacillus casei* LC122 or *Bifidobacterium longum* BL986 | Improved muscle strength and function | Improved intestinal barrier function and reduced inflammatory response |
| Chen et al. [130] | Mice | Supplementation of *Lactobacillus paracasei* PS23 | Reduced risk of sarcopenia | Improved mitochondrial function and decreased secretion of proinflammatory cytokines |
| Huang et al. [131] | Mice | Colonization of *Eubacterium rectale* or *Clostridium coccoides* | Endurance swimming time was increased | / |
| Scheiman et al. [132] | Mice | Inoculation of *Veillonella atypica* | Treadmill running exhaustion time was increased | *Veillonella atypica* converts lactic acid metabolism to propionic acid |
| Fielding et al. [52] | Mice | Fecal samples from older adults | The grip strength of mice in the high-function group increased significantly | Altered gut microbiome and strengthened intestinal barrier in high-functioning mice |
| Munukka et al. [133] | Mice | Supplementation of *Faecalibacterium prausnitzii* | Muscle mass was increased | Enhanced mitochondrial respiration, reduced inflammatory response, altered gut microbiota composition, and improved intestinal integrity |
| Lee et al. [134] | Mice | Supplementation of SA-03 | Significant improvement in muscle strength and endurance performance | Increased liver and muscle glycogen stores, decreased levels of lactate, blood urea nitrogen, ammonia, and creatine kinase |
| Lee et al. [135] | Mice | Supplementation of OLP-01 | Increased grip strength and endurance in mice | Increased SCFA, liver, and muscle glycogen |
| Hsu et al. [54] | Mice | Supplementation of *Bacteroides fragilis* | Increased muscle mass and endurance swimming time | Serum superoxide dismutase activity was lower than GF mice |
| Lahiri et al. [23] | Germ-free mice | Supplementation of SCFA | Increased muscle mass and function and grip strength | SCFA reduces the expression of Atrogin-1 and Murf-1 |
| Walsh et al. [138] | Mice | Supplementation of butyrate | Prevention of hind limb muscle atrophy in mice | Increase in muscle fibers, prevention of intramuscular fat accumulation, improvement of mitochondrial function and glucose metabolism |
| Buihues et al. [139] | Elderly people (≥65 years) | Supplementation of prebiotic:inulin plus fructooligosaccharides | Improved muscle strength and endurance, less fatigue | Prebiotics promote the growth of beneficial bacteria and reduce proinflammatory cytokines |

Table 1: Continued.

| References | Objects | Methods | Results | Remarks |
|---|---|---|---|---|
| Huang et al. [140] | Triathletes | Supplementation of *Lactobacillus plantarum* PS128 | Significantly improves triathletes' endurance | Regulate gut microbiota composition and increase SCFA content |
| Huang et al. [141] | Healthy adults | Supplementation of LP10 | Increased muscle mass and fatigue resistance | LP10 improves aerobic endurance performance |
| Barger et al. [142] | Older men | High dietary fiber diet | Higher grip strength and physical performance indicators | High dietary fiber promotes butyrate production |
| Morita et al. [143] | Older women | 12 weeks of aerobic training | Increased trunk muscle strength | Increased gut microbiota diversity and fecal SCFA content |
| Shing et al. [144] | Male runners | Supplementation of probiotic capsules | Prolonged fatigue exercise at high temperatures | / |
| Salarkia et al. [145] | Female swimmers | Supplementation of probiotic yogurt | Improved aerobic performance | Improved maximum oxygen uptake |

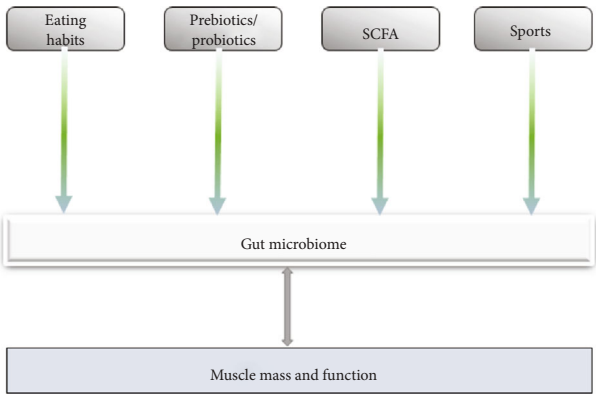


Figure 6: Diet, exercise, prebiotics/probiotics, and SCFA supplementation can alter the gut microbiota and improve muscle mass and function.

a downstream target gene of PPAR$\beta/\delta$ [107]. The expression of PPAR$\beta/\delta$ also increases the level of PGC-1$\alpha$, which affects fatty acid oxidation and glucose metabolism [108]. These results also show that PPAR agonists can improve the deficiency of myotonic proteins, compensate for the loss of muscle fibers, and improve myotonic dystrophy [109]. Experiments using antibiotics to treat mice with changes in muscle peripheral biological clock mechanisms and metabolic regulators (PPAR$\gamma$) have suggested that disturbances in the gut microbiota are associated with the expression of genes that regulate muscle peripheral circadian mechanisms and metabolism [26].

PPAR primarily interacts with the gut microbiota in inflammation and metabolism [110]. PPAR$\alpha$ protects the intestine from an inflammation-induced increase in intestinal permeability by preventing neutrophil infiltration, and the microbiota activates PPAR$\alpha$ through TLR4 signaling, thereby acting to reduce inflammation [111]. Previously, it was reported that treatment of mice with type I diabetes with a PPAR$\alpha$ agonist (bezafibrate) resulted in improved skeletal muscle insulin sensitivity through activation of PI3K/AKT signaling [112]. Similarly, PPAR$\beta/\delta$ and PPAR$\gamma$ play a role in reducing inflammation in the intestines, thereby regulating the composition of the intestinal flora [113]. PPAR$\beta/\delta$ suppresses the inflammatory response and enhances insulin sensitivity by activating the AMPK signaling pathway and inhibiting the extracellular regulated protein kinase ERK1/2 [114]. PPAR$\gamma$ in muscle promotes glucose utilization by muscle through activation of glucose transporter protein (GLUT) 1 and GLUT4 [115].

*3.5. Mitochondrial Function and Neuromuscular Connectivity.* Skeletal muscle mitochondrial dysfunction is also a cause of decreased muscle mass and function [116]. Skeletal muscle mitochondrial function and content decrease with age, and electron microscopy shows abnormally expanded mitochondrial segments [117]. The production of IGF-1 by the gut microbiota connects mitochondrial skeletal muscle to the gut microbiota. It was discovered that IGF-1 levels in GF mice were lower than in PF mice, and that the expression of genes encoding mitochondrial oxidative phosphorylation complexes was lower in GF mouse skeletal muscle, resulting in a loss in mitochondrial function [23].

The central nervous system controls skeletal muscle function via neurotransmission at the neuromuscular junction [118]. Acetylcholine, a key neurotransmitter for signaling between muscles and nerves, was reduced in GF mice when compared to PF mice, as was the expression of the acetylcholine receptor subunit Rapsyn and low-density lipoprotein receptor-related protein 4; both of which are important for neuromuscular junction assembly [23] (Figure 5).

## 4. Interventions

To date, there have been many preclinical and human studies that have directly or indirectly demonstrated a link between gut microbiota and muscle mass/function (Table 1). Various interventions have been proposed for the gut microbiota, and probiotics and/or prebiotics, SCFAs, dietary supplementation, and exercise have all been effective in enhancing muscle mass and host function (Figure 6). Dietary habits influence the composition of the gut microbiota and can induce changes in

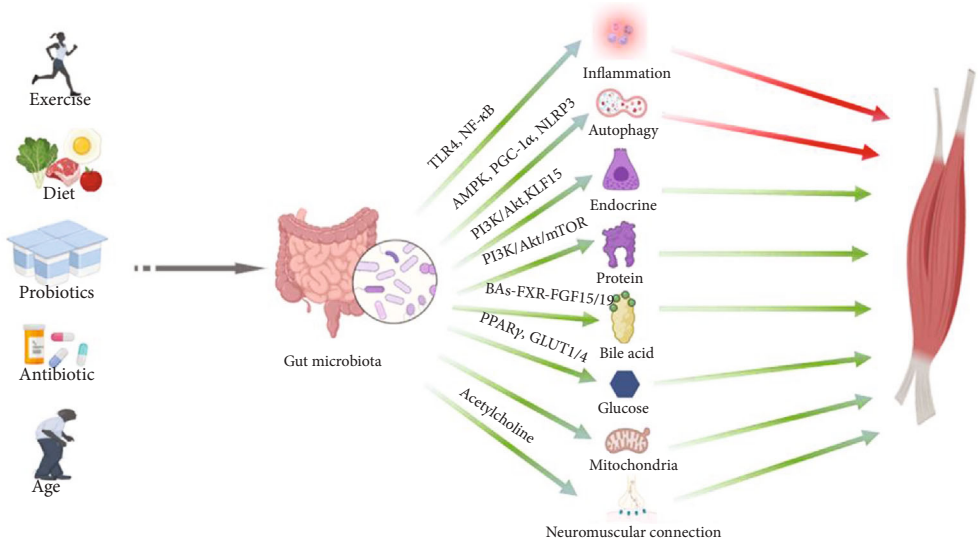


Figure 7: The gut–muscle axis under physiological and pathological conditions. Red arrows represent negative effects on muscles, and green arrows represent positive effects on muscles.

the microbiota that are important for the function of the organism [119]. In the context of skeletal muscle aging, eating disorders cause reduced microbial diversity and increased intestinal permeability, which inhibit cytokine-mediated protein anabolism [120]. Supplementation of prebiotics and/or probiotics improves intestinal homeostasis and promotes skeletal muscle metabolism and synthesis [121]. Exercise or physical activity is also a factor in regulating the gut microbiota [122].

In a mouse model, forelimb grip strength and endurance swimming time were significantly increased after 6 weeks of supplementation with *Lactobacillus plantarum* TWK10 (LP10), which increased glucose utilization and reduced the inflammatory response by increasing the number of types I muscle fibers in the gastrocnemius muscle, thereby increasing endurance exercise time [123]. In a study of *Drosophila*, *Lactobacillus plantarum* can increase protein synthesis and upregulate mTOR, thereby promoting MPS and enhancing muscle anabolism [124]. Curcumin as a prebiotic can alter the composition of gut microbiota and improve endurance, swimming time, and forelimb grip strength in mice, possibly due to a significant increase in tissue glycogen content in mice after supplementation with nanobubble curcumin extract (NCE) [125]. Inulin combined with microbial transplantation improves endurance in mice on a low microbiome-accessible carbohydrate (LMC) diet, but no improvement in muscle mass was found [126]. Myotube diameter was significantly reduced after treatment of mouse skeletal muscle C2C12 myotubes with dexamethasone, whereas *Lactobacillus curvatus* CP2998 (CP2998) restored mouse myotube diameter by inhibiting glucocorticoid receptor activation and prevented muscle atrophy [127]. After oral administration of kefir supplementation, the forelimb grip strength scores, endurance swimming time, and muscle mass of mice were significantly higher than in controls, and the composition of the gut microbiota of mice was changed (reduced *Firmicutes*/*Bacteroidetes* ratio) and tissue glycogen content was also significantly increased after kefir supplementation [128]. After oral administration of *Lactobacillus casei* LC122 or *Bifidobacterium longum* BL986 for 12 weeks, these two probiotics improved intestinal barrier function, increased muscle strength, and reduced oxidative stress and inflammation in peripheral tissues [129]. *Lactobacillus paracasei* PS23 restores mitochondrial dysfunction due to aging in mice, reduces inflammatory factor activity, and has potential therapeutic implications for decreased skeletal muscle function and quality [130]. Colonization of *Eubacterium rectale* or *Clostridium coccoides* in mice increases endurance swimming fatigue time [131]. *Veillonella atypica* was isolated from fecal samples of marathon runners. Inoculation of this strain into mice significantly increases treadmill running exhaustion time, and *Veillonella atypica* improves running time by converting exercise-induced lactate metabolism to propionic acid [132]. Transferring fecal samples from older adults (high-functioning/low-functioning group) into GF mice found significantly increased grip strength in high-functioning mice compared to low-functioning mice [52]. Treatment with *Faecalibacterium prausnitzii* increased muscle mass in high-fat-fed mice, which may be associated with enhanced mitochondrial respiration, altered intestinal microbiota composition, reduced inflammatory response, and improved intestinal integrity [133]. *Lactobacillus salivarius subspecies salicinius* (SA-03) was isolated from the gut microbiota of gold medal weight lifters and then orally fed to mice for 4 weeks, resulting in a significant improvement in muscle strength and endurance performance and an increase in liver and muscle glycogen stores [134]. Similarly, *Bifidobacterium longum* (OLP-01), isolated from gold medal winners in weightlifting, was supplemented into mice and found that OLP-01 supplementation improved grip strength and endurance in mice and significantly increased liver and muscle glycogen levels [135]. Compared with GF mice, mice in the *Bacteroides fragilis* group showed increased endurance swimming time,

reduced physical fatigue, and lower serum superoxide dismutase activity than GF mice [54].

Many studies have demonstrated that the gut microbiota can produce SCFA by fermenting indigestible carbohydrates [136]. SCFAs consist of three primary components: acetate, propionate, and butyrate; all of which are absorbed in the intestinal lumen and influence muscle and fat metabolism [137]. After feeding SCFA to GF mice, it was found that GF mice showed greater gastrocnemius muscle mass and strength, and the grip strength of GF mice was increased, which was consistent with the fact that SCFA increased muscle density, muscle mass, and function in GF mice by regulating the expression of Atrogin-1 and Murf-1 [23]. Butyrate prevents the loss of skeletal muscle mass and function during aging. After butyrate treatment, aged mice were found to have increased muscle fibers, prevented intramuscular fat accumulation, decreased fat mass in mice, and improved glucose metabolism and mitochondrial function in skeletal muscle [138].

After 13 weeks of oral administration of prebiotics consisting of a mixture of inulin plus fructooligosaccharides to elderly people aged 65 and over with frailty syndrome, these participants were found to have improved muscle strength and reduced fatigue, possibly because the prebiotics affected the body's immune function by promoting the growth of beneficial bacteria, inhibiting the growth of pathogens, and reducing other proinflammatory cytokines [139]. In triathletes, *Lactobacillus plantarum* PS128 increased endurance running performance, which was linked to changes in microbiota composition and greater levels of SCFAs [140]. *Lactobacillus plantarum* TWK10 has been shown in previous studies to improve exercise performance in mouse models, and LP10 has also been shown to do the same in human experiments. In healthy adults taking LP10 daily, it was found that LP10 significantly increased human exercise capacity in a dose-dependent manner, as well as improved fatigue-related performance and significantly increased muscle mass [141]. An observational study of older men found that a diet high in dietary fiber had higher physical performance indicators, higher scores on the short physical performance battery (SPPB), and higher grip strength, and that a diet high in dietary fiber may have a positive effect on the body's production of butyrate [142]. In a test of 32 sedentary older women over the age of 65, 12 weeks of aerobic training altered the participants' gut microbiota diversity and increased trunk muscle strength, and fecal SCFA level content has also been increased [143]. After supplementing 10 male runners with probiotic capsules daily for 4 weeks, it was found that probiotic supplements significantly increased runners' fatigued exercise time in the heat [144]. In a test of young adult female swimmers, it was found that after 8 weeks of supplementation with probiotic yogurt, the athletes' aerobic performance improved [145].

## 5. Conclusion and Future Perspectives

The role of the gut microbiota–muscle axis plays a crucial role in both humans and animals. The gut microbiota interacts with skeletal muscle through inflammatory immunity, autophagy, protein anabolism, energy, lipids, neuromuscular connectivity, oxidative stress, mitochondrial function, and endocrine and insulin resistance, thus affecting the physiological functions of the body (Figure 7). Specifically, the host's diet provides nutritional resupply to the gut microbiota, which maintains the structural integrity and the health of the gut, and participates in and mediates nutrient absorption and metabolism in the gut, which provides the material basis for muscle growth and development. Substances such as neurotransmitters, SCFAs, and bile acids produced by the metabolism of the gut microbiota regulate energy consumption and storage through the nervous and circulatory systems, providing energy for muscle development. The gut microbiota also influences the secretion of insulin, glucocorticoids, and leptin through the endocrine system, hormones that are important regulators of muscle growth and development. In addition, disturbance of the gut microbiota and invasion of exogenous harmful substances can lead to the impaired intestinal barrier and increased secretion of proinflammatory cytokines, which can negatively affect muscle growth and development.

Dietary supplementation, probiotics and/or prebiotics, SCFAs, and exercise can influence the composition of the gut microbiota, improving skeletal muscle mass and function. Although there is now a large body of research demonstrating a strong link and communication between gut microbiota and muscle tissue, there are no clear experiments showing which type or types of probiotics and/or prebiotics, SCFA, promote muscle growth and development, and there is also a lack of research on the quantitative nature of supplements.

To validate the above influencing factors and the mechanisms involved, a large number of high-quality interventional experimental studies are needed to demonstrate how dietary supplementation, probiotics and/or prebiotics, SCFAs, and exercise affect the gut microbiota. It is believed that as research methods continue to advance, the understanding of the gut microbiota–muscle axis will become more advanced. By regulating the gut microbiota, people can improve several diseases caused by reduced skeletal muscle mass and function.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Guangyao Li and Binghui Jin contributed equally to this work.

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