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The role of gut microbiota in bone homeostasis

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Abstract

The gut microbiota (GM) is referred to as the second gene pool of the human body and a commensal, symbiotic, and pathogenic microorganism living in our intestines. The knowledge of the complex interaction between intestinal microbiota and health outcomes is a novel and rapidly expanding the field. Earlier studies have reported that the microbial communities affect the cellular responses and shape many aspects of physiology and pathophysiology within the body, including muscle and bone metabolism (formation and resorption). GM influences the skeletal homeostasis via affecting the host metabolism, immune function, hormone secretion, and the gut-brain axis. The premise of this review is to discuss the role of GM on bone homeostasis and skeletal muscle mass function. This review also opens up new perspectives for pathophysiological studies by establishing the presence of a 'microbiota-skeletal' axis and raising the possibility of innovative new treatments for skeletal development.

Keywords

Gut microbiota; Bone formation; Probiotics; Immune system; Osteoporosis; Skeletal muscle function

Introduction

The whole of commensal, symbiotic and pathogenic living microorganisms residing in the gastrointestinal tract, typically lining the mucosal surfaces of the host, is known as the gut microbiota (GM).[1] The gut microbiome is composed of trillions of microorganisms that live in the gut and building mutually beneficial relationships with the host.[2,3] The GM is acquired entirely from the mother during birth, and is subject to change under different environmental factors such as diet, age, travel, use of certain medications and disease conditions.[1,4] Furthermore, this microbiota can function as multicellular organ which influences hosts in a wide variety of biological processes in various ways,[2] including

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gut physiology,[5] nutrient production and absorption,[6] host growth,[7] metabolic functions,[8] immune system function and inflammatory processes,[9] energy balancing,[10] and brain-behavior.[11] Additionally, alteration of gut microbiota composition has been associated with the pathogenesis of several complex diseases, such as type 1 and type 2 diabetes,[12,13] irritable bowel syndrome,[14] colorectal cancer[15], transient ischemic attack[16], and rheumatoid arthritis.[17]

Metabolic bone diseases like osteoporosis are characterized by increased microstructural destruction of bone tissue and low bone mass, which leads to bone fragility.[18] It is reported that osteoporotic fractures are linked with increased mortality and substantial economic loss. Over the age of 50, 10.2 million Americans affected by osteoporosis and while 43.4 million Americans have low bone mass, according to the National Osteoporosis Foundation[19]. As per World Health Organization (WHO) recommendation, the common diagnostic criteria solely depends on the lumbar vertebrae 1~4 and femoral neck as measured by dual- energy X- ray bone densitometer.[2] It is well documented that bone mass increases after birth and peaks in adulthood. This increase of bone mass occurs from mid-late adulthood, and is greatly affected by lifestyle factors as well as age and biological sex.[20] Bone metabolism is balanced by the bone formation and resorption. When the bone formation is dominant, it is characterized by bone formation and anabolism, otherwise, it's directed to resorption and catabolism.[2,18] It is understood that host metabolic pathways, immune systems, and the hormonal environment can affect this bone metabolism balance, and the gut microbiota could also able to affect these pathways [21]. For easy maintenance of healthy bone, the intestinal absorption of calcium and vitamin D is essential. The work of Wallace et al., (2017) suggested that probiotics can improve calcium absorption and maintain gut pH.[22] Similarly, fructose, oligosaccharides, and soluble corn fibers (SCF) can improve the calcium absorption efficiency.[23]

Emerging evidence suggesting that the use of probiotics is beneficial to the host system in various ways.[2] The majority of experimental data showed that modulation of GM by the use of probiotics can promote bone homeostasis in different pathological settings, such as sex steroid-associated bone loss in mice.[2,23–25] Others have shown using yogurt that contains different probiotics (*Lactobacillus* species) that these probiotics can protect bone health, indicating that dairy product consumption may lead to a higher peak bone mass.[26] The natural prebiotics (typically high-fiber foods) is enriched in vegetables, fruits, and grains that mainly contain high fiber. Among them, fructo- oligosaccharides and inulin- type prebiotics can improve the beneficial bacteria in the gut and promote the absorption of minerals by lowering the pH of the intestines. This led to an increase in bone formation.[27]

This current review summarizes the evidence on the GM may play a vital role in bone turnover and density by influencing the host metabolism, immune system, endocrine milieu, and gut-brain axis and how GM manipulation employing probiotics, prebiotics and antibiotics treatment may influence bone health. Furthermore, the review covers some aspects of knowledge on probiotics intervention that could improve skeletal muscle function.

The gut microbiome regulation on bone homeostasis by influencing host metabolism

The growing concern that recent lifestyle innovations, notably long-term dietary intake of the typical western diet (high fat/high sugar) alters the structure and metabolic activity of microbiome that resides in the human gut.[28] These diets caused a change in the microbial communities that contribute to growing epidemics of chronic illness/afflictions such as obesity and inflammatory bowel disease.[29,30] David et al., (2014) also suggested that the population of *Bifidobacteria* species was indeed increased following a high-fiber and fructo-oligosaccharides diet rather than an animal-based diet. In contrast, exogenous supplementation of probiotics can affect host metabolism and may protect the gut epithelial cell and maintain gut-barrier integrity.[28] In this section, we emphasize several aspects of GM mediated host metabolism to influence bone turnover.

1. Short-Chain Fatty Acids (SCFAs)

The GM produces several metabolites through fermentation of undigested prebiotic food by either modifying host products or *de novo* synthesis. Among these molecules, short-chain fatty acids (SCFAs, propionate, and butyrate) are the most widely investigated and have anti-inflammatory effects in the intestinal mucosa.[1, 31] In contrast, intestinal bacteria (*Clostridia and Peptostreptococci* species) also produce SCFAs through amino acid fermentation.[31] SCFAs can activate several signaling cascades such as AMP kinase and free fatty acid receptors 2/3 (FFAR2/3) or G- protein- coupled receptors 43 and 41.[32] Likewise, a high oligosaccharide diet indeed changes the microbial composition and increases SCFAs production. The butyrate also activates the GPR109A/HCA2 signaling in immune cells. Both butyrate and propionate regulate gene expression by inhibiting histone deacetylases (HDAC3, HDAC4), which led to the activation of Treg function and gut protection.[33, 34] The work of Yan et al., (2017) reported that SCFAs produced by microbiota induces the hormone-insulin-like growth factor 1 (IGF-1), which promotes bone growth, suggesting a mechanism by which microbiota affects bone health.[3] Others have shown that SCFAs are regulators of osteoclast metabolism and bone mass *in vivo*. Treatment of mice with SCFA (propionate and butyrate) significantly increases bone mass and prevents post-menopausal and inflammation-induced bone loss. Mechanistically, propionate and butyrate induce metabolic reprogramming of osteoclasts resulting in the downregulation of osteoclast bone resorption activity.[35] Tyagi et al., (2018) show that the administration of butyrate in the mouse model increases bone mass by activating Wnt signaling in osteoblasts.[36] Therefore, SCFAs may represent new methods of intervention for the prevention of bone disease, osteoporosis (Table 1). The mechanisms of GM derived SCFAs on bone homeostasis are shown in Figure 1.

2. Bile acid

Bile acids are small metabolic molecules that are produced in the liver. They are secreted to the small intestine to participate in the absorption of dietary lipids. Emerging evidence has suggested that the gut microbiome plays a significant role in bile acid metabolism.[2, 37] In the gut, primary bile acids undergo biological transformation into secondary bile acids

(primarily lithodeoxycholic acid and deoxycholic acid) via anaerobic bacteria.[38] During enterohepatic recirculation, bile acids spread over in the systemic circulation and can reach every organ in the body, including the bone. A growing body of evidence suggests that bile acids regulate skeletal homeostasis through various signaling on osteoblasts and osteoclasts. In particular, *in vitro*, activation of FXR signaling by bile acids (chenodeoxycholic acid) or FXR agonists (Fexaramine) significantly enhanced osteoblastic mineralization through the upregulation of Runx2 and enhanced extracellular signal-regulated kinase (ERK) and β-catenin signaling.[39] Upon bacterial transformation of secondary bile acids, it acts as an agonist of the membrane- bound G- protein- coupled receptor (TGR5) of intestinal cells and increases the production of glucagon- like peptide- 1 (GLP- 1). This leads to the secretion of calcitonin by thyroid cells via paracrine action, thus inhibiting bone resorption. GLP- 1 also can stimulate the proliferation and differentiation of osteoblasts.[40] Monohydroxylated secondary lithocholic acid (LCA), a derivative bile acid produced by 7- dehydroxylation of intestinal bacteria, acts as vitamin D receptor (VDR) ligand,[41] and affects bone metabolism.[42] Excessive deposition of LCA can damage osteoblast mitochondrial activity and reduce cell viability.[42] Besides, LCA reduces vitamin D effects in osteoblasts and associated with decreased osteocalcin and RANKL gene expression.[43]

3. Calcium absorption and intestinal barrier integrity

GM can affect the absorption of nutrients such as calcium and vitamin D that is required for skeletal development. Calcium is the dominant mineral for bone health and its absorption is facilitated by vitamin D. Calcium is absorbed by the intestinal cells via an active transcellular pathway or passive paracellular diffusion, depending on the level of calcium present in the cell, and is deposited as calcium hydroxyapatite (Ca10[PO4]6[OH]2) in bones and teeth.[44] Either of calcium or vitamin D deficiency leads to severe skeletal abnormalities.[45] Clinical study has revealed that high consumption of dietary calcium (47.4 mmol/day compared to the recommended 22.5 mmol/day) showed decreased bone resorption in adolescent girls. In addition to these findings, the study suggests that a low calcium diet alone is sufficient to enhance bone resorption and impaired trabecular bone formation in the rat model.[46]

It has been reported that fermentation of dietary prebiotics to SCFAs by the gut microbiota results in higher calcium absorption.[47] Studies in adolescents found that consumption of different prebiotics diets such as galacto-oligosaccharides (GOS) and soluble corn fiber (SCF), both of which can be fermented to SCFA, led to increased calcium resorption (Table 1). This increased calcium absorption is associated with relative abundances of *Parabacteroides*, *Bifidobacterium*, *Bacteroides*, *Butyricicoccus*, *Oscillibacter*, and *Dialister* species measured in feces.[48–50] Additionally, a clinical trial reported that SCF consumption in post-menopausal women showed a positive-response effect on bone calcium retention and observed a significant increase in bone-specific alkaline phosphatase activity.[51] In another study, GOS feeding in the experimental rat model resulted in increased calcium absorption and higher trabecular volumetric bone mineral density (vBMD) in both the distal femur and proximal tibia with consequently greater bone strength.[52] Prebiotic inulin also produced an enhancement of calcium absorption and cortical and cancellous bone density compared to oligosaccharides in the growing rat model.[53] Additionally, a specific probiotic bacterium

Lactobacillus salivarius stimulated calcium uptake by enterocytes in a Caco-2 cell culture model [54] and probiotics bacterial species has a beneficial function on bone mass function (Table-1).

The intestinal epithelial barrier is a one-cell-thick internal lining of different types of epithelial cells of the gut.[55] Several tight junction proteins seal the paracellular pathway and conduct gate and fence functions in epithelial cells. Underneath this layer, a thin layer of connective tissue called the lamina propia is present, which cherishes the healthy communication between the microbiome and the immune system. Besides, the mucosal layer is a chemical barrier that provides the first layer of defense in the epithelium, is formed by a layer of mucus and limits the contact between the microbiome and epithelial cells. The absence of the mucus layer leads to intestinal inflammation and the onset of various metabolic diseases.[55,56] Hamilton et al., (2015) demonstrated that alteration of the gut microbiota composition causes intestinal permeability and metabolic disorders.[57] The dysfunction of the intestinal mucosal barrier may lead to an increase in serum levels of lipopolysaccharide (LPS), resulting in metabolic endotoxemia.[58] Early studies have suggested that LPS promotes bone loss in the femur *in vivo* and the survival of osteoclasts *in vitro*.[2,59] Chongwatpol P. et al., (2015) illuminated that LPS substantially decreased trabecular bone volume, lumbar vertebra bone mineral density, and the number of the vertebral bodies in the mouse model.[60]

The gut microbiome regulation on bone homeostasis by influencing the immune system

Recent studies have suggested that a close interplay between the immune system and bone metabolism, a term called “osteoimmunology,” which represents the role of immune cells or immune-related factors in modulating skeletal development.[45] Further, GM is required for the function and maturation of the immune system and also influences host health.[61, 62] Sjogren et al., (2012) discovered for the first time the relation between microbiota and bone development.[63] This study also confirmed that altered immune status in germ-free mice (decreased pro-inflammatory cytokines, fewer CD4+ T cells and reduced osteoclast/precursor cells in bone marrow) may account for the higher bone mass formation than in CONV-R mice.[63] Other studies have shown that intestinal filamentous bacteria were able to increase IFN-γ production and IL-17, which play an essential role in bone formation *in vivo* and rescues osteoporosis in mice following ovariectomy (OVX).[64, 65] These studies suggest that the gut microbiota regulates bone metabolism by altering host immune status.

Th17 cells are a subset of CD4+T cells, which produces IL-17 and IL-22 which are important for innate immunity. It induces the intestinal epithelial cells to produce antimicrobial peptide against the pathogens.[2] Following transplantation of segmented filamentous bacteria in GF mice increased the number of Th17 cells and maintain the antimicrobial action in the epithelium.[66] $CD4^{+}FOXP3^{+}$Treg cells are associated with systemic immunity and stable at the intestinal mucosa. Also, many have shown the close relationship between intestinal microflora and Treg cells.[63] Indigenous *Clostridium* species colonization in gnotobiotic mice resulted in increased accumulation of colonic

Tregs.[67] Besides, CD4+CD25+Foxp3+Treg cells are able to suppress osteoclast maturation/differentiation via cytotoxic T-lymphocyte associated protein 4 (CTLA-4) mediated pathway.[68] The work of Dar et al., (2018) suggested that Bacillus clausii is known to promote bone formation via increasing Treg cells in the OVX mouse model.[69] The probiotic bacteria Lactobacillus acidophilus inhibits the bone loss in OVX mice via modulating Treg-Th17 cell balance.[70] Additionally, the report also revealed that the microbial population indeed affects B-cell development, which produces osteoprotegerin, an inhibitor of osteoclasts for effective bone resorption.[71]. Wnt signaling plays an important role in early embryonic development, organogenesis and tissue morphogenesis. Rubinson et al., (2013) and Wu et al., (2003) reported that intestinal bacteria such as *Fusobacterium nucleatum* and *Bacteroides fragilies* are known to activate the Wnt/β- catenin signaling.[72, 73] Others have shown that loss of beta-catenin is associated with decreased bone formation.[74] Tyagi et al., (2018) also reported that Lactobacillus rhamnosus GG (LGG) treatment regulates bone anabolism via Treg cell-mediated regulation of CD8+T cell Wnt10b production.[36] The mechanisms of GM mediated bone homeostasis via immune regulation are shown in Figure 1.

The gut microbiome regulation on bone homeostasis by influencing the endocrine system

The intestinal microbiota acts as a virtual endocrine organ of the body and can engage in an interplay with the endocrine system and have a possible effect on bone homeostasis. Lack of these hormones leads to an increase in bone loss and affecting bone formation.[71] Sex hormone deficiency causes intestinal permeability and osteoclastic bone resorption in a TNF and RANKL dependent manner. The work of Li et al., (2016) demonstrated that supplementation of probiotics LGG prevents sex steroid-induced bone loss by restoring intestinal permeability in the OVX mouse model.[75] Other have shown that when estrogen deficiency or OVX mice were treated with *Lactobacillus acidophilus*, the level of bone resorption markers decreased and bone formation was improved.[24] Besides, the administration of probiotic L. reuteri prevents bone loss in both estrogen deficiency (OVX) and type 1 diabetes mouse models.[76, 77] The work of Yan et al., (2016) also reported that gut microbial colonization in GF mice significantly induced the serum insulin-like growth hormone 1 (IGF-1) level and promote bone formation and growth.[78] Therefore, more human studies are needed to confirm that intestinal microbiota can affect bone metabolism via the activity of various hormones.

Role of the microbiome on bone health via the gut-brain axis

It has been reported that GM has an essential role in the nervous system to synthesize the hormone and neurotransmitters such as serotonin (5- hydroxytryptamine, 5- HT).[11] Therefore, 5-HT signaling is important in the regulation of bone development and growth. Ducy and Karsenty et al., (2010) suggested that 5-HT produced in circulation has a negative action on bone metabolism. By contrast, when it is produced from the brain as a neurotransmitter, it promotes bone development.[79] Recent studies have reported that intestinal microbiota has a role in regulating the blood levels of 5- HT (2). The bacteria

such as *Streptococcus*, *Corynebacterium*, and *E. coli* have been proved to produce 5-HT in animal culture conditions.[80] Additionally, Sjogren et al., (2012) have shown that decreased 5-HT levels and increased trabecular bone volume/tissue volume were observed in GF mice.[63] Another study demonstrated that 5-HT levels are indeed lowered in GF mice and transplantation of intestinal microbiota can restore the levels of 5-HT in serum and colon.[81] Yadav et al., (2008) demonstrated that certain spore-forming microbes can regulate gut serotonin which in turn regulates osteoblast proliferation and bone formation via Htr1b/PKA/CREB/cyclins signaling. The detailed understanding of GM-derived serotonin action on bone metabolism is described in Figure 2.

The adipocyte-specific hormone leptin is known to regulate the many physiological processes including bone development, energy homeostasis, etc.[82] The growing body of evidence suggested that the gut microbiota regulates different physiological processes through interaction with the brain, which is called the gut-brain axis.[83] Queipoortuno et al., (2013) revealed that microbiota such as *Lactococcus*, *Mucispirilum*, *Lactobacillus*, and *Bifidobacterium* positively regulate the systemic level of leptin.[84] This released leptin production binds to leptin receptor (ObRb) that expressed in brainstem neurons, which led to decreased release of brain serotonin levels and may influence bone homeostasis.[85, 86] Therefore, the GM affects bone metabolism via diverse potential mechanisms by influencing the host metabolism, immunity, endocrine environment, and gut-brain axis. This could potentially lead to the development of a new era for effective treatment of bone disease as well as future work to be validated in the human model using GM as potential therapeutics in promoting bone health.

The gut microbiota regulation on bone mechanical function

As we discussed that gut microbiota is indispensable for maintaining bone mass and its alternation has been associated with changes in bone mass and microstructural deterioration. Bone strength is defined as the capacity of bone to respond to mechanical demands, is ultimately determined by bone distribution, microarchitecture, material composition and quantity.[44] The primary function of bone or skeleton in the body is to resist mechanical forces and impairment of the mechanical strength of bone is therefore challenged by clinical bone disease and may also lead to fragility fracture.[87] Bone mineral density (BMD) typically explains bone strength and also have been preferred phenotype to study the bone and gut interaction.[44] A recent study by Gus et al., (2017) suggested that alternation of gut microbiota not only changes bone mass but also impairs bone mechanical properties.[88] In this study, they examined bone strength using two different mouse models such as toll-like receptor 5-deficient mouse [TLR5KO]) and WT (C57Bl/6) mice were treated selected antibiotics (ampicillin and neomycin) to deplete the microbiota (ΔMicrobiota) independently. Interestingly, the data have shown that femur bending strength was less in ΔMicrobiota mice than untreated WT mice. However, there were small differences in whole bone bending strength were observed between WT and TLR5KO mice.[88] In the other study, demonstrated that probiotics strain *Lactobacillus helveticus* administration was reported to improve the bone strength in a three-point bending test of Ovariectomy rat model.[89]

Relationship between gut microbiota and dietary phosphorus metabolism

Diet is an essential environmental factor that modulates or support the digestive system and other organ function, but also shape a healthy microbial ecosystem in the GI tract.[90] Among the dietary components, phosphorus (P) is an essential nutrient that helps in both microbial and host metabolism, for example in bone development, cellular signaling, energy metabolism, membrane protein synthesis and also provides a barrier against pathogens in the gut.[91,92] Emerging evidence suggested that P can be stored as polyphosphates in bacterial cells and used as energy and metabolic processes.[90, 93] Other have shown that P acts as a coenzyme for bacterial synthesis of fibrolytic enzymes, which is essential for dietary fibre degradation in gut.[94] Komisarczuk et al. demonstrated that P supply through diet is essential for intestinal SCFA production. Further, they demonstrated that P deficiency caused a reduction in SCFA synthesis due to reduced fermentation of cellulose in the GI tract of ruminant animals in sheep.[96] Others have also shown that dietary calcium phosphate (CaPi) has a positive effect on gut eubiosis (by increasing the number of ileal and fecal Lactobacillus acidophilus) and strongly protect against Salmonella enteritidis infection in a rat model.[96] In a recent investigation by Mann et al., reported that feeding CaP-rich diets promoted bacterial growth and proliferation of Lactobacillus at the gastric pars non-glandularis of the stomach in pigs.[97] Generally, Lactobacillus is said to produce very effective bacteriocidin and organic acid, which inhibit the growth of potential pathogens such as Escherichia coli.[98–100] Therefore, CaP mediated increase of Lactobacillus in the GI tract might be essential for promoting gut microbial eubiosis and gut barrier function. However, no direct evidence is yet available demonstrating that the relationship between gut microbiota and P metabolism in bone development in mice and humans. Therefore, future research is warranted to study the role of dietary P metabolism on gut microbiota eubiosis, gut barrier integrity for better bone mass formation in osteoporotic patients.

Role of gut-microbiome on skeletal muscle mass and function

Skeletal muscle function is regulated by central nervous function via a neuro-muscular transmission.[101] and it displays marked plasticity and can respond to several environmental stimuli such as exercise and nutrition. Skeletal muscle is one of the important organs involved in glucose homeostasis and fatty acid oxidation.[102] Dysfunction in the skeletal muscle is associated with sarcopenia, muscle atrophy and consequently, it causes metabolic disorders.[103] It has been established that the GM is involved in the onset of several pathophysiological conditions such as diabetes, cancer, obesity, and osteoporosis. However, it is still not clear how GM influences skeletal muscle function. The work of Nay et al., (2019) demonstrated that gut bacteria are indispensable for the host optimal skeletal muscle function.[104] In the study, they found that GM-depleted mice following administration of broad-spectrum antibiotics, muscle endurance capacity was severely affected. Also, glucose metabolism was severely affected by GM-depletion in mice[104] Others have shown that the gut microbiota is required for skeletal muscle mass and function by comparing the skeletal muscle of germ-free (GF) mice that lacked a GM to the skeletal muscle of pathogen-free (PF) mice that has a GM[102] supporting the notion that gut bacteria is essential for maintaining skeletal muscle mass. In the study, they found that GF mice skeletal muscle showed atrophy, reduced expression of IGF-1, and skeletal muscle growth and mitochondrial

function-related genes. GF mice also showed reduced serum choline and altered amino acid mentalism in comparison to PF mice. Transplanting the GM from PF mice into GF mice increased skeletal muscle mass and improved oxidative metabolic capacity.[102] Taken together, these studies strongly support the role of GM that influences skeletal muscle mass and function in mice. One implication that might be inferred from these studies is that maintaining a healthy gut microbiota is important to the health of the muscles. The detailed study of GM's role in skeletal muscle mass and function is shown in Figure 3.

Translational potential and concluding remarks

The intimate association between the gut microbiota and skeletal metabolic processes suggests that the identification of important gut microbiota to be characterized and that have features for great clinical potential.[105] In the current scenario, the majority of studies have focused on the preventive or protective effect of probiotics, prebiotics, and antibiotics in different diseases.[106] These types of treatment have been successfully tested in the clinical conditions in many human diseases such as hypercholesterolemia, ulcerative colitis, and obesity, etc.[107–110] Additionally, the antibiotic based treatment that targets microbiome has provided greater promise for effective therapy.[106] However, not much focus has been directed toward understanding the potential of GM regulation as a treatment for musculoskeletal disease. However, this finding needs to be further validated in human clinical studies. In the future, the identification of therapeutic microbes could provide a promise for effective regulation of bone metabolism in musculoskeletal disease. Furthermore, pathogenic mutations that cause genetic bone diseases and its relation with intestinal microbial flora remain warranted and it needs to be further investigated.

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Highlights

✓ The gut microbiota promotes bone formation by influencing intestinal short-chain fatty acids metabolism.

✓ The gut microbiota promotes bone homeostasis by maintaining gut-barrier integrity and the immune system.

✓ Supplementation of prebiotic diet improves the microbial composition and short-chain fatty acids production leads to activation of regulatory T-cells function and gut protection.

✓ Supplementation of probiotics prevents bone loss in both sex steroid-deficiency and type-1 diabetic mouse model.

✓ The gut microbiota regulates osteoblast proliferation and bone formation via gut serotonin dependent Htr1b/PKA/CREB/cyclins signaling

✓ The gut microbiota promotes skeletal muscle mass and function through increased **Insulin-like growth factor-1** expression and mitochondrial function.

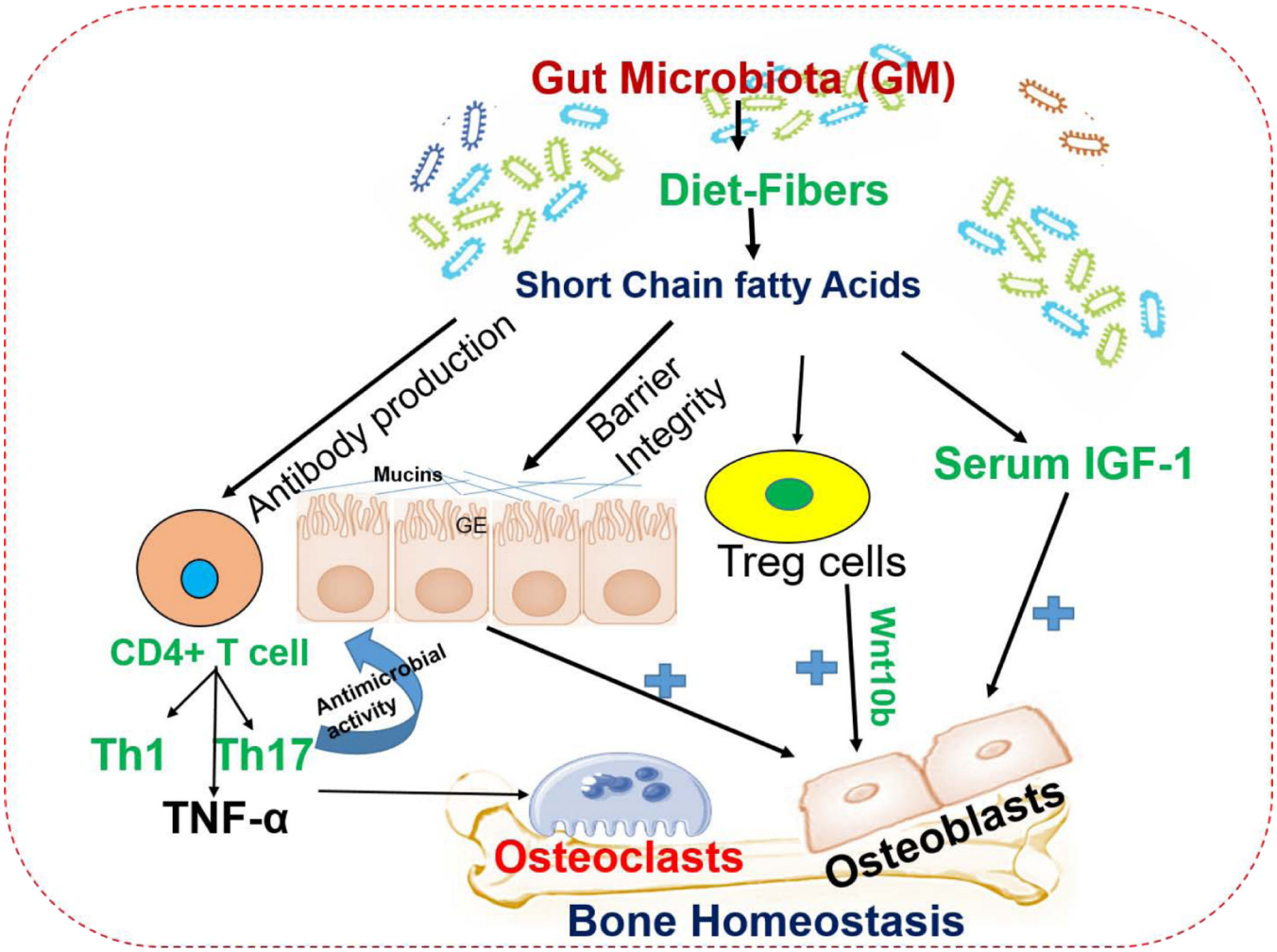

Figure 1: The influence of gut microbiota on bone metabolism through metabolite mediated barrier integrity and immune system.

SCFAs have the ability to influence Tregs development and promote bone metabolism via Wnt10 action. The production of SCFAs may be a mechanism by swhich microbial community increased the serum level of IGF- 1 which led to bone growth and homeostasis. Gut microbiota (GM) induced an increase in intestinal barrier integrity. The alternation of GM composition leads to a result in metabolic disorders. The dysfunction of the intestinal mucosal barrier may lead to an increase in serum levels of lipopolysaccharide (LPS), which could in turn increase membrane permeability, resulting in metabolic endotoxemia. Th17 cells are essential for estrogen- deficient bone loss and it produces IL-17 cytokines. The elimination of IL17 or the use of an anti- IL17 antibody may prevent bone loss from estrogen deficiency. GE: Gut epithelial cells, Treg cells: T regulatory cells, Th1: T helper-1, Th17: T helper-17 cells, SCFAs: short-chain fatty acid, IGF-1: Insulin-like growth factors.

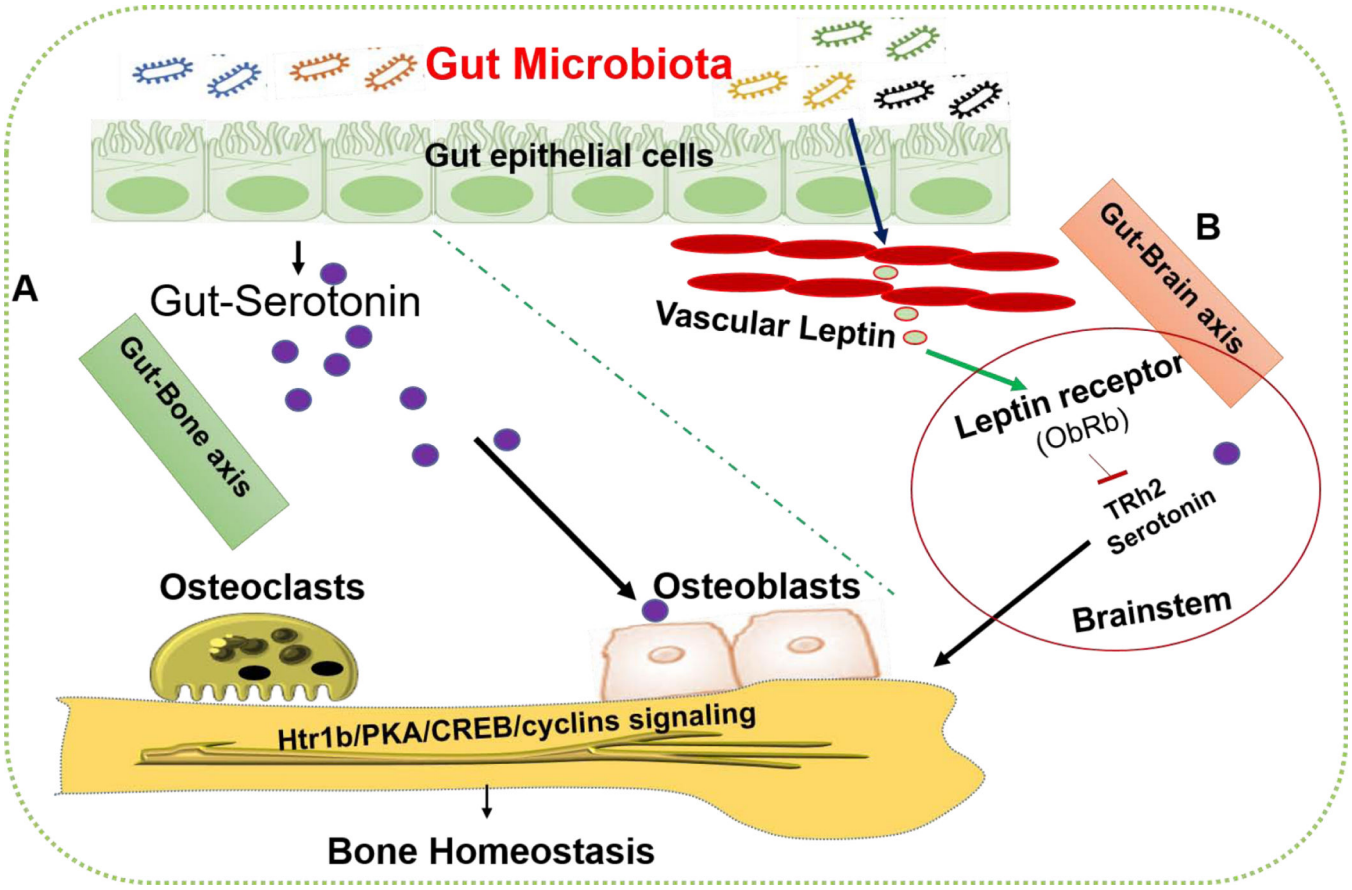

Figure 2. Effect of gut microbiota on bone homeostasis via biphasic action of serotonin.

(A) Certain microbiota such as spore-forming microbes regulates the level of serotonin in gut, serum and fecal matters. This released serotonin binds to the htr1b receptor of osteoblasts membrane and regulates its proliferation via Htr1b/PKA/CREB/cyclins signaling. **(B)** Another group of bacterial species (Lactococcus, Mucispirillum, Lactobacillus, and Bifidobacterium) can positively regulate peripheral/vascular leptin level which in turn regulates bone homeostasis via brain serotonin action. htr1b: 5-hydroxytryptamine receptor 1B, PKA: protein kinase A, CREB: cAMP response element-binding protein.

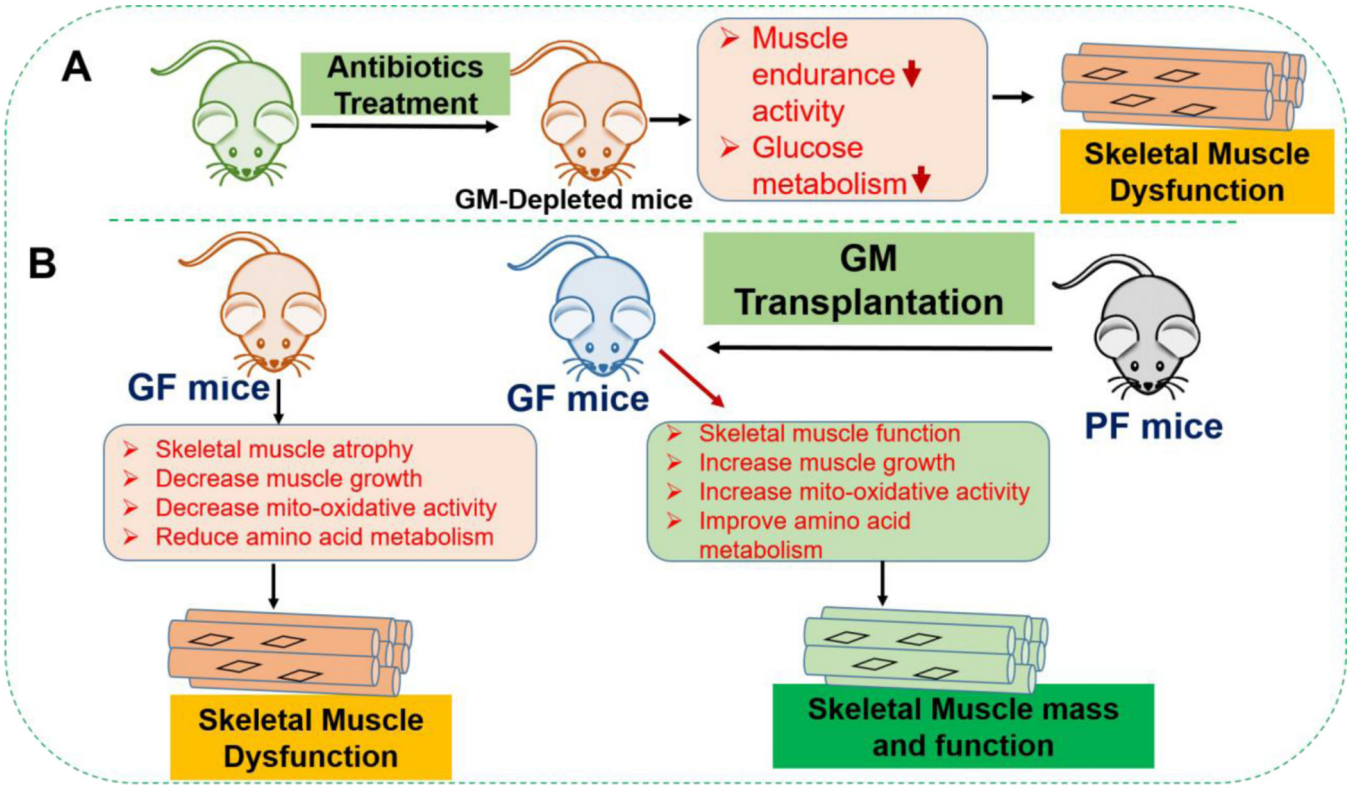

Figure 3: The gut microbiota influences skeletal muscle mass and function in mice.

(A) The consequence of gut microbiota depletion after antibiotic treatment for 21 days, results in muscle endurance activity as well as glucose metabolism was severely affected. **(B)** It compares the skeletal muscle phenotype and function between GF mice and PF mice. Compared to PF mice, GF mice have increased muscle atrophy and reduced muscle growth, mitochondrial oxidative activity and amino acid metabolism. Transplantation of GM from PF mice to GF mice improved the skeletal muscle mass and oxidative metabolic function. GM: Gut microbiota, GF: Germ-free, PF: Pathogen free mice.

Table 1

Modulation of gut microbiota (GM) via probiotics, prebiotics, and antibiotics treatment affects the musculoskeletal parameters

Therapeutic agent	Animal study	Mechanism of action	Effect on the skeletal system	References
Prebiotics				
SCFA	C57BL/6J mice	Change in the metabolic state of pre-osteoclasts	Increased bone BMD and bone mass	77
SCFA	BALB/c mice	Increase in serum IGF-1	Acute bone resorption and further normalize bone mass	36
GOS	C57BL/6J mice	Increased calcium retention	Increased bone BMD and bone strength	53
Inulin- type prebiotics	C57BL/6J mice	Absorption mineral contents and improve the beneficial bacteria in the gut	Bone formation increases	28
Probiotics				
Lactobacillus GG	C57BL/6J mice (OVX)	-Attenuates intestinal inflammation - Improves TJ destruction and gut epithelial permeability	Bone formation increases	23
Lactobacillus paracasei	C57BL/6 OVX mice	Decreases inflammatory cytokines	Increases bone mass	25
Lactobacillus reuteri ATCC PTA 6475	BALB/c mice	Decreased bone resorption	Promotes bone formation	75
Lactobacillus Plantarum WJL	BALB/c mice	Increase in serum IGF-1	Increased femur length	78
Lactobacillus acidophilus ATCC4356	BALB/c mice	Increased number of Treg cells and decreased number of Th17	Promotes bone mass	70
Lactobacillus helveticus ATCC 27558	Sprague-Dawley rat model	BMD and bone strength increases	Promotes bone mass	89
Bifidobacterium longum	OVX Sprague-Dawley rat model	BMD increases	Promotes bone mass	26
L. paracasei and L. plantarum	C57BL/6 OVX mice	Decreases inflammatory cytokines	Inhibits bone loss	25
VSL#3	C57BL/6 OVX mice	Attenuates intestinal and BM inflammation.	completely inhibits bone loss	23
Bacillus clausii	BALB/c mice	Increased number of Treg cells and decreased number of Th17	Inhibition of bone loss	69
Antibiotics				
penicillin, vancomycin, penicillin plus vancomycin, chlortetracycline	C57BL/6 young mice	Increase intestinal incretin, GIP and adiposity	Increases BMD	107

BMD: Bone mineral density, SCFA: Short-chain fatty acid, GOS: Galacto-oligosaccharides, GIP: Glucose-dependent insulinotropic Polypeptide, TJ: Tight junction, IGF-1: Insulin-like growth factor-1.

VSL#3 contains a mixture of bacteria such as *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus, and Streptococcus thermophiles*.