Role of the gut microbiota in the pathogenesis and therapeutic approach to osteoporosis (Review)

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Abstract. Osteoporosis (OP) is a chronic systemic metabolic bone disease characterized by an imbalance between bone resorption and bone formation, resulting in a decrease in bone mass and density. Additionally, the deterioration of bone tissue microstructure increases the risk of fracture. This condition not only impairs the quality of life of patients but also threatens their safety. With the gradual aging of the population of China, the incidence of OP has been increasing annually, becoming a major public health issue threatening the health of middle-aged and elderly individuals, particularly women in these age groups. OP has garnered increasing attention due to its high prevalence and severe complications. OP has many triggering factors and a complex pathogenesis, which are currently considered to be related to hormones, the endocrine and immune systems, and gut microbiota (GM). With the continuous development of modern medical research, the association between GM and OP has emerged as a hotspot in the field of orthopedics in recent years. Current evidence confirms that GM can influence systemic regulation through various metabolic pathways, either directly or indirectly, employing a multi-channel, multi-target effect on both preventing and controlling OP. However, current research still faces limitations such as the homogeneity of animal models, insufficient investigation of multi-pathway interactions, and inadequate clinical translational evidence. Future studies should leverage multi-omics technologies to elucidate the GM-bone axis mechanisms in depth, thereby advancing the clinical application of traditional Chinese medicine (TCM) in the prevention and treatment of OP. The present review summarizes the associations between GM and the development of OP, as well as the application of TCM, probiotics and prebiotics in regulating GM for OP management, aiming to provide novel therapeutic strategies for its clinical prevention and treatment.

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Abbreviations: OP, osteoporosis; GM, gut microbiota; BM, bone metabolism; OC, osteoclast; OB, osteoblast; GFM, germ-free mice; TMAO, trimethylamine N-oxide; NF-κB, nuclear factor kappa B; 5-HT, 5-hydroxytryptamine; TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharides; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin; TLR4, toll-like receptor 4; IL, interleukin; Th, type helper; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; SCFAs, short-chain fatty acids; Tregs, regulatory T cells; GPR, G protein-coupled receptor; FMT, fecal microbiota transplantation; TCM, traditional Chinese medicine

Key words: osteoporosis, gut microbiota, traditional Chinese medicine, short-chain fatty acids, probiotics

Contents

1. Introduction

Osteoporosis (OP) is a systemic metabolic bone disease characterized by degradation of bone microstructure, bone fragility, and increased fracture risk (1). OP affects 10.2% of adults >50 years of age and is expected to increase to 13.6% by 2030 (2). Annually, >150 million individuals suffer from OP, and the disease poses a great threat to the quality of life for >50% of postmenopausal women, with the most severe risk being osteoporotic fractures (3). OP is influenced by the process of bone loss, and its therapeutic goal is to halt bone mass reduction and rectify the imbalance in bone remodeling. Currently, certain drugs are effective in mitigating bone loss or increasing bone calcium content. The commonly employed drugs for OP treatment include bisphosphonates, estrogens, norepinephrine, and teriparatide acetate. However, the

prolonged and high-dose administration of these agents may cause adverse effects such as hypercalcemia, gastrointestinal reactions, and an increased risk of breast cancer and heart disease (4,5). With the in-depth study into the pathogenesis of OP, the relationship between intestinal microecology and bone metabolism (BM) has become a worldwide research hotspot. Studies indicate that the gut microbiota (GM) is associated with the reduction of bone mass and the development of OP in humans (6-8).

The intestinal microecosystem is the largest ecosystem within the human body, harboring $>10^{14}$ orders of magnitude of bacteria, with the total number of genes in the gut microbiome genome being ~150-fold that of the human genome (9). The GM is mainly dominated by *Firmicutes* and *Bacteroidetes*, which together account for over 90% of the relative abundance, followed by *Actinobacteria*, *Proteobacteria*, and *Fusobacteria* (10). The GM exhibits variabilities between individuals and dynamic changes throughout the lifetime of an individual and is influenced by a variety of factors, including diet, age, lifestyle, medications, and disease states. The GM plays a regulatory role in multiple physiological functions, encompassing the enteric nervous system, enteroendocrine system, immune system, and intestinal permeability (11,12). Specific GM can modulate immunity, improve defense function, promote skeletal health, and inhibit bone calcium loss. Additionally, it can improve intestinal permeability, reduce inflammatory responses, and facilitate nutrient absorption (13). Imbalance of intestinal flora affects calcium absorption, subsequently triggering inflammatory and autoimmune changes, leading to bone loss and reduced bone formation (14-18). Therefore, GM is closely associated with the occurrence of OP, and the restoration of GM imbalance has become a key approach to treating OP, as revealed in Fig. 1. In the present review, the latest advancements in the association between GM and OP are examined, elucidating the role of GM in the pathogenesis and treatment of OP. The aim of the review is to explore novel therapeutic strategies and research directions for OP and related diseases.

Articles on the treatment of OP with traditional Chinese medicine (TCM) through regulation of GM were collected using the keywords 'osteoporosis', 'gut microbiota', and 'traditional Chinese medicine' by searching in PubMed (ttps://pubmed.ncbi.nlm.nih.gov/) and Web of Science (https://www.clarivate.com.cn/). The search period was from the inception of each database up to August, 2025. To ensure the quality of literature and accuracy of data extraction, the search was limited to articles published in English.

2. Association between gut microbiota and osteoporosis

Intestinal microflora can affect the energy homeostasis of the host organism and serve as a source of energy for the differentiation and formation of osteoblasts (OBs) and osteoclasts (OCs). In cases of aberrant energy metabolism, the OB precursors will shift to adipose differentiation (19). The composition of bacterial structures, characterized by varying quantities and ratios of different bacterial genera, forms diverse GM structures, which in turn have differential effects on BM (13,20). Reduced bone mass in the lumbar vertebrae and femoral neck is associated with excessive growth of gut bacteria, suggesting that overgrowth of the GM may constitute a significant risk factor for OP (21). Another study further validated this finding (22). The administration of antibiotics in the diet was shown to modulate the GM, thereby promoting growth and skeletal development (11). The increase in bone mass in germ-free mice (GFM) was associated with a decrease in the number of OCs (12). Bone marrow cultures revealed that the number of OCs was reduced in GFM, accompanied by a significant reduction in the formation of $CD11b^+/Gr1^-$ and $CD4^+$ T cells in the precursor OC population (13). Microbial transplantation of GFM led to an increase in the number of bone marrow $CD4^+$ T cells and OC precursors, along with a decrease in bone mass, suggesting the key regulatory role of the GM in the process of BM. Additionally, studies have identified associations between GM and both postmenopausal (PM) OP and senile OP (23,24). Elevated levels of trimethylamine N-oxide (TMAO), a metabolite of GM, have been shown to have a strong negative correlation with the degree of bone mineral density (BMD) in OP. TMAO has been demonstrated to regulate the cell function of BMSCs by activating the NF-κB signaling pathway, which affects the balance of BM, leading to acceleration of bone loss and further progression of OP (25). Furthermore, estrogen-deficient GFM exhibited reduced bone mass, while probiotic interventions effectively prevented loss of bone mass (26). 5-Hydroxytryptamine (5-HT) levels were increased after the introduction of specific *Escherichia coli* in GFM (27). *Lactobacillus reuteri* not only reduced the level of bone resorption markers and inhibited OC formation, but also suppressed the expression of tumor necrosis factor-α (TNF-α) and modulated the level of Wnt10b RNA in OBs cultured *in vitro* (28,29).

In clinical research, changes have been observed in the abundance and diversity of the GM, as well as in their associated metabolites, in patients with OP (30). Distinct differences exist in the GM between patients with OP and healthy populations (31). A previous meta-analysis encompassing 12 studies included fecal data from 2,033 subjects (604 patients with OP and 1,429 healthy controls). It observed increased relative abundances of *Lactobacillus* and *Ruminococcus* in the OP group, along with a higher proportion of *Bacteroides* (32). With an expanded sample size, research has identified that an increase in *Bacteroidetes* and a decrease in *Firmicutes* may be important factors in the GM imbalance triggering OP (33). In a previous study, *Klebsiella*, *Escherichia-Shigella*, and *Akkermansia* were identified as biomarkers in patients with OP. Among them, the abundance of *Akkermansia* was negatively correlated with lumbar BMD, while *Klebsiella* and *Escherichia-Shigella* were negatively correlated with femoral neck and hip BMD (34). In another study, an increase in the abundance of *Actinomyces*, *Eggerthella*, *Clostridium* Cluster XlVa and *Lactobacillus* was noted in the OP group, suggesting that alterations in GM abundance may serve as an independent risk factor for bone mass loss in the elderly (35). Research has indicated a positive correlation between the relative abundance of *Actinobacillus*, *Blautia*, *Oscillospira*, *Bacteroides*, *Phascolarctobacterium* and OP, while a negative correlation was identified with other Veillonellaceae, *Collinsella*, and other Ruminococcaceae (36). Another study evaluated the causal relationship between GM and bone development by determining specific causal bacterial taxa using Mendelian

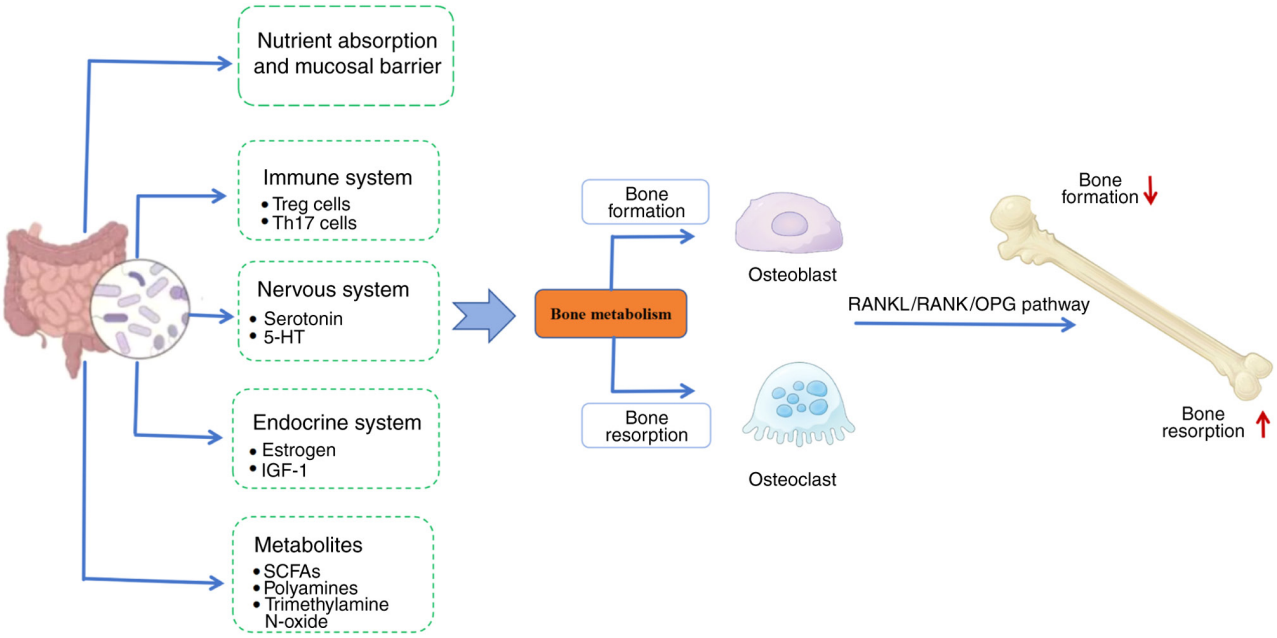

Figure 1. Relationship between GM and BM. GM affects bone formation and resorption by acting on the nervous, immune, and endocrine systems, thereby regulating BM. GM, gut microbiota; BM, bone metabolism; Th17, type helper 17; 5-HT, 5-hydroxytryptamine; IGF-1, insulin-like growth factor-1; SCFAs, short-chain fatty acids; RANKL, receptor activator of nuclear factor-κB ligand; OPG, osteoprotegerin.

randomization (MR). The study revealed that *Clostridiales* and Lachnospiriaceae regulate bone mass variation, indicating a causal relationship between GM and bone development (37). Previous research revealed that increased abundances of the family Pasteurellaceae, order Pasteurellales, and genus *Ruminococcaceae UCG004* were linked to an increased risk of OP. Conversely, the family Oxalobacteraceae, unknown family (ID.1000006161), the genus *Lachnospiraceae NK4A136* group, an unknown genus (ID.1000006162), and order *NB1n* were associated with a reduced risk of OP (38). Additionally, other research found that changes in the GM, including the *Lactobacillus* genus, are associated with osteoporosis (39).

Other studies further demonstrated the association between GM and PMOP, while also exploring the phenomenon of GM regulating BM (30). Notably, healthy postmenopausal women exhibited higher abundances of *Clostridia* and Methanobacteraceae within their GM, whereas women with osteopenic/osteoporotic conditions showed a greater richness of *Bacteroidetes* in their fecal microbiota. Therefore, alterations in GM composition are considered closely associated with OP (40). The abundance of *Fusicatenibacter*, *Lachnoclostridium*, and *Megamonas* species was significantly higher in PMOP women compared to women with osteopenia (41). Researchers have proposed GM as a potential new target for the treatment of PMOP, highlighting that GM can influence BM through various mechanisms, such as regulating the host's immune system, particularly affecting inflammation and autoimmune responses. For example, GM can promote the production of regulatory T cells (Tregs) by producing short-chain fatty acids (SCFAs) such as butyrate and propionate, thereby exerting anti-inflammatory effects (42). Reduced α-diversity in GM has been shown to be associated with PMOP (43). Previous research has revealed that GM has an essential role to perform as a target for TCM intervention in bone disease treatment (44). Certain TCMs with natural prebiotic properties may help combat OP by facilitating the development of healthy probiotics. Hence, the gut-bone axis may provide an explanation for the multi-target regulation of TCM in treating OP (45).

3. Mechanisms of gut microbiota-mediated osteoporosis

In recent years, the association between GM and OP has increasingly become a research hotspot. The mechanisms by which GM influences OP are highly complex. The pivotal role of GM in bone regulation is elaborated in this section through a multifaceted approach, including nutrient absorption, intestinal mucosal barrier permeability, the immune, endocrine, and nervous systems, as well as metabolites of GM, as illustrated in Fig. 2.

Nutrient absorption. Calcium, the major mineral element in human skeletal tissue, plays an important role in bone formation and is absorbed in the form of calcium ions. Only 30% of dietary calcium intake is absorbed by the bones in healthy individuals (46), underscoring the importance of enhancing calcium absorption to promote bone production. The GM facilitates the formation of bone calcium and reduces bone loss, thereby promoting BM (13). The impact of calcium intake on bone health is closely related to the quantity and type of flora. Calcium supplements or a high-calcium diet can increase the population of beneficial bacteria and maintain the integrity of the GM ecosystem (47). Calcium absorption is associated with a lower pH in the cecum (20), and alterations in the microbiota can decrease intestinal pH, impede the intestinal calcium-acid complexes, enhance calcium solubility, and increase the amount of calcium available for absorption (48). The expression of calcium transporter proteins has been shown to be

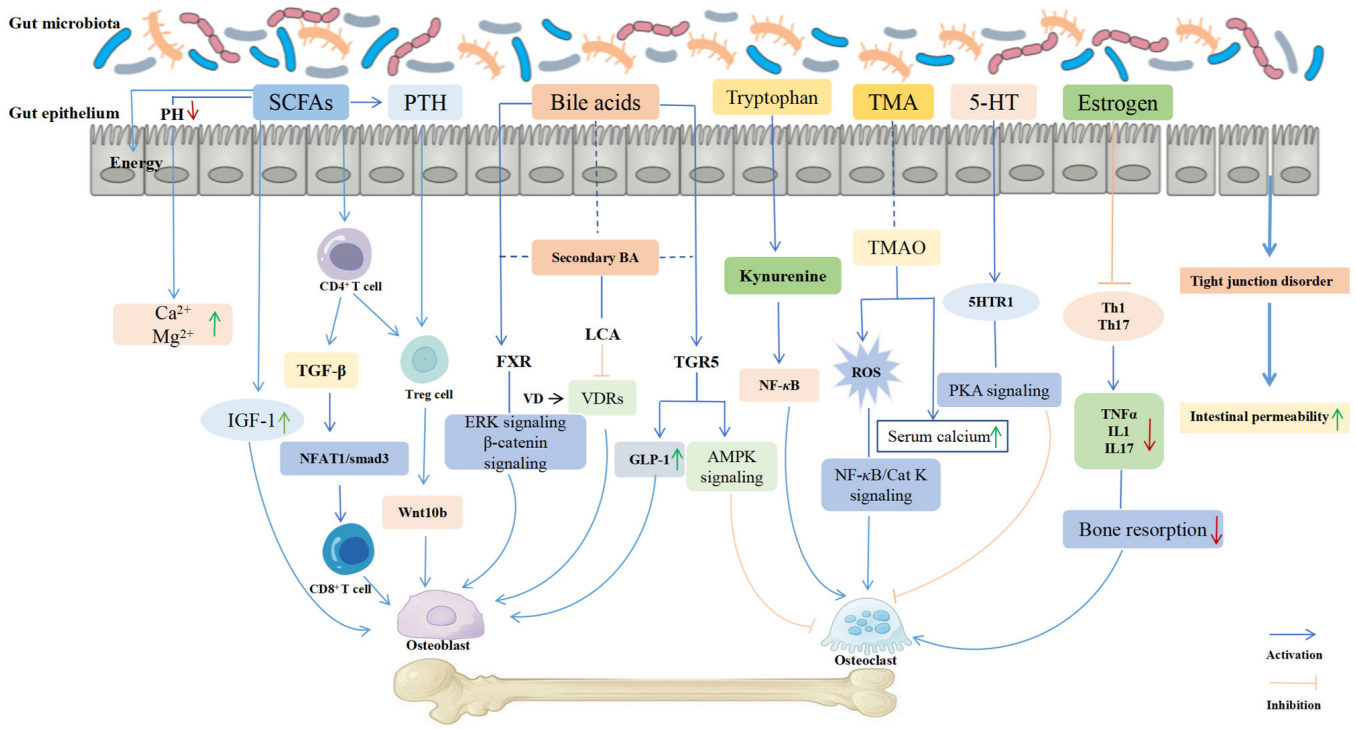

Figure 2. Relevant mechanisms of GM in the regulation of osteoporosis. GM mainly regulates the immune system, produces beneficial metabolites such as SCFAs, regulates the absorption of minerals such as calcium and phosphorus, affects the gut-brain axis and hormone secretion, and affects bile acid metabolism through multiple mechanisms that affect bone metabolism and maintain bone health. GM, gut microbiota; SCFAs, short chain fatty acids; PTH, parathyroid hormone; TMA, trimethylamine; TMAO, Trimetlylamine oxide; 5HTR1, 5-hydroxytryptamine receptor 1; IGF-1, insulin like growth factor-1; TGF-β, transforming growth factor-β; FXR, farnesoid X receptor; VD, vitamin D; LCA, lithocholic acid; Th1, type helper 1; GLP-1, glucagon-like peptide-1; TGR5, Takeda G protein-coupled receptor 5; AMPK, adenosine 5-monophosphate (AMP)-activated protein kinase; ROS, reactive oxygen species; PH, potential of hydrogen; 5-HT, 5-hydroxytryptamine; TNF-α, tumor necrosis factor-α; VDRs, Vitamin D Receptors; PKA, protein kinase A; Cat K, Cathepsin K; IL-1, interleukin-1; ERK, extracellular-regulated protein kinase; NFAT1, nuclear factor of activated T-cells 1.

upregulated, enhancing calcium absorption and mitigating the downstream effects of bone resorption on bone (49). Vitamin D is one of the important substances maintaining bone homeostasis (50). When insufficient calcium intake occurs, the GM can produce active vitamin D, promoting calcium absorption in the gut (51). In addition, intestinal microorganisms are considered one of the primary sources of vitamins B and K, both of which play important roles in bone homeostasis (52).

Intestinal mucosal barrier permeability. The intestinal mucosal barrier constitutes the interface between the human body and the external environment, and its integrity is critically important for preventing the invasion of harmful substances, such as toxins and bacteria, into the body (53).

The intestinal barrier is composed of a mucus layer, GM, immune cells, and a monolayer of intestinal epithelial cells (54). Lipopolysaccharides (LPS), the main component of the bacterial cell wall of GM, induce chronic inflammatory responses, leading to bone loss. Additionally, LPS promotes the formation and activation of OCs, thereby further contributing to bone loss (55). Conversely, muramyl dipeptide functions to reduce bone resorption and increase bone mass by downregulating the ratio of the receptor activator of NF-κB ligand (RANKL)/osteoprotegerin (OPG), thereby indirectly suppressing OC differentiation (56). Barrier damage is universally observed in all types of OP models, mainly manifesting as disruption of tight junction proteins, alteration in intestinal villus morphology, and increased intestinal permeability (57,58). This compromised intestinal barrier facilitates the translocation of microbes or potential antigens to subepithelial mucosa, thereby activating immune cells and triggering aberrant intestinal and systemic immune responses, ultimately resulting in bone loss (26). GM affects BM by influencing mucosal barrier function, with increased membrane levels of Toll-like receptor 4 (TLR4) on the membrane observed in primary cells treated with RANKL or LPS. TNF-α is secreted via the endotoxin/TLR4 signaling pathway and modulates RANKL-induced osteoclastogenesis (59). The integrity of the GM prevents bacterial LPS from contacting macrophage TLR-4 in the lamina propria and prevents OP (60). The impact of GM on the intestinal mucosal barrier is involved in glucocorticoid-induced OP (61).

Immune system. OP is characterized as a systemic disease with chronic low-grade inflammation. Changes in GM can elicit systemic or localized immune responses, which are closely implicated in the development of OP (62). The immunological impact on bone conversion is often manifested by B- and T-cell activation, along with an increase in osteoclastogenic factors such as interleukin (IL)-17, IL-6, RANKL, and TNF-α. These factors enhance the activity of OCs while simultaneously inhibiting the formation and function of OBs, ultimately exacerbating the imbalance in BM (63). GM maintains contact with dendritic cells and immune cells at the vascular endothelial boundary, stimulating the immune system to release inflammatory factors.

These factors subsequently influence the immune cell population within bone tissue, thereby modulating the bone remodeling process (64). Hematopoietic stem cells in bone tissue have the potential to differentiate into OCs and immune cells. GM regulates the BM microenvironment by promoting the maturation of the host immune system (65). The harmful flora in GM can produce endotoxins. These endotoxins initiate inflammatory responses by binding to the TLRs on the surface of host immune cells, subsequently resulting in bone mass reduction (18). Type helper 17 (Th17) cells, an integral subset of the $CD4^+$ T-cell OC population, can be generated and differentiated under GM dysregulation, and secrete IL-17a, IL-1, IL-6, along with low levels of interferon-γ and TNF. These cytokines contribute to the release of RANKL and the formation of OCs (66). Regulatory T cells are capable of stimulating bone marrow $CD8^+$ T cells to produce the osteogenic Wnt signaling pathway ligand Wnt10b, thus promoting osteogenic differentiation (67). It has been found that the expression of inflammatory factors such as TNF-α and IL-6 is reduced in mice that maintain a balanced composition of GM. In addition, the number of T cells in the body is reduced, accompanied by a decrease in the number of OCs and an improvement in bone quality (12,68).

Endocrine system. Endocrine hormones act on various organs within the body, playing a role in the progression of a variety of musculoskeletal disorders, including OP. Beneficial bacteria in the GM can stimulate the secretion of incretins from intestinal cells (17). Incretins, a group of gastrointestinal hormones secreted by the intestine that exert glucose concentration-dependent insulinotropic effects, include glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (69). GIP can bind to surface receptors on OBs, enhancing the expression of type I collagen genes, promoting the maturation and mineralization of collagen matrix, increasing alkaline phosphatase activity, and promoting TGF-β secretion, all of which contribute to bone formation. GIP interacts with receptors on pro-OCs to suppress the generation and activity of OCs, thereby reducing bone resorption. Furthermore, GLP-1 facilitates insulin secretion from pancreatic β-cells, which in turn promotes bone formation; GLP-1 also promotes calcitonin secretion from thyroid C-cells, suppressing bone resorption (70). Insulin-like growth factor-1 (IGF-1), as a growth-promoting endocrine hormone, plays a key role in cell proliferation, differentiation, and the cell cycle, exerting its regulatory function through endocrine and paracrine/autocrine mechanisms. IGF-1 was the first identified substance mediating the association between GM and OP. Furthermore, it was the earliest confirmed molecule involved in the interaction between intestinal flora and OP (71). Research has demonstrated that IGF-1 promotes the proliferation and differentiation of OBs, as well as the mineralization of the bone matrix. It has been shown to play an important role in the maturation of the growth plate and the formation of the secondary ossification center (72).

Estrogen, a steroid hormone secreted mainly by the ovaries, promotes the generation of OCs, while also exerting regulatory effects on the BM. Under normal physiological conditions, estrogen, as a product of enterohepatic circulation, needs to undergo enzymatic hydrolysis by the GM before re-entering the internal circulation. When the GM is imbalanced, the enterohepatic circulation is weakened, and the reabsorption capacity of estrogen decreases, accelerating the loss of bone mass and the course of OP (73,74). In a previous study it was found that in GFM or mice subjected to prolonged antibiotic treatment, the absence of estrogen did not lead to significant bone loss (26). By contrast, in normal animals, the deficiency of estrogen led to increased intestinal permeability, allowing a large number of pathogenic bacteria to invade the intestine, which in turn triggered OP (75). *Lactobacillus rhamnosus* GG ameliorated estrogen deficiency-induced osteoporosis by regulating the gut microbiome and intestinal barrier and stimulating Th17/Treg balance in the gut and bone (76). Estrogen has been demonstrated to play a crucial role in maintaining bone health by reducing bone resorption through the maintenance of systemic and bone marrow T-cell homeostasis and also to directly regulate the formation of OB and OC (76-78).

Nervous system. The central nervous system and GM mediate the transmission of information between the brain and the gut by chemical signals (such as acetylcholine, γ-aminobutyric acid, and 5-HT), a pathway known as the 'brain-gut axis' (79). GM can communicate directly with the brain through various signaling molecules or indirectly through the brain-gut axis. Similarly, the brain's regulation of GM can be achieved either directly or indirectly by altering the intestinal environment (80). The nervous system regulates the secretion of gastric acid, mucus, bicarbonate, intestinal peptides, and antimicrobial peptides through intestinal cuprocytes, and influences the thickness and quality of the intestinal mucus layer (81). In addition, physiological changes in the gut can alter the microbial habitat, thus regulating the composition and activity of the microbial community (82). It has also been found that *Lactobacillus* and *Bifidobacterium* are positively correlated with serum leptin, while *Clostridium*, *Prevotella* and *Bacteroides* are negatively correlated with leptin levels (83). ObRb, as a receptor in the brainstem nervous system, inhibits the release of 5-HT and the expression of 5-HT receptor 2C in the ventromedial hypothalamic nucleus upon binding to leptin. Activation of the β2-adrenergic receptor on OBs promotes bone resorption (84). Conversely, when leptin concentrations decrease, the release of 5-HT is diminished, leading to a reduction in sympathetic activity, which affects the homeostasis of BM. 5-HT, as a neurotransmitter, has been confirmed to regulate BM function through the gastrointestinal system (85). Tryptophan hydroxylase 1 has been identified as the enzyme catalyzing serotonin synthesis, and its inhibitors hold potential therapeutic utility in the treatment of low bone mass (86). It has been revealed through knockdown of the serotonin receptor gene in OBs that a variety of serotonin receptors can be expressed in these cells (87,88). A correlation between serotonin levels and BM in women has been observed, in which increased serotonin levels were accompanied by decreased bone formation and increased bone turnover (89).

Metabolites. During the metabolic process of an organism, the GM can produce numerous active substances, such as SCFAs, hypocholestatic acid, TMAO, indole derivatives, and polyamines. These metabolites are relatively stable and can diffuse into the body circulation through the intestinal tract,

thereby indirectly revealing the role of the GM on BM (90). SCFAs are saturated fatty acids with a chain length of one to six carbon atoms, which are metabolized from the fermentation of indigestible carbohydrates by GM, including acetate, propionate, and butyrate (91). Additionally, the fermentation of amino acids in GM also produces SCFAs, which were found to inhibit the activity of histone deacetylase, induce the generation of Tregs, and maintain the immune system. By binding to G protein-coupled receptors (GPRs) on the cell surface, particularly free fatty acid receptor 2, SCFAs have been shown to reduce intracellular cyclic adenosine monophosphate levels, activate the immune system, promote the proliferation and differentiation of Tregs, inhibit intestinal inflammation and OC differentiation, and regulate BM (92). SCFAs were shown to induce the development of peripheral Tregs by acting through GPR43 (acetate and propionate receptors). Additionally, the precursor OCs derived from differentiated bone marrow cells express GPR41 (propionate and butyrate receptors) and GPR109 (butyrate and nicotinic receptors), confirming that SCFAs affect OB and OC activity through GPR activation (93). SCFAs in tryptophan, such as butyrate, acetate, propionate, and indole, were revealed to promote muscle synthesis, inhibit catabolism, exhibit anti-inflammatory and antioxidant effects, and regulate BM, thereby helping to prevent OP (94). SCFAs can directly act on bone cells, increasing bone density and bone strength (95). Experiments have demonstrated that SCFAs can increase bone density and bone strength in mice. Under pathological conditions (such as inflammation), a reduction in gut probiotic populations in mice has been observed, leading to a reduction in SCFAs levels, which contributes to the onset of OP (96). SCFAs can also lower intestinal pH, reducing the formation of inorganic salts such as calcium phosphate that bind to calcium ions. In addition, SCFAs have been shown to selectively increase the phosphorylation of mitogen-activated protein kinase p38, which mediates the phosphorylation of the downstream substrate heat shock protein 27 at Ser78 and Ser82, thereby affecting the absorption of minerals and improving bone quality (97). SCFAs have also been demonstrated to induce metabolic reprogramming of OBs, resulting in enhanced glycolysis at the expense of oxidative phosphorylation, thereby downregulating essential OC genes to directly inhibit OC activity (98).

Bile acids, serving as metabolites of cholesterol, are synthesized and secreted by hepatocytes. Within this process, 95% of bile acids enter the gut-liver axis. Under the influence of GM, primary bile acids can be converted into secondary bile acids, such as lithocholic acid, and deoxycholic acid (99). GM contributes to the processing and metabolism of bile acids, and metabolism regulates BM mainly through the sphincter farnesoid X receptor (FXR) and GPRs (100). LPS, a distinctive chemical component of the outer membrane layer of Gram-negative bacteria, can cause a chronic inflammatory response, promoting OC formation and inducing bone loss (101). *In vitro* experiments found that LPS inhibited RANKL activity by reducing the expression of RANK and M-CSF receptor and stimulated osteoclastogenesis in RANKL-pretreated cells via TNF-α (102). Lactic acid significantly reduced TNF-α and IL-6 production in LPS-stimulated macrophages, thereby decreasing the release of RANKL from OBs and inhibiting the differentiation of bone marrow-derived macrophages to OCs (103).

4. Mechanism of action of traditional Chinese medicine affecting gut microbiota in the treatment of osteoporosis

As an important stage in drug absorption, the progression of GM is closely associated with OP and its variations in metabolites. With deepening research, the efficacy of traditional Chinese medicine in preventing and treating OP through the modulation of GM, has been highlighted. Single-flavor Chinese medicinal herbs and their active ingredients, as well as herbal compounds, play an anti-inflammatory role, enhance the mucous barrier, improve the immune system, and regulate metabolism (104-134). Specific mechanisms are detailed in Table SI.

Effects on calcium absorption. Glycosylation in the structure of total flavonoids of Epimedii folium affects the number and activity of GM, which in turn increases the thickness of bone trabeculae, elevates bone mineral density, enhances OB activity, and promotes calcium deposition (104). *Fructus Ligustri* Lucid has been shown to promote the generation of SCFAs, and regulate calcium absorption and calcium homeostasis by upregulating the serum levels of 1,25-dihydroxyvitamin D3 and vitamin D-dependent calcium transporter gene expression (128,129). *Fructus Ligustri* Lucidi aqueous extract may preserve bone quality through regulation of the calcium balance and intestinal SCFA production in ovariectomized rats (130). Traditional herbal formula Gushukang (GSK) was clinically applied to treat primary osteoporosis. GSK exerted beneficial effects on trabecular bone of ovariectomized mice by improving calcium homeostasis through regulation of paracellular calcium absorption in the duodenum and transcellular calcium reabsorption in the kidney (131).

Anti-inflammatory effects and improvement of the mucosal barrier. Puerarin has been demonstrated to repair intestinal mucosal integrity by regulating the species and abundance of GM, increasing the levels of tight junction proteins (ZO-1 and occludin and their related mRNAs), and decreasing the release of inflammatory factors (TNF-α, IL-6, IL-1β, LPS and their related mRNAs). Concurrently, it was shown to increase the content of SCFAs in the colon, influencing host metabolic pathways with anti-inflammatory responses, such as amino acid metabolism, lipid metabolism and butyrate. This action was revealed to maintain intestinal homeostasis, alleviate inflammatory responses, and exert anti-osteoporotic effects (112). Berberine has been shown to enhance the intestinal barrier function by upregulating butyrate production from GM, ameliorate both systemic and local inflammation, and prevent alveolar bone loss associated with estrogen deficiency (115).

Improvement of immunization. The aqueous extract of Epimedii folium has been shown to increase the abundance of *Candidatus Saccharimonas*. It was demonstrated to be positively correlated with the production of T-cell cytokines (IL-2, IL-4, IL-10 and IFN-γ) by mesenteric lymphocytes (132). *Lycium barbarum* polysaccharide has been shown to promote the production of SCFAs by adjusting the structure of GM (110). Among them, butyric acid was demonstrated to increase the number of Tregs in both intestine and bone marrow, which in turn activated the Wnt signaling pathway

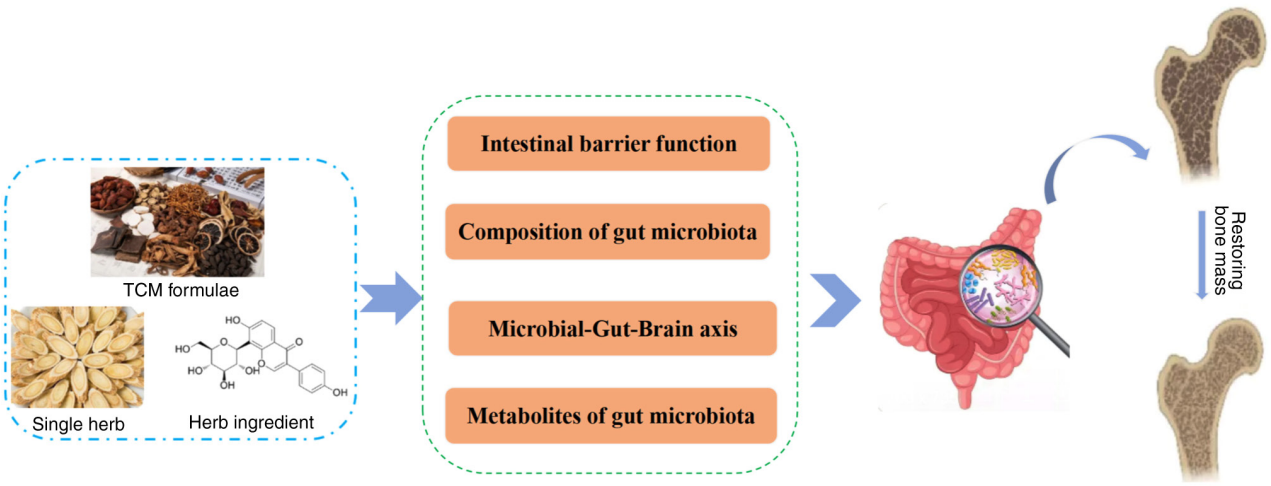

Figure 3. TCM regulates GM in the treatment of osteoporosis. TCM, including single herbs, single herbs, and compound formulas, acts on GM and its metabolites, playing a role in anti-inflammatory effects, improving mucosal barriers, enhancing immunity, and regulating metabolism. TCM, traditional Chinese medicine; GM, gut microbiota.

in OBs and stimulated osteogenesis (133). Jian Gu granule effectively prevented bone loss and enhanced bone strength by restoring the abundance of GM, increasing the levels of SCFAs, reducing the permeability of colonic epithelium, increasing the proportion of Tregs in the spleen, and altering cytokines associated with bone immunomodulation (120).

Metabolic regulation. The aqueous extract of Epimedii folium can improve the ratio of dominant flora, such as *Muribaculaceae* and *Lactobacillus*, and regulate the level of parathyroid hormone in the body, which affects BM (104). *Eucommia ulmoides* extract was shown to change the composition of intestinal microbiota and enhance the production of SCFAs, thereby improving OP (106). Extracts of *Sambucus williamsii* Hance, administered at a dose of 140 mg/kg, significantly reduced the abundance of *Ruminococcaceae UGC-014*, inhibited the synthesis of 5-HT, and increased bone density (134).

In conclusion, herbal combinations, single-flavor herbs, their extracts, and herbal monomers regulate the intestinal microbiota and their metabolic homeostasis, thereby exerting an anti-OP effect, as shown in Fig. 3. Although numerous studies on this subject have been conducted, the current understanding is limited to the association between the GM and the herbal medicines or active ingredients. Specific strains of microorganisms, genes, or metabolic enzymes involved remain unidentifiable. Therefore, it is still necessary to employ multi-omics technologies to elucidate the mechanism by which Chinese medicines act on which intestinal microorganisms to exert their therapeutic effects, and then to elucidate the metabolic mechanism of Chinese medicines in the intestinal tract of patients with OP in subsequent studies.

5. Microbiota-based therapy

Research focusing on targeting the GM for the treatment of OP has emerged as a hot topic. Studies have demonstrated that interventions such as fecal microbiota transplantation (FMT), probiotic and prebiotic supplementation, or dietary modification can improve the composition of the GM. These changes not only modulate local processes, but also systemic responses, including BM, affecting host metabolism, the immune system, and hormone secretion (135-175), as shown in Table SII.

Probiotics. Probiotics are a group of active microorganisms that colonize the intestinal tract and reproductive system. They confer benefits on the host, producing precise health effects and contributing to the microecological balance of the host (151). The regulation of OP by probiotics represents an emerging field, where the mechanisms underlying their enhancement to BM, prevention, and treatment of OP have become a focal point in BM research, yielding certain advancements. Research indicates that probiotics can maintain skeletal health by inhibiting bone resorption, promoting bone formation and mineralization, enhancing bone density and improving bone microstructure (152). The specific mechanisms include: i) Increasing calcium content in bone: Feeding hens with *Bacillus subtilis* significantly increased the calcium content of the tibia in hens (153) and prevented the reduction of bone mass in chicks caused by *Salmonella enteritidis* infection (154). ii) Inhibition of OC activity: Under estrogen deficiency, *Lactobacillus reuteri* ATCC PTA 6475 exerted a protective effect by reducing bone loss, bone resorption markers and osteoclastogenesis (28). *Lactobacillus reuteri* ATCC PTA 6475 inhibited bone resorption and TNF-α production, and significantly improved bone density in male mice, suggesting that the effect of bone regulation may be related to sex (139). iii) Inhibition of inflammatory response to reduce bone resorption: Mice were treated with *Lactobacillus paracasei* DSM13434 and the amount of cortical bone mineral content was increased, while the serum levels of bone resorption marker C-terminal telopeptides and the urinary fractional excretion of calcium were reduced (137). *Lactobacillus rhamnosus* GG and probiotic supplement VSL #3 have been demonstrated to reduce intestinal permeability and inhibit intestinal inflammation, thereby preventing estrogen deficiency-induced bone loss (26). iv) Promoting OB activity: *Lactobacillus reuteri* attenuated bone loss in type 1 diabetic

mice by regulating GM, preventing the decline in osteocalcin levels and mineralization deposition rates, suggesting a positive effect of probiotics on osteoanabolism (30). v) *Bifidobacterium longum* increased the bone mineral content in rats during experimental trials (155). *Lactobacillus rhamnosus* stimulated the secretion of insulin-like growth factor, which in turn promoted bone mineralization (140). vi) Regulation of BM pathways: *Lactobacillus rhamnosus* promoted the maturation and differentiation of OB and osteocytes by upregulating the expression of genes associated with the mitogen-activated protein kinase 1 and 3 pathway (156). In addition, *Bifidobacterium longum* increased bone mineral density by upregulating the expression of SPARC and Bmp-2 genes (157).

Prebiotics. Prebiotics are a class of indigestible food ingredients, mainly non-digestible oligosaccharides, including isomaltooligosaccharides, inulin, oligofructose, soybean oligosaccharides, lactuloses, and pyrodextrins. They can selectively stimulate the growth of one or more types of intestinal flora, which can be utilized by GM, and promote the health of the host (158). It has been found that prebiotics can promote the absorption of mineral elements, increase bone mineralization and enhance bone density (152). i) Promoting calcium absorption: Galactooligosaccharides increased the population of *Bifidobacteria* in the gut, which was conducive to the utilization of calcium and magnesium, thus improving bone mass (145). A three-week oral administration of galactooligosaccharides to adolescent females significantly increased the proportion of intestinal *Bifidobacteria* and the rate of calcium absorption (141). Agave fructans were shown to promote the absorption of minerals in the intestinal tract, and improve the content of bone minerals (144). In addition, oligofructose effectively promoted calcium absorption in rats fed a high-calcium diet (147). ii) Influence on bone conversion: The combination of lactulose galactooligosaccharides/oligofructose with calcium supplementation has been shown to elevate bone mineralization, bone mineral density, and increase the surface area of OBs in rats (159). Calcium supplementation combined with short-chain fructooligosaccharides has been shown to mitigate the rate of systemic and spinal bone loss in postmenopausal women (160). iii) Improvement of bone strength: Galactooligosaccharides, oligofructose, and inulin have demonstrated bone-strengthening effects in both healthy and ovariectomized rats (161,162). iv) Enhancement of other bone health agents: The synergistic prebiotic combination of fructo-oligosaccharide and soy isoflavones was shown to improve the bone strength of ovariectomized rats. The prebiotic mixture had a more pronounced enhancement in bone strength when soy isoflavone content was relatively low, underscoring that this prebiotic blend can yield synergistic effects. These effects enhanced the efficacy of prebiotics, with superior bone-strengthening outcomes compared with the use of prebiotics alone (152). Therefore, prebiotics play an important role in regulating GM and maintaining bone homeostasis.

FMT. FMT is the transfer of GM from a healthy donor to an intestinal dysbiosis recipient, aiming to restore intestinal microbial homeostasis and ameliorate intestinal dysbiosis (163). A significant increase in bone mass was observed 4 weeks after GFM received FMT using cecal contents from conventionally housed mice (12). Additionally, the bones of conventional mice chronically infused with broad-spectrum antibiotics reproduced the phenotype of GFM (164). The transplantation of fecal bacteria from osteoporotic mice into normal mice resulted in a significant decrease in the bone mass of the recipient (61). Transplantation of fecal bacteria from young rats into aged rats improved intestinal homeostasis at the portal and familial levels, while increasing the bone volume, trabecular volume fraction, trabecular number, and trabecular thickness in aged rats. This phenomenon suggests the direct influence of that GM on BM within the organism (57). The transplantation of segmented filamentous bacteria into GFM increased the number of Th17 cells, leading to increased levels of IL-17, TNF-α, and IL-1, and induced the expression of RANKL, thereby promoting osteoclastogenesis (165). The transplantation of *Clostridium* clusters IV and XIVa, which were isolated from mice, into GFM resulted in an increase in the number of systemic and lamina propria Tregs (166). SCFAs also induced the differentiation of Tregs, which regulated osteoclastogenesis through the secretion of IL-4, IL-10, and TGF-β (167). Malnutrition in infants and young children has been shown to lead to the dysbiosis of the GM, subsequently disrupting the maturation of the immune system and the normal growth of the skeletal system. It was demonstrated that the transplantation of the GM from malnourished children into healthy mice resulted in a significant reduction in bone mass over time (168). Animal experiments have shown that bone mass and immune factor levels are restored to normal through metabolites and immunomodulation when GM is transplanted into germ-free or depleted mice (12). Certainly, the adverse effects caused by the allochthonous enterobacteria post-transplantation, as well as the compliance issue arising from the differences in administration routes between the upper and lower gastrointestinal tract, remain areas for improvement and consideration. Long-term and effective FMT therapy may radically improve the diversity of GM in patients and become an effective method to treat OP (169).

Dietary regulations. Dietary structure can influence the type of GM and the function of the gut. Low dietary fibre intake may disrupt the integrity of the intestinal mucosal barrier, causing a relative increase in the levels of *Firmicutes* and *Proteobacteria* and a relative decrease in the levels of *Bacteroidetes* in the intestines of dietary fibre-deficient mice (170). A previous study indicated that Tanzanian hunter-gatherers, and Malawian and Venezuelan farmers consuming agri-foods had a greater diversity of flora than populations from the United States (171). Higher dietary fibre consumption may exert a positive influence on the progression of OP by altering the structural composition of GM (172). High-fibre diets and oligofructose may increase the number of *Bifidobacterium* species, optimize the microbial composition of the GM, increase the content of SCFAs, and lower the pH in the gut, thus promoting calcium absorption. Dairy consumption encourages bone formation and inhibits bone resorption (173). Studies have also found that consumption of dairy products by adults aged 60 years and above can reduce the risk of OP. This beneficial effect is partly attributed to the lactulose content of lactulose derivatives.

This interaction is capable of lowering serum parathyroid hormone levels, thereby decreasing levels of bone resorption markers (174,175).

6. Conclusions and prospects

GM is closely related to the occurrence and development of OP and related metabolic diseases. Treating OP by improving GM and its metabolites has been a hot and challenging topic in medical research in recent years. On one hand, the GM can directly or indirectly participate in bone mass regulation by modulating host metabolism, calcium absorption, hormone levels, the immune system, and the central nervous system. On the other hand, GM-associated metabolites can also reliably and effectively reflect the impact of GM on BM, potentially serving as novel targets for the prevention and treatment of OP. The present review summarized that TCM, probiotics, and prebiotics can ameliorate the composition of GM and promote microbial-metabolite balance by regulating the 'gut-bone axis', thereby exerting therapeutic effects against OP. These findings provide theoretical support and reference for clinical treatment of OP and the development of innovative therapeutic agents.

Currently, TCM has demonstrated potential in treating OP by regulating GM. However, several challenges and limitations remain in its practical applications: i) Research primarily relies on laboratory animal studies, lacking high-quality clinical trials to objectively reflect TCM clinical efficacy and clarify its mechanisms of action; ii) TCM interventions in OP studies often focus on single signaling pathways, failing to systematically elucidate multi-pathway interactions; iii) Research on GM profiles has primarily focused on common bacterial communities, lacking studies on the mechanisms underlying complex microbial interactions; and iv) The therapeutic effects of TCM on OP currently remain confined to the levels of marker microorganisms, microbial diversity, and differential metabolic products, making it difficult to elucidate the biotransformation processes of herbal active components through GM. Therefore, further investigation is still required to elucidate how TCM optimizes the structure and function of the host GM, how the host GM converts the active components of TCM into metabolites that act on target organs, and the mechanisms by which microbial metabolites exert biological effects on the host. Moreover, research should leverage modern molecular biology techniques, such as macro-genomics, functional genomics and metabolomics, to elucidate the mechanisms of TCM treatment for OP from a multi-component, multi-target, and holistic regulatory perspective. Simultaneously, techniques such as FMT, supplementation of deficient microbiota and metabolites, and co-incubation of TCM with GM should be employed to bridge the gap in understanding the 'TCM active components-GM-microbial metabolites' interaction pathway. Furthermore, it is imperative to enhance clinical trials to substantiate the efficacy and safety of these treatments, while methodologies such as molecular dynamic simulations should be employed to identify critical therapeutic targets of TCM in the treatment of OP.

As awareness of the impact of GM on health grows, an increasing number of studies are focusing on the relationship between gut microbial metabolism and BM. Regulating GM through supplementation with probiotics and prebiotics to improve BM has emerged as a new therapeutic target for OP. However, whether moderate supplementation of prebiotics or probiotics is effective in humans needs to be supported by large-scale multi-centre clinical studies. In addition, it remains to be elucidated whether the effects of different types of prebiotics or probiotics are consistent, the differential effects of age, sex, and etiology on the efficacy of OP as well as the optimal dosage, duration of treatment, timing of initiation and cessation, and the specific mechanisms underlying their actions. FMT as a treatment for *Clostridium* infection has become more mature, but there are still some unresolved problems in the treatment of OP. Therefore, in clinical practice, the use of enterobacterial preparations administered via capsule for transplantation can improve patient compliance and reduce adverse reactions. Timely and long-term FMT in patients with OP can fundamentally improve their GM composition and intestinal barrier function, potentially emerging as an effective therapeutic approach for OP in clinical treatment.

In summary, future research should focus on the core area of the mechanisms by which GM and their metabolites treat OP. By skillfully integrating cutting-edge technologies such as artificial intelligence, genomics, and high-throughput screening, and closely aligning with clinical practice needs, this approach aims to lay a solid foundation for exploring and innovating anti-OP treatment strategies.

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Competing interests

The authors declare that they have no competing interests.

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