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Gut microbiota: an overlooked factor that plays a significant role in osteoporosis

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Abstract

Gut microbes are known as the body's second gene pool. Symbiotic intestinal bacteria play a major role in maintaining balance in humans. Bad eating habits, antibiotic abuse, diseases, and a poor living environment have a negative effect on intestinal flora. Abnormal intestinal microbes are prone to cause a variety of diseases, affecting life expectancy and long-term quality of life, especially in older people. Several recent studies have found a close association between intestinal microorganisms and osteoporosis. The potential mechanism of intestinal flora affecting bone formation or destruction by mediating nitric oxide, the immune and endocrine systems, and other factors is briefly described in this review. All of these factors may be responsible for the intestinal flora that causes osteoporosis. Studying the relationship between intestinal flora and bone health not only provides new ideas for studying the role of intestinal microorganism in osteoporosis, but also provides a new therapeutic direction for clinically refractory osteoporosis. Study of the relationship between intestinal microbiota and osteoporosis is important for maintaining bone health and minimizing osteoporosis.

Keywords

Intestinal flora, osteoporosis, nitric oxide, immunity, endocrine system, homocysteine

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Introduction

There is an average of 100 trillion microorganisms in the human intestinal tract, including bacteria, archaea, fungi, and viruses. Among the many categories of microbes that have been identified, thick-walled bacteria, *Bacteroides*, microalgae, actinomycetes, and *Proteus* account for the majority of microbes. These microorganisms have far more genetic phenotypes than humans themselves, but their role in maintaining the health of the body has been largely overlooked.[1] Intestinal symbiotic bacteria play a major role in maintaining balance in humans by aiding food digestion and absorption, secreting microbial metabolites, and protecting mucosal barrier function. However, an imbalance in microorganisms releases proteins, peptides, and metabolites that act against organs, causing systemic multiple organ dysfunction.[2]

As the load-bearing organ of the body, bone has active metabolism and is of great significance in routine activities. Repeated falls, muscular atrophy, muscle-strength deficiency, limited activity, and weight loss can easily cause loss of bone mass, microstructure destruction, and other pathological changes, such as osteoporosis.[3] A dynamic imbalance between osteoblast and osteoclast populations is the decisive factor for developing osteoporosis. Osteoporosis affects more than 200 million people worldwide, with more than one third of them suffering from osteoporotic fractures.[4] Therefore, osteoporosis places enormous economic and social pressure on society and individuals.[5] As the population of older people in society increases, so will the incidence of osteoporosis and the rates of disability and death due to osteoporosis. Early intervention during development of osteoporosis can reduce the burden on families and society and improve the long-term survival rate and quality of life of patients.

The symbiosis between gut microbiota and the host requires a delicate balance, which once disrupted, may increase the risk of osteoporosis. David et al. found that intestinal microorganisms affect bone through inflammation, nutrition, and calcium. The significance of inflammation was highlighted.[6] A review also proposed that the effect of intestinal flora on bone is mediated by effects on inflammation and immunity.[7] Under stress, the intestinal tract can cause changes in vasoactive substances,[8] which has an adverse effect on bone formation. The autoimmunity of germ-bearing mice affected bone formation compared with sterile mice.[9] The intestinal tract is also involved in endocrine metabolism, which is associated with bone homeostasis. Studying the relationship between intestinal flora and bone health provides a new theoretical basis for delaying osteoporosis and provides a new therapeutic direction for clinically refractory osteoporosis. This article reviews the potential mechanism by which gut microbes mediate osteoporosis.

Nitric oxide

Intestinal smooth muscle and intestinal capillary endothelial cells express inducible nitric oxide synthase (iNOS).[10] Nitric oxide synthase (NOS) is a rate-limiting enzyme for biosynthesis of nitric oxide (NO). Microbes can drive the binding of pathogenic bacteria or bacterial lipopolysaccharide-inducible transcription factor nuclear factor (NF-κB) to the *iNOS* promoter, thus upregulating *iNOS* transcription.[11,12] The number of osteoblasts are increased by iNOS under mechanical stress, but iNOS also promotes osteoclast production by increasing levels of RANKL (receptor activator of NF-κB).[13]

Intestinal microbes also promote the release of endothelial NOS (eNOS).[14] *eNOS* mRNA regulates production of osteoblasts and osteoclasts, as well as the

release of inflammatory mediators, such as tumor necrosis factor-α, interleukin (IL)-1, and IL-6. In NOS-deficient rats, trabecular bone mineralization and chondrocyte function are reduced, impeding lateral and lateral bone growth in 8- to 10-week-old mice. Low NO concentrations might affect bone growth. In a previous study, iNOS-deficient rats developed more severe osteoporosis than did eNOS-deficient rats, which indicated that intestinal microbes mainly maintained bone homeostasis by affecting iNOS activity.[15] However, after 12 to 18 weeks of age, the negative effect of NOS deficiency on bone homeostasis in mice was eliminated. This might be related to the negative effect of NOS deficiency on bone growth being counteracted by sex hormone-associated bone protection in mice.

High NO concentrations can impede osteoclast differentiation and activate the cGMP-dependent protein kinase G pathway, thus reducing acid secretion and adhesion.[13] However, at high concentrations, NO can compete for binding to RANKL, thus hindering the interaction of RANKL with NF-κB receptor agonists and impeding osteoclast activity.[16] These results indicate that the concentration of NO has opposite effects on bone. Moreover, studies have shown an association between NO and vitamin D. Vitamin D, as a direct transcriptional regulator of endothelial NOS, can positively regulate NO.[17] However, epidemiology suggests otherwise. NO can also interact with vitamin D, affecting the number of osteoblasts, as well as their response under fluid-flow shear stress.[18] NO indirectly affects bone homeostasis by affecting vitamins. Changes in plasma NO concentrations can also affect changes in bone blood flow, which may affect bone homeostasis. NO plays a role in the endpoint of osteoporosis and this can be seen in various types of osteoporosis.

Immune system

The immune system is a host defense system. Under normal conditions, intestinal microbes and the immune system maintain the health of the body. However, imbalance of gut flora due to various factors can also promote development of disease through the immune system. In sterile mice, transplantation of complex microorganisms can alleviate depletion of myeloid progenitor cells and obstruction of monocyte proliferation.[19] This finding suggests that intestinal flora are related to development of immune cells. The immune system is inextricably linked to development of osteoporosis. In mice, thick-walled bacteria, particularly *Clostridium*, promote accumulation of regulatory T cells (Tregs) in the lamina propria of the colon. Tregs inhibit osteoclast differentiation and hinder osteoclast formation. Decreased abundance of *Clostridium* strains cause a decrease in factor-β and $Foxp3^+$ Treg levels and an increase in bone loss.[20] However, Treg cells secrete cytotoxic T-lymphocyte antigen 4, which binds to CD80/CD86 molecules on osteoclast precursors, and thus inhibits inflammatory responses.[21] Britton et al.[22] found that *Lactobacillus reuteri* can decrease the number of T lymphocytes and inhibit osteoclast formation. However, $CD4^+$T cells interact with CD11c dendritic cells and develop into functional osteoclasts.[23] Bacterial colonization experiments in animals have also confirmed that $CD4^+$T cells interact with CD11c dendritic cells to probably aggravate osteoporosis.[9]

An imbalance in intestinal microbes inhibits differentiation of type 1 and 2 T helper cells (Th1 and 2), as well as Tregs, thus inducing osteoclast differentiation and proliferation, and aggravating bone loss. An imbalance in intestinal flora also induces differentiation of Th17 cells, which belong to the $CD4^+$T-cell osteoclast population. Th17 cells secrete IL-17a, tumor

necrosis factors, IL-1, and IL-6, as well as low levels of interferon-γ, which stimulate osteoblasts to release RANKL.[24] Kim et al.[25] found that mouse commensal segmented filamentous bacteria and human commensal bacteria can promote Th17 differentiation, providing new guidelines for intestinal microbiota-targeted therapy for osteoporosis.

B lymphocytes control the RANKL/osteoprotegrin (OPG) (osteotrophin) ratio through the phosphoinositide 3-kinase/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signal transduction pathway, and consequently regulate the growth rate of bone cells. Intestinal flora affect the expression of mTOR transcription factors in the posterior midgut. Knocking out *TSC1* expression (a negative regulator of mTOR) causes an increase in the RANKL/OPG ratio, thus accelerating osteoclast proliferation.[26] Gut microbiota can also affect B-cell development and OPG production by B cells.[27] The decoy receptor OPG can directly inhibit RANKL.[28] Furthermore, OPG can inhibit osteoclast differentiation and bone resorption by modulating autophagy-related genes and AMP-activated protein kinase/mTOR/p70S6K signaling.[29] The immune system may be involved in the pathogenesis of osteoporosis mediated by intestinal microorganisms. Immune system abnormalities often result in secondary osteoporosis.

Endocrine system

Endocrine hormones act on various organs of the body. These hormones are also involved in development of many diseases. Estrogen is directly related to the diversity of gut flora. Among gut microbes, those encoding β-glucuronidase are involved in the enterohepatic circulation of estrogen.[1] Estrogen accelerates osteoblast differentiation and survival through the NO/cGMP-dependent phosphorylation pathway[30] and the Fas/Fas-ligand system.[31] Moreover, estrogen increases the OPG/RANKL ratio that is dependent on the low-density lipoprotein receptor-related protein 5 pathway, reduces osteoclast differentiation, and maintains bone homeostasis.[32] Intestinal microbes affect the enterohepatic circulation of thyroid hormones by modulating glucuronidase and sulfate activities.[33] Among the thyroid hormones, calcitonin (secreted by thyroid C cells) can inhibit parathyroid hormone, thus reducing bone mobilization, promoting osteoclast apoptosis, and delaying the onset of osteoporosis. However, some studies have shown that calcitonin accelerates bone circulation and inhibits osteoblast proliferation, which is mediated by osteoclasts and bone cells after initiation of bone formation mechanisms.[34] Thyroid hormone membrane-bound receptors also increase eNOS activity,[35] indirectly stimulating osteoblast proliferation and survival through the cGMP-dependent protein kinase G pathway. Insulin-like growth factor-1 (IGF-1) is a growth factor that promotes the quality of life of chondrocytes and osteoblasts.[36] IGF-1 is regulated through the Akt–mTOR-dependent pathway and induces a sustained increase in eNOS levels.[37] Intestinal microbial imbalance indirectly affects osteoblast function and number by causing a decrease in IGF-1 levels.[38]

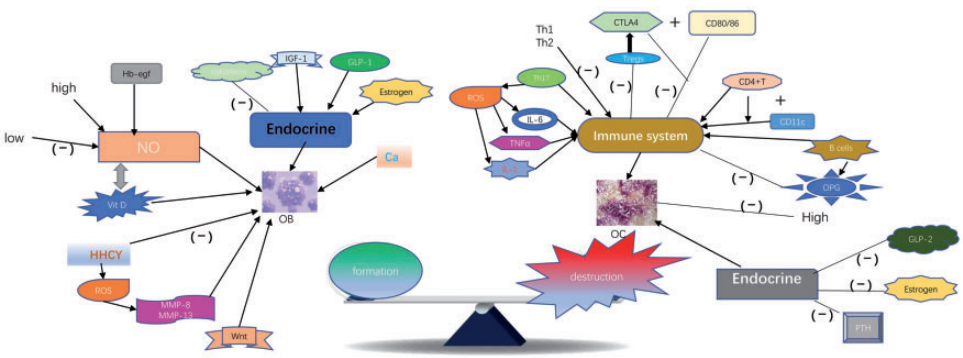
Glucagon-like peptide 1 (GLP-1), which is secreted by intestinal L cells, promotes osteogenic differentiation and inhibits mesenchymal stem cells, with conversion to fat.[39] GLP-2 is also secreted by L cells in the intestinal mucosa. GLP-2 has a strong intestinal affinity and can inhibit proliferation of osteoclasts through the transforming growth factor-β–SMAD2/3–iNOS–NO–caspase3–B-cell lymphoma 2 signaling pathway.[40] GLP-2 can also upregulate butyric acid levels in the intestine, thus decreasing levels of stress

byproducts and delaying apoptosis.[41] GLP-2 also maintains stability of various cells in the intestine. Testosterone and metabolites that protect bone homeostasis are also modulated by the gut microbiome.[42] Insulin-like factor 3 in testosterone affects the musculoskeletal system through the insulin-like factor 3/relaxin family peptide receptor 2 axis.[43] Osteoporosis caused by endocrine dysfunction is often secondary osteoporosis (Figure 1).

Others

Homocysteine is a non-protein amino acid that is produced during metabolism of methionine. Folic acid is an important cofactor in the metabolism of homocysteine. Intestinal flora disorders lead to reduced absorption of folic acid in the jejunum, leading to hyperhomocysteinemia. Hyperhomocysteinemia not only causes degradation of the extracellular matrix and a decrease in bone blood flow, but also has a harmful effect on bone health.[44] This condition also affects osteoblast precursors, namely human mesenchymal stem cells, which attenuate osteoblast differentiation.[45] Moreover, hyperhomocysteinemia causes an increase in reactive oxygen species production and activation of matrix metalloproteinases, among which matrix metalloproteinase-9 and matrix metalloproteinase-13 play a role in cartilage osteogenesis.[46] Additionally, hyperhomocysteinemia leads to activation of protein phospholipase 2A. This disrupts the FOXO1 and mitogen-activated protein kinase signaling cascades and alters the redox regulation mechanism of osteoblasts.

Bifidobacteria in the intestine can significantly enhance transcription of the lactase gene promoter and promote lactose absorption,[47] thus reducing hypocalcemia-induced bone mobilization. *Lactobacillus helveticus* fermentation further promotes the release of free calcium and accelerates calcium deposition.[48] Intestinal microbes are also involved in development of inflammatory bowel disease. This causes a decrease in calcium and vitamin D absorption in the intestine, and indirectly increases the risk of



Abbreviations: OB: osteoblast; OC:osteoclast; Wnt:Wnt singal molecule; MMP-8:matrix metalloproteinases 8; MMP-13: matrix metalloproteinases 13; ROS: reactiveosygenspecies ; HHCY:hyperhomocysteinemia; Vit D:vitamin D; NO:nitric oxide; High:high levels of NO; Low:low levels of NO; Hb-egf:heparin-binding epidermal growth factor; IGF-1:insulin like growth factor 1; GLP-1:glucagon-likepeptide1; Ca:calcium; IL-1:Interleukin 1; TNFα:Tumor necrosis factor α; IL-6: Interleukin 6; Th17:type 17 T helper cells ; Th1:helper T cell 1; Th2:helper T cell 2; Tregs:regulatory T cells; CTLA4:cytotoxic T-lymphocyte antigen 4; B cells:B lymphocyte; OPG:osteoprotegerin; GLP-2: glucagon-likepeptide 2; PTH:parathyroid hormone;

Figure 1. Association between intestinal microorganisms and osteoporosis. The figure shows differences in nitric oxide concentrations, endocrine hormones, immune system-related cells, and antigens in osteoblasts and osteoclasts. High homocysteine, calcium, and vitamin D levels are also involved in gut microbes and osteoblasts

osteoporosis. Intestinal pH values also change because of intestinal flora, thus affecting calcium absorption.[49] *L. reuteri* can inhibit Wnt10b.[50] Wnt, which is a highly conserved signaling molecule, effectively stimulates osteoblast differentiation, inhibits adipogenesis, and impedes bone degeneration.[51] Wnt shows osteoprotective effects by affecting the microenvironment necessary for differentiation of mesenchymal cells into bone precursor cells and preventing osteoblasts from transforming into chondrocytes.[52]

Therapy

High-fiber probiotic supplements, short-chain fatty acid (SCFA) diets, and fecal transplants are the most studied therapies. They are anti-inflammatory and maintain balance of the gut microbiota. Each of these therapies has its own characteristics. Among them, SCFAs not only increase calcium absorption, but also have anti-inflammatory effects and maintain intestinal microbial balance.[6] Additionally, SCFAs can reduce intestinal pH and inhibit formation of calcium chelates. SCFAs also increase calcium absorption by increasing the level of calcium binding, protein transcription, and upregulation of the vitamin D receptor.[53] These fatty acids also promote Treg development and stimulate the Treg immune response, which weaken immune system abnormalities caused by intestinal microbes.[54] However, SCFAs play a role in the pathogenesis of hepatic encephalopathy, and an excessive increase in SCFAs may aggravate hepatic coma in patients with liver disease. Even in patients without liver disease, octanoic acid (a SCFA) is associated with experimental hepatic encephalopathy.[55] Further research on the role of SCFAs in liver disease is required. In recent years, heparin-binding epidermal growth factor has been shown to significantly increase NO and may provide a new treatment for osteoporosis.[56]

Conclusions and prospects

Many studies have confirmed that there is a close relationship between gut microbiota and osteoporosis. Endocrine dysfunction plays an important role in osteoporosis. Immune system abnormalities and NO also affect osteoporosis. Various pathways work together and eventually lead to osteoporosis. The relationship of gut microbes with osteoporosis requires further research, especially the role of specific microorganisms in osteoporosis. This article is a preliminary discussion on the relationship between intestinal flora and osteoporosis. This review provides a theoretical basis for new prevention and treatment strategies for osteoporosis and suggests new ideas for immunomodulation and targeted treatment of osteoporosis.

Author contributions

Meng-lei Hao and Guang-yao Wang were major contributors in writing and submitting the manuscript. Dong-lai Wang made substantial contributions to conception of the article. Xiao-qin Zuo, Chan-juan Qu, and Bo-chen Yao provided assistance and suggestions during the submission process. All authors read and approved the final manuscript.

Declaration of conflicting interest

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