The impact of the intestinal microbiome on bone health

Jian Zhang[1,2,§], Yanqin Lu[1,2,§,*], Yanzhou Wang[3], Xiuzhi Ren[4], Jinxiang Han[1,2,*]

[1] *Key Laboratory for Biotech-Drugs Ministry of Health, Key Laboratory for Rare & Uncommon Diseases of Shandong Province, Shandong Medicinal Biotechnology Centre, Shandong Academy of Medical Sciences, Ji'nan, China;*
[2] *School of Medicine and Life Sciences, University of Ji'nan-Shandong Academy of Medical Sciences, Ji'nan, China;*
[3] *Department of Paediatric Surgery, Shandong Provincial Hospital, Ji'nan, China;*
[4] *Department of Orthopaedic Surgery, The People's Hospital of Wuqing District, Tianjin, China.*

Summary	Intestinal microbial flora, known as the second gene pool of the human body, play an important role in immune function, nutrient uptake, and various activities of host cells, as well as in human disease. Intestinal microorganisms are involved in a variety of mechanisms that affect bone health. Gut microbes are closely related to genetic variation, and gene regulation plays an important part in the development of bone-related diseases such as osteoporosis. Intestinal microorganisms can disrupt the balance between bone formation and resorption by indirectly stimulating or inhibiting osteoblasts and osteoclasts. In addition, intestinal microorganisms affect bone metabolism by regulating growth factors or altering bone immune status and can also alter the metabolism of serotonin, cortisol, and sex hormones, thereby affecting bone mass in mice. Moreover, probiotics, antibiotics, and diet can change the composition of the intestinal microbial flora, thus affecting bone health and also potentially helping to treat bone disease. Studying the relationship between intestinal flora and osteoblasts, osteoclasts, and bone marrow mesenchymal stem cells may provide a basis for preventing and treating bone diseases. This paper reviews recent advances in the study of the relationship between intestinal microflora and bone disease.

Keywords: Intestinal microbial flora, hereditary bone disease, bone health, bone-related cells

1. Introduction

Microbial groups, which are symbiotic in the human body or on the surface of the human body and cause various diseases under certain conditions, are collectively known as the human microbiome. This concept was first proposed by Lederberg *et al.* (*1*). There are many types of bacteria that populate the intestines of humans and animals. For example, the number of microorganisms in the body and the proportion of cells can reach a ratio of 10:1, and there are more than 10 trillion bacteria (*2*) that encode 100-fold more genes than those in the human genome (*2*). Thus, the intestinal microbial flora are known as the "second gene pool" of the human body (*2*).

[§]These authors contributed equally to this work.
*Address correspondence to:
Dr. Jinxiang Han and Dr. Yanqin Lu, Shandong Medicinal Biotechnology Centre, Shandong Academy of Medical Sciences, 18877 Jingshi Road, Ji'nan 250062, China.
E-mail: samshjx@sina.com (JH); yanqinlu@yahoo.com (YL)

Like human organs, human intestinal microflora have significant effects on immune function, nutrient uptake, and various life activities of host cells (*3*) as well as on various diseases and conditions in the human body. The microbial flora of the gut affect an organism by changing the balance of bacteria and metabolites (*4*), which can lead to changes in metabolic processes and induce the development of various diseases such as ulcerative colitis in inflammatory bowel disease (*5*) and Crohn's disease (*6*). Microbial flora can also cause many other common diseases including obesity (*7*), diabetes (*8*), and other endocrine system diseases, as well as cardiovascular diseases (*9*). Intestinal flora also affect diseases such as immune system-induced rheumatoid arthritis (*10*) and systemic lupus erythematosus (*10*). A study on cancer found that *Bifidobacterium* can improve anti-tumor immunity in mice (*11*). Conversely, *Fusobacterium nucleatum* can promote colorectal cancer resistance chemotherapy in cancer treatment (*12*). In addition, changes in intestinal microbial flora can induce inflammatory reactions in the host and change neurotransmitter metabolism, resulting

in neurological dysfunction (*13*), depression, mental decline, and other problems (*14,15*). An imbalance in intestinal flora affects many diseases, whereas intestinal flora in homeostasis can prevent diseases. For example, intestinal microbial flora attach to the intestinal mucosa to form a protective barrier to defend against invasion by exotic pathogenic microorganisms (*10*). Intestinal flora can also stimulate an organism to produce an immune response to microbial antigens and to produce more lymphocytes (*16*). The resulting immunoglobulin G (IgG) antibodies can induce the organism to eliminate pathogenic microorganisms by identifying Gram-negative bacteria and neutralizing toxins and viruses (*17*), thereby promoting maturation of the immune system. This protects against inflammation and infection and improves immune function in the host (*6,17*). The current review provides updated information on the intestinal microbial flora and their affect on bone health.

2. Intestinal microbial flora and bone disease

2.1. Intestinal microbial flora and genetic factors

The relationship between human intestinal microbes and host genetic variation (*18-22*) is one in which the latter affects the composition of human intestinal microbes (*19,20*). A single-nucleotide polymorphism (SNP) (C/T-13910) of the European lactase (*LCT*) gene and SNPs (G/C-14010, T/G-13915, and C/G-13907) in the African *LCT* gene are associated with the abundance of *Bifidobacterium* in the gastrointestinal tract. These SNPs were found to significantly enhance transcription of the *LCT* gene promoter *in vitro* and to facilitate the hydrolysis of lactose in the gastrointestinal tract by lactase, thus directly affecting the persistence of LCT (*23*). *Bifidobacterium* in the gastrointestinal tract can also metabolize host lactose (*24*). *LCT* gene mutations may indirectly regulate the abundance of *Bifidobacterium* in the gastrointestinal tract by altering its lactose levels. Ruminococcaceae and Lachnospiraceae are the two main families of human intestinal microorganisms and are more similar in identical twins than in fraternal twins (*25*). The host genotype can regulate the abundance of many microbial flora.

Microbial diversity is controlled by both environmental and host genetic factors and is related to multiple diseases. Toll-like receptor (TLR) 5 gene-deficient mice have signs of metabolic syndrome including hyperlipidemia, hypertension, and obesity. After intestinal microflora were transferred from TLR5 gene-deficient mice to wild-type aseptic mice, the aseptic mice also exhibited the characteristics of metabolic syndrome, which were related to changes in microbial flora in the gut (*26*). Next-generation sequencing of genes in the gastrointestinal tract and

quantitative trait loci (QTL) mapping have revealed that some host genes can change gut immunological profiles and modulate the balance between gut microbial communities. For example, the interferon gene-rich QTL region located on chromatin 4 modulates *Firmicutes* and *Bacteroidetes*, which are dominant BXD strains among gut microbes. Interleukin-1 receptor-associated kinase (IRAK) 4 modulates Rikenellaceae while TGF-β3 modulates Prevotellaceae (*27*).

Genetic variations in the genome may occur during the process of adapting to the environment, causes specific changes in microbial groups. Some loci can control a single microbial species, the associated taxa of certain microorganisms, and some of the associated microbial populations that are presumed to be more efficient and widely distributed (*27*). In addition, the gut flora have certain effects on host gene mutations. Some strains of *Escherichia coli* have a polyketide synthase (*PKS*) gene island that induces DNA mismatch repair in host intestinal epithelial cells by encoding gene toxins that cause tumors (*28*). Moreover, the superoxide anion produced by *Enterococcus* also causes host DNA damage and genomic instability, resulting in intestinal epithelial cell mutations that trigger colorectal cancer (*29*).

2.2. Intestinal microbial flora and osteoporosis

Osteoporosis is a metabolic bone disease that results in decreased bone mass and bone mineral density and that induces changes in bone microstructure (*30*). This disease is mainly influenced by heredity and environmental factors, and many studies have confirmed that there is a certain relationship between intestinal microbial flora and osteoporosis (*30*). An estrogen deficiency leads to bone loss-induced osteoporosis, and the use of surgical ovarian resection (OVX) or sexual hormone inhibition causes a lack of estrogen in mice (*31*). When OVX mice were treated with *Lactobacillus acidophilus*, the level of bone resorption markers decreased and osteoclast formation was inhibited (*31*). In addition, the number of T lymphocytes in OVX mice decreased and osteoclast formation was inhibited by *Lactobacillus reuteri* (*32*). Type I diabetes can also induce osteoporosis. A study reported that *L. reuteri* can inhibit the expression of tumor necrosis factor (TNF) and Wnt10b, preventing bone loss and bone marrow adiposity in a mouse model of type I diabetes (*33*).

Recent studies have found that intestinal microflora can regulate growth factor insulin-like growth factor (IGF)-1 levels, and thus regulate bone formation and absorption in young and middle-aged mice. The levels of IGF-1 decreased when antibiotics were used to destroy intestinal microflora in young mice (*34*). In addition, intestinal microbes were able to mediate the regulation of bone metabolism by altering bone
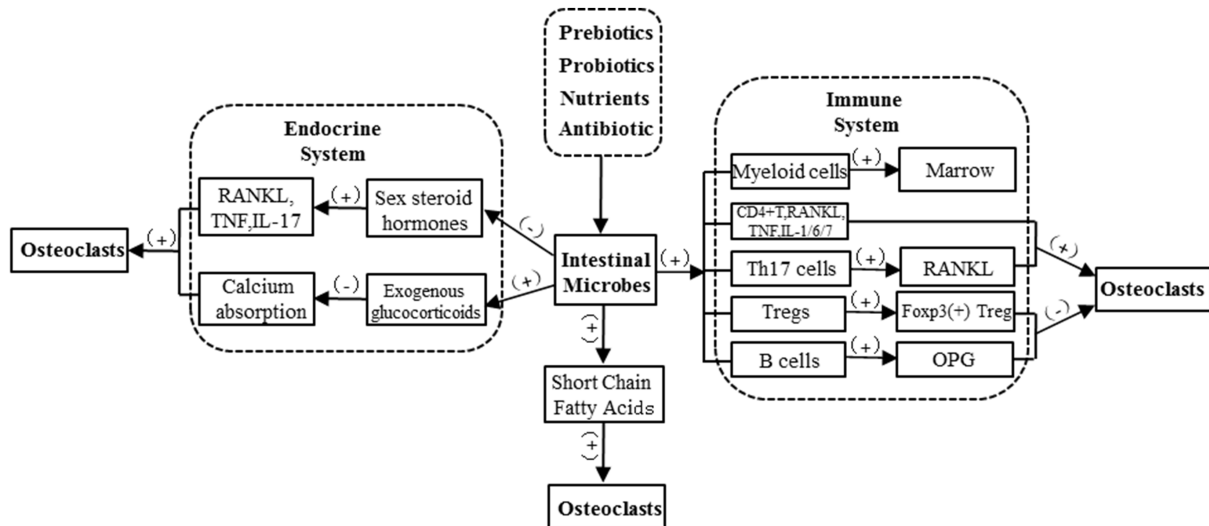
Figure 1. Mechanisms by which intestinal microbes affect bone health.

immune status (*35*). Sterile mice had a significant increase in bone mass and a significant decrease in the number of osteoclasts, the number of CD4+ T cells derived from the bone marrow, and the number of osteoclast precursor cells compared to normal mice. While the number of osteoclasts, CD4+ T cells, and osteoclast precursor cells returned to normal in 3-week-old sterile mice treated with intestinal microbial flora and the quality of bone trabecular and cortical bone decreased, the expression of inflammatory cytokines in bone decreased significantly (*35*).

Intestinal disorders can cause inflammatory bowel disease (*36*), which can increase the risk of osteoporosis and related brittle fractures (*37*). The reduction in bone associated with inflammatory bowel disease is mainly due to insufficient calcium absorption and a decrease in the cycling levels of vitamins D and K (*38*). In addition, the inflammatory reaction caused by the lack of sex steroids can promote bone resorption, resulting in the loss of trabecular bone. The probiotic *Lactobacillus rhamnosus GG* (LGG) can reduce inflammation in the intestine and bone, improve intestinal permeability, and prevent bone loss (*39,40*).

When an imbalance of intestinal flora homeostasis occurs during intestinal inflammation, the intestinal absorption of vitamin D can help to treat enteritis, thereby reducing intestinal permeability and avoiding the impact of that imbalance on bone health (*41*). Vitamin D has beneficial effects in bones (*42*). When calcium supply is sufficient, the absorption of vitamin D and its metabolites in the gut can maintain the homeostasis of intestinal flora, thus improving the calcium balance and promoting mineral deposition in the bone matrix. In the absence of calcium, vitamin D can enhance bone resorption while inhibiting bone mineralization, thus maintaining blood calcium homeostasis at the expense of bone mass. Therefore,

adding a proper amount of calcium to the diet can help treat inflammation and maintain the homeostasis of intestinal microbes to prevent osteoporosis mediated by vitamin D (*42,43*).

3. The mechanism(s) by which intestinal microbes affect bone health

3.1. *Immune-mediated mechanisms*

Intestinal microflora affect bone remodeling, bone mass accumulation (*44*), and bone health in a variety of ways (Figure 1). They can impact bone health *via* the immune system (*35*), which regulates the development of bone marrow cells and inflammatory cytokines. In germ-free (GF) mice, the number of myeloid cell progenitors decreased and the response to *Listeria monocytogenes* was impaired. These defects were remedied by transplantation of complex microbiota. Therefore, gut bacteria mediate innate immune cell development by increasing hematopoiesis (*45*). GF mice have fewer granulocyte-monocyte progenitors (GMPs). GF mice also have fewer CD11b^{+} Ly6C^{+} mononuclear cells and CD11b^{+} Ly6G^{+} granulocytes in the bone marrow than do specific pathogen-free (SPF) mice, but the formation of bone marrow was improved by the administration of serum isolated from SPF mice (*46*).

In addition, many studies have recently revealed the close relationship between intestinal microflora and regulatory T cells (Tregs) and helper T cells (Th cells) (*31,47-49*). *Clostridium* colonization in gnotobiotic mice resulted in preferential accumulation of Tregs in colonic lamina propria (*50*). The presence of intestinal bacteria might affect both the number and function of Tregs. Transforming growth factor beta (TGF-β), a key regulator of Treg development, is abundant in the colon in its active form. Similar findings were obtained with

regard to other Treg-inducing molecules within the colon (*50*). Moreover, the induction of interleukin (IL)10-expressing Foxp3+ Tregs was specifically restricted in *Clostridium* but not in other bacteria. In addition, CD4+, CD25+, and Foxp3+Treg cells suppress macrophage colony-stimulating factor and osteoclast formation. This action is mainly through cytotoxic T-lymphocyte associated protein 4, an anti-osteoclastogenic molecule that binds osteoclast precursor cells and that inhibits its differentiation (*51-53*).

The bacterial segment in the gut is the driving force for Th17-assisted cell differentiation, which plays an important role in bone loss induced by rheumatoid arthritis (*54,55*). Intestinal flora disorders disrupt the balance of the pro-osteoclastogenic pathway and induce osteoclast-mediated bone loss in multiple ways, including the differentiation and inhibition of anti-osteoclastogenic Th1, Th2, and Treg subsets. This induces the differentiation of Th17 cells, which produce and increase RANKL expression on stromal cells, much like inflammatory cytokines (*52,56-58*). In addition, the number of osteoclast precursor cells also increases, thus promoting osteoclast differentiation (*47*). Microbial populations affect B cell development as well as bone resorption by osteoprotegerin, an inhibitor of osteoclasts produced by B cells (*59*).

3.2. Endocrine-mediated mechanisms

Intestinal microflora as a virtual "endocrine organ" (*60*) have certain effects on bone health. Sex hormones are vital in maintaining bone homeostasis, and a lack of these hormones can result in a decrease of microbial flora in the gut, thus increasing bone loss and affecting bone formation (*40*). In addition, a lack of sexual hormones increases the activity of osteoclasts and osteoblasts, although osteoclasts are more affected and bone loss is significant (*61*).

Sex steroid deficiency increases intestinal permeability and levels of the osteoclastogenic cytokines TNF, RANKL, and IL-17 in a murine model while GF mice were protected from bone loss (*39*). Supplementing the indigenous microbiota with the probiotic *LGG* prevents sex steroid-induced bone loss by inhibiting intestinal permeability. However, ingestion of nonprobiotic or mutant *LGG* eliminates this protective action. These findings indicate that gut microbiota serve as a dual role in sex steroid deficiency-induced bone loss (*39*). Moreover, excess glucocorticoid reduces the number of both osteoblasts and osteoclasts, it prolongs the lifespan of osteoclasts, and promotes apoptosis of osteoblasts and osteocytes (*62,63*).

3.3. Changes in intestinal flora affect bone health

Changes in intestinal microbial flora and their metabolites affect bone health directly or indirectly (*47*). The effects of microbial flora (*64*), probiotics (*65*), antibiotics (*66*), and dietary nutrition (*67*) also affect bone health. Probiotic dietary supplements containing *Bacillus licheniformis* and *B. subtilis* increased the diversity of intestinal flora, causing the tibial density in chickens to significantly increase compared to chickens fed a normal diet (*68*). Antibiotics can affect intestinal flora, and these effects are related to age, sex, and duration of treatment. The bone density of 3-week-old weaning mice dramatically increased when they were exposed to low doses of penicillin, chlortetracycline, or vancomycin compared to 7-week-old weaning mice (*69*). As a result of continuous treatment with low-dose penicillin (LDP), bone mass decreased in all male mice, regardless of whether or not they were exposed to LDP post-weaning or if the pregnant mother was exposed. These controversial results were replicated in several types of female mice administered LDP. LDP treatment in early life transiently disturbed the microbiota and altered the composition of the microbial community (*66,69*).

In addition, intestinal flora and different levels of nutrition affect bone health. Several studies transplanted the intestinal microflora of healthy and malnourished infants into sterile mice and found that malnourished infants with transplanted intestinal microorganisms had delayed development and abnormal skeletal muscle development (*70*). The effect of intestinal flora on bone health can by mediated by metabolites. The intestinal flora can digest soluble grain fiber in the diet into short-chain fatty acids (SCFAs), which lower the pH of the gut, contribute to calcium uptake, and inhibit the formation of osteoclasts (*71*). The G-protein-coupled receptors GPR40 and GPR120 superficially bind medium- and long-chain fatty acids, and a GPR40/120 agonist inhibits fatty acids in osteoclastogenesis. GPR120 acts as a mediator of the anti-osteoclastogenic action of C16 and C18 fatty acids (*72*).

4. Intestinal microbial groups and osteogenic/osteoclasts and bone marrow mesenchymal cells

Bone marrow mesenchymal stem cells are pluripotent stem cells that can differentiate into various mesodermal lineages (*73*). Osteoblasts are the main cells that promote bone development and bone remodeling (*74*). Osteoblasts directly interact with bone cells, osteoclasts, and hematopoietic stem cells (*74*), thereby maintaining homeostasis between bone formation and bone resorption (*74,75*).

Studies have described the interaction between intestinal microflora and bone-related cells. Intestinal microflora influence the expression of peripheral and central serotonin (*76*). Peripheral serotonin is primarily produced in the gut, and intestinal flora such as indigenous spore-forming bacteria may play a catalytic role in the production of serotonin (*77*). Peripheral

serotonin functions as a hormone to inhibit osteoblast proliferation through 5-hydroxytryptamine receptor 1B (5-HT1BR) and cAMP response element binding protein (CREB) (*78*), thereby reducing bone density and bone formation. The level of serotonin expression is lower in the intestinal tract of sterile mice and can be increased by transplanting *E. coli* Nissle 1917 into ileal tissue ex vivo, where it interacts with compounds secreted by host tissues (*79*). The use of probiotics can improve intestinal flora, reduce serotonin content, and alleviate bone disease (*65,80*). Central serotonin acts as a neurotransmitter to increase bone mass by calmodulin kinase (CaMK)-dependent signaling involving CaMKKβ and CaMKV, which are mediated by CREB (*76*).

Osteoclasts are functionally related to osteoblasts and are involved in bone resorption (35). Intestinal bone signaling pathways and microbial populations play an important role in regulating bone health (64). Decreases in estrogen in postmenopausal women result in an increase in inflammatory factors and osteoclast formation (*81*). In a model of osteoporosis in OVZ rats, expression of the inflammatory cytokines TNFα and IL-1 decreased, osteoprotegerin levels increased, and osteoclast formation was inhibited (*35*) when *Lactobacillus* strains and other probiotics were given to OVZ rats (*35*).

A reduction in the diversity of the intestinal microbiota has been noted and specific taxonomic preferences have been identified in Crohn's disease and ulcerative colitis (*6*). In a mouse model of ulcerative colitis induced with 2, 4, 6-trinitrobenzene sulfonic acid, bone marrow stromal cells (BMSCs) were implanted in intestinal mucosa, where they repaired damaged intestinal tissue. This may be because BMSCs differentiate into colonic stromal cells and express vascular endothelial growth factor and TGF-β1 in the injured region (*82,83*). BMSCs also have immune-regulatory effects on antigen-specific T cells in Crohn's disease through direct cell-cell contact (*84*), inhibiting allogeneic antigen-specific responses and mitogen-induced proliferation (*85*), preventing the production of cytotoxic T lymphocytes. In general, there is growing evidence that BMSCs could be used to treat inflammatory bowel disease caused by a disorder in intestinal flora, but the precise molecules and mechanisms responsible for immune regulation need to be ascertained (*86*).

5. Conclusion

Understanding the correlation between intestinal microbial flora and bone health paves the way for further studies on the treatment of bone diseases by intestinal microbes. Intestinal microbial flora influence bone health through the immune system, endocrine system, and various bone-related cells. An increasing number of studies on osteoporosis have suggested the beneficial effects of probiotics in terms of increasing bone mass.

This provides a basis for treating hereditary bone diseases, which involve abnormal bone, cartilage, joint, and other related tissues due to pathogenic mutations in genes. The relationship between pathogenic mutations that cause genetic bone diseases and intestinal microbial flora remains unclear and needs to be studied further.

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