frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 28 May 2026
DOI 10.3389/fimmu.2025.1719445

Check for updates

OPEN ACCESS

EDITED BY
Taylor Sitarik Cohen,
AstraZeneca, United States

REVIEWED BY
Kanami Orihara,
Tokyo Institute of Technology, Japan
Harold Van Bosse,
Saint Louis University, United States

*CORRESPONDENCE
Zheng Liu
liuzheng1707@163.com

RECEIVED 06 October 2025
REVISED 09 November 2025
ACCEPTED 01 December 2025
PUBLISHED 28 May 2026

CITATION
Liu M, Gong J, Liu Y, Yu J, Hu Z and Liu Z (2026) Multi-omics reveals circadian regulation of bone homeostasis by gut microbiota metabolites: mechanisms and chronotherapeutic implications. *Front. Immunol.* 16:1719445. doi: 10.3389/fimmu.2025.1719445

COPYRIGHT
© 2026 Liu, Gong, Liu, Yu, Hu and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

# Multi-omics reveals circadian regulation of bone homeostasis by gut microbiota metabolites: mechanisms and chronotherapeutic implications

Mingdong Liu[1], Jiaqi Gong[2], Yaqi Liu[3], Jiayao Yu[2], Zepeng Hu[2] and Zheng Liu[2]*

[1]Department of Orthopaedics, Affiliated Hospital of Shaoxing University, Shaoxing, Zhejiang, China, [2]Department of Pharmacology, School of Medicine, Shaoxing University, Shaoxing, Zhejiang, China, [3]Department of Nursing, School of Medicine, Shaoxing University, Shaoxing, Zhejiang, China

The gut-bone axis plays a pivotal role in skeletal health, yet the integration of multi-omics approaches to elucidate circadian metabolite-bone interactions remains limited. This review synthesizes evidence from metagenomics, metabolomics, and germ-free models to uncover how microbiota-derived metabolites—including short-chain fatty acids (SCFAs), bile acids, tryptophan derivatives, and gaseous molecules—orchestrate bone remodeling in osteoporosis, osteoarthritis, and bone malignancies. Many studies demonstrate that SCFAs inhibit osteoclastogenesis via GPR43/HDAC signaling and promote osteoblast metabolic reprogramming, while bile acids enhance osteogenesis through FXR/Wnt/β-catenin activation. Tryptophan metabolites repair intestinal barrier integrity and modulate osteoimmunity via the AhR pathway. Single-cell omics reveal circadian oscillations of metabolite receptors (e.g., GPR43, FXR) in bone stromal cells, linking microbial diurnal rhythms to epigenetic regulation of bone turnover. We propose a novel "metabolite-immune-bone triad" model, highlighting microbiome-driven immunometabolic reprogramming as a central regulator of skeletal homeostasis. These insights advance precision microbial therapeutics and chrono-nutritional strategies, bridging multi-omics discoveries with clinical applications for bone disorders.

KEYWORDS

gut microbiota, bone diseases, circadian rhythm, multi-omics, microbial therapeutics

## 1 Introduction

As the global population ages, skeletal diseases such as osteoarthritis (OA) and osteoporosis (OP) have emerged as major public health challenges, collectively affecting over 550 million individuals worldwide (1, 2). These disorders are characterized by disrupted bone remodeling dynamics, yet current therapies targeting osteoblast-osteoclast equilibrium

remain suboptimal due to incomplete mechanistic insights. Recent advances in the gut-bone axis (GBA) have revealed that gut microbiota-derived metabolites—including short-chain fatty acids (SCFAs), bile acids, and tryptophan derivatives—orchestrate bone homeostasis through immunometabolic crosstalk (3–5). Moreover, an imbalance in the gut microbiota may lead to bacterial and metabolite translocation, which can affect bone health (6–9). Although research in this field is still in its early stages, there is evidence to suggest that modulating the gut microbiota to improve bone health may be a promising therapeutic strategy.

Existing studies predominantly focus on static metabolite-bone interactions, neglecting the dynamic reciprocity between microbial circadian outputs and host bone cells. For instance, although SCFAs exhibit time-dependent effects on osteoclastogenesis, the mechanisms underlying circadian oscillations of G Protein-Coupled Receptor 43 (GPR43) in bone marrow stromal cells—and their disruption in OP—are yet to be elucidated (10). Similarly, bile acid-Farnesoid X Receptor (FXR) signaling shows phase-specific activity in osteoblasts (11), but how microbial metabolite rhythms align with skeletal transcriptional programs remains unknown. These gaps hinder the development of chronotherapeutic strategies targeting the GBA.

To address these challenges, this review underscores the transformative potential of multi-omics integration in elucidating the dynamic crosstalk between gut microbiota-derived metabolites and skeletal health. By leveraging metagenomics, metabolomics, and single-cell RNA sequencing (scRNA-seq), we delineate how microbial metabolites—such as SCFAs, bile acids, and tryptophan derivatives—orchestrate bone remodeling through receptor-mediated signaling (e.g., GPR43, FXR, AhR), epigenetic reprogramming, and immunometabolic regulation. Furthermore, we reveal circadian oscillations in metabolite receptor expression within bone stromal cells, linking microbial diurnal rhythms to osteoblast-osteoclast dynamics. These insights culminate in a novel "metabolite-immune-bone triad" model, which integrates circadian biology with microbiome-driven skeletal regulation. Our synthesis not only resolves prior knowledge gaps regarding temporal host-microbe interactions but also provides a mechanistic foundation for chronotherapeutic and nutritional interventions aimed at treating bone disorders. This work sets the stage for future translational studies, enabling precision medicine approaches that harness microbial metabolites for skeletal disease management.

# 2 Bone homeostasis and the role of GBA in bone diseases

## 2.1 Fundamentals of bone homeostasis

The skeleton is a dynamic organ that maintains its strength through continuous remodeling, a process that relies on the delicate balance between bone formation mediated by osteoblasts and bone resorption mediated by osteoclasts. Osteoblasts originate from mesenchymal stem cells, and their differentiation is driven by key signaling pathways such as Wnt/β-catenin and bone morphogenetic proteins; osteoclasts, on the other hand, derive from hematopoietic stem cells, and their formation is centrally regulated by the RANKL/RANK/OPG system. Osteocytes embedded in the bone matrix act as central regulators, coordinating the activities of the aforementioned two types of cells by sensing mechanical signals and secreting factors such as sclerostin (which inhibits the Wnt pathway) and RANKL. Additionally, mesenchymal stem cells in the bone marrow microenvironment compete between osteogenic and adipogenic differentiation pathways, and the tilt of this balance (i.e., the accumulation of bone marrow adipose tissue) is an important mechanism driving bone loss. Finally, systemic hormones such as estrogen play a key role in maintaining bone homeostasis by inhibiting osteoclastogenesis and promoting osteogenesis. Understanding this fundamental framework is crucial for exploring how gut microbiota and their metabolites remotely regulate this complex network through immune, metabolic, and endocrine pathways.

## 2.2 The role of GBA in bone diseases

Recent advances in microbiology and skeletal disease research have brought increasing attention to the emerging concept of the GBA. This concept delineates a bidirectional communication system wherein gut microbiota influence bone metabolism and health through diverse pathways. Specifically, gut microbes modulate bone density, formation, and resorption. The complex mechanisms underlying the GBA include the production of SCFAs, modulation of the immune system, and direct or indirect effects of gut microbial metabolites on bone cells (5, 12, 13).

OP is a disease characterized by reduced bone density and altered microstructure, which significantly increases the risk of fractures. Studies have shown that gut microbes can regulate bone density by affecting vitamin D synthesis and calcium absorption (5, 8, 14), and SCFAs (such as butyrate and propionate) produced by gut microbes can stimulate the activity of osteoblasts and inhibit the formation of osteoclasts, thereby helping to maintain and enhance bone mass (15–19). However, an imbalance in gut microbes can trigger chronic low-grade inflammation, which is associated with the development of OP (5, 20–22). Inflammatory cytokines such as TNF-α and IL-6 can enhance osteoclast activity, leading to bone loss. Some studies found that modulating the gut microbiota can improve bone density, further confirming the potential regulatory role of gut microbes in OP (23, 24).

OA, characterized by articular cartilage degradation and joint inflammation, may be influenced in its development by gut microbiota through the gut-joint axis. Research indicates that lipopolysaccharides (LPS) derived from gut microbes can induce systemic inflammation, thereby exacerbating joint inflammation and accelerating disease progression (25, 26). Furthermore, alterations in gut microbiota composition can modulate immune responses. For instance, probiotics may alleviate OA symptoms by regulating T-cell activity (5, 27).

The development of bone tumors is intricately linked to chronic inflammation, immune dysregulation, and gut microbiota dysbiosis through GBA. Emerging evidence indicates that gut microbiota-derived metabolites modulate bone tumorigenesis and progression through multiple mechanisms. Dysbiosis-driven systemic inflammation (e.g., elevated circulating LPS) and impaired immune surveillance disrupt bone microenvironment homeostasis, facilitating tumor cell survival and osteolytic activity. Experimental studies demonstrate that gut microbiota depletion reduces bone metastasis burden in murine models, while fecal microbiota transplantation (FMT) from tumor-bearing hosts accelerates osteosarcoma growth, underscoring microbiota's causal role in bone malignancy (28, 29) (Figure 1).

The gut microbiota modulates bone tumors through three main pathways. First, microbial metabolites such as SCFAs, notably butyrate, exert direct anti-tumor effects by inhibiting histone deacetylases (HDACs) in osteosarcoma cells, promoting cell cycle arrest and apoptosis via p21/WAF1 upregulation. Butyrate also enhances CD8+ T-cell infiltration and polarizes tumor-associated macrophages (TAMs) toward anti-tumor M1 phenotypes via GPR43 signaling (30, 31). Clinically, reduced fecal SCFAs correlate with accelerated bone tumor progression (32, 33). Second, dysbiosis impairs immune surveillance by reducing IL-17A+ γδ T cells and NK cell activity, hindering the elimination of metastatic cells in bone marrow, as shown in germ-free models (34, 35). Tryptophan metabolites (e.g., indole-3-aldehyde) activate the aryl hydrocarbon receptor (AhR) in dendritic cells, boosting anti-tumor CD8+ T-cell responses (36). Third, gut barrier disruption enables pathobiont translocation (e.g., Fusobacterium nucleatum), increasing systemic LPS. This activates TLR4/NF-κB signaling in

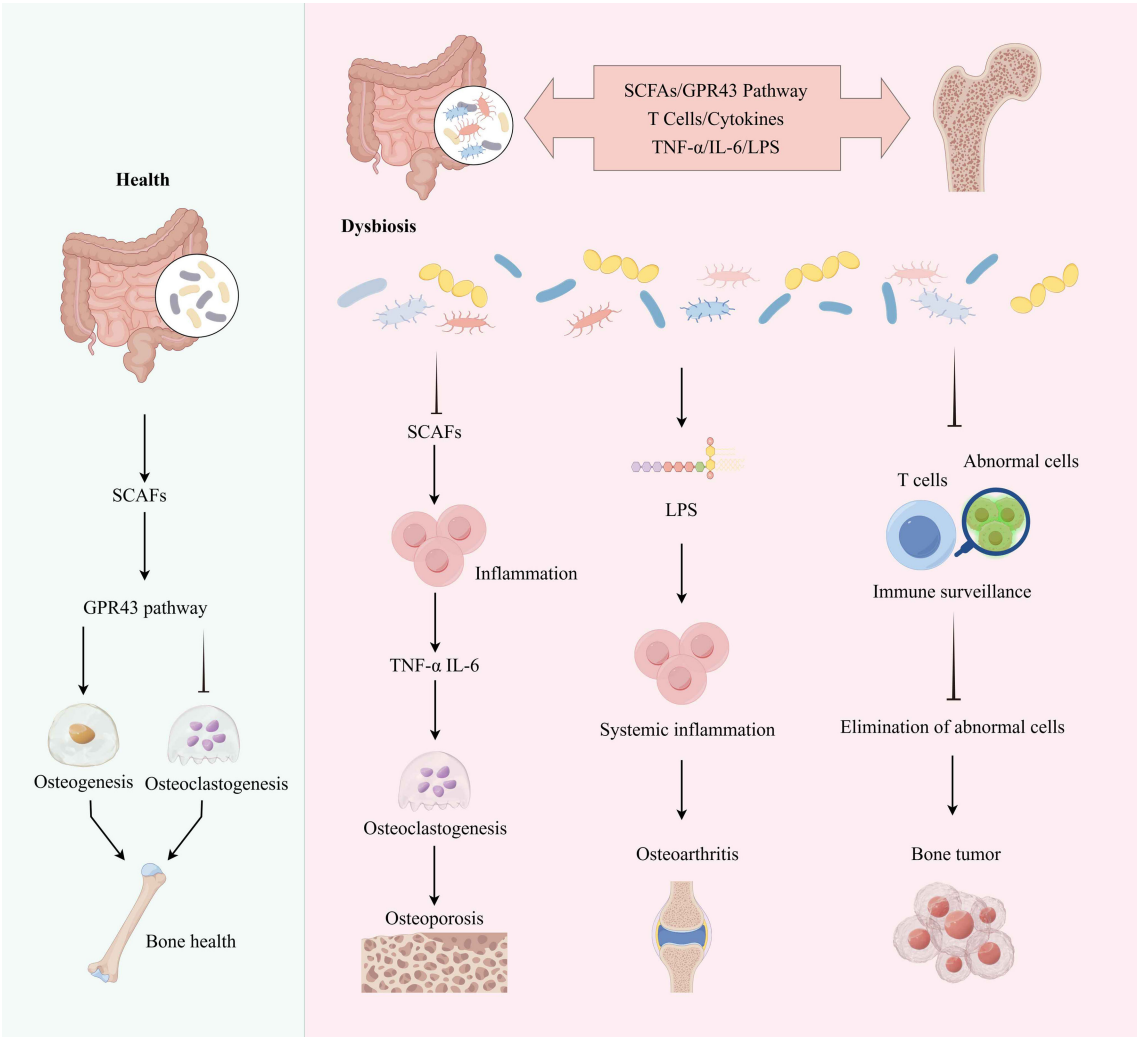



FIGURE 1
A panoramic view of the regulatory mechanisms of the microbiota-gut-bone axis in skeletal diseases. Under healthy conditions (left side), short-chain fatty acids (SCFAs, e.g., butyrate/propionate) produced by the gut microbiota activate the GPR43 signaling pathway, directly promoting osteogenesis and inhibiting osteoclastogenesis to maintain bone health. Under gut dysbiosis conditions (right side), decreased SCFA production and microbial imbalance drive skeletal pathogenesis through three distinct pathways: (1) Osteoporosis: Reduced SCFAs and increased pro-inflammatory cytokines (e.g., TNF-α/IL-6) disrupt the bone formation/resorption balance, favoring bone loss; (2) Osteoarthritis: Lipopolysaccharide (LPS) triggers systemic inflammation that spreads to joints, promoting local inflammation and cartilage degradation; (3) Bone Tumor: Dysbiosis impairs immune surveillance (e.g., T cell dysfunction), allowing abnormal cell proliferation and bone tumor formation due to failed elimination. ⊥: Inhibits/decreases.

bone stromal cells, elevating IL-6 and TNF-α, which enhance osteoclastogenesis and form a pre-metastatic niche (37, 38).

# 3 Role of microbiota metabolite on the cell metabolsim of bone diseases

## 3.1 Gut microbiota and bone metabolism

The gut microbiota, a complex ecosystem of trillions of microorganisms, significantly influences bone metabolism. Studies demonstrate that probiotics (e.g., Lactobacillus and Bifidobacterium) can enhance bone density (39), while opportunistic pathogens (e.g., Enterobacteriaceae) are linked to bone loss (23). In individuals and models with a conventional gut microbiota, reduced microbial diversity has been observed to correlate with decreased bone density. Notably, the relationship between microbial presence and bone mass is complex and non-linear. Germ-free (GF) mice, which entirely lack microbiota, exhibit a unique skeletal phenotype characterized by suppressed osteoclastogenesis and increased trabecular bone volume compared to conventionally raised mice. This apparent paradox is attributed to the underdeveloped immune system in GF mice, particularly the reduction in osteoclast precursors and pro-osteoclastic immune cells (e.g., CD4+ T cells), leading to diminished bone resorption. This highlights that the complete absence of microbiota creates a distinct immunometabolic state not directly comparable to simply having 'low diversity' within a conventional microbiome (23). Additionally, gut microbiota indirectly regulate bone metabolism through immune modulation and inflammatory responses. For example, OP patients exhibit diminished gut microbiota diversity alongside reduced bone density.

A key mechanism involves SCFAs—microbial metabolites derived from dietary fiber fermentation. SCFAs (e.g., acetate, butyrate) mediate bone homeostasis through epigenetic regulation (e.g., histone deacetylase inhibition) and receptor signaling (e.g., GPR activation). Their roles in osteoblast promotion, osteoclast suppression, and circadian synchronization of bone remodeling are detailed in Section 3.2 (14, 17, 40, 41).

## 3.2 Metabolite signaling pathways in bone homeostasis and diseases

### 3.2.1 Bidirectional regulation by SCFAs

SCFAs are primary products of gut microbial fiber fermentation. Absorbed via the colon and transported systemically, they serve as energy substrates or signaling molecules. Acetate circulates widely, while propionate is liver-metabolized (17, 42–44). SCFAs exert their effects on bone metabolism through multiple interconnected mechanisms. SCFAs activate GPR41, GPR43, GPR109A and free fatty acid receptors (FFAR2/3), initiating intracellular signaling pathways that regulate hormone secretion, inflammation, and immune function. These receptors exhibit rhythmic expression patterns in bone marrow stromal cells, with GPR43 peaking at midnight, aligning with circadian metabolite flux and suggesting a role for SCFAs in synchronizing bone remodeling through temporal regulation (17, 45). Furthermore, butyrate and propionate drive metabolic reprogramming in osteoclasts by shifting their energy metabolism toward glycolysis and away from oxidative phosphorylation. This metabolic shift downregulates osteoclastogenesis-related genes such as TRAF6 and NFATc1, thereby suppressing bone resorption and enhancing bone density (14, 17). Epigenetically, SCFAs modulate histone acetylation (e.g., H3K27ac) by inhibiting HDACs, influencing osteoblast differentiation and osteoclast activity. For instance, butyrate directly targets TRAF6 and NFATc1 expression to maintain bone homeostasis (40, 41). Advances in multi-omics approaches, including single-cell transcriptomics (scRNA-seq) and metabolomics, have uncovered circadian oscillations of metabolite receptors (e.g., GPR43, FXR) in osteoprogenitor cells. These findings provide a framework for understanding how microbial metabolites like SCFAs synchronize bone remodeling through dynamic interactions between epigenetic modifications (e.g., histone acetylation) and metabolic pathways, as evidenced by temporal alignment of receptor expression and metabolite flux (17) (Figure 2).

Although low concentrations of SCFAs (especially butyrate) have been shown to promote osteoblast differentiation and bone formation, *in vitro* experiments have clearly demonstrated that higher concentrations of butyrate can inhibit the proliferation and differentiation of osteoblasts. The underlying mechanism is related to the induction of cell cycle arrest. For example, studies have indicated that histone deacetylase inhibitors (HDACi) such as butyrate and trichostatin A (TSA), at concentrations that can cause high levels of histone acetylation, will inhibit the expression of osteoblast marker genes (such as Osteocalcin) and matrix mineralization. The mechanism is that HDACi upregulate the expression of the cell cycle-dependent kinase inhibitor p21 Cip1/WAF1, leading to cell cycle arrest of osteoblast precursors in the G1 phase and blocking their differentiation into mature osteoblasts (46). This suggests that the bidirectional regulation of osteoblasts by SCFAs follows the "hormesis" effect (beneficial at low doses, harmful at high doses). Moreover, under specific pathological conditions, SCFAs may exacerbate inflammation and produce complex effects through different immune pathways in specific microenvironments (such as rheumatoid arthritis synovium). For example, studies have shown that SCFAs can promote the differentiation of Th17 cells, which are key subsets of cells that promote inflammation and bone erosion, potentially leading to adverse consequences in autoimmune bone diseases (47).

### 3.2.2 Bile acid-FXR/Wnt cascade

Bile acids, synthesized by the liver, are essential for the digestion and absorption of lipids and are reabsorbed in the enterohepatic circulation. They also influence bone metabolism through the FXR, which is highly expressed in osteoblasts and plays a significant role in their proliferation (48, 49) FXR, as a major bile acid receptor and a member of the nuclear receptor superfamily, plays a central role in

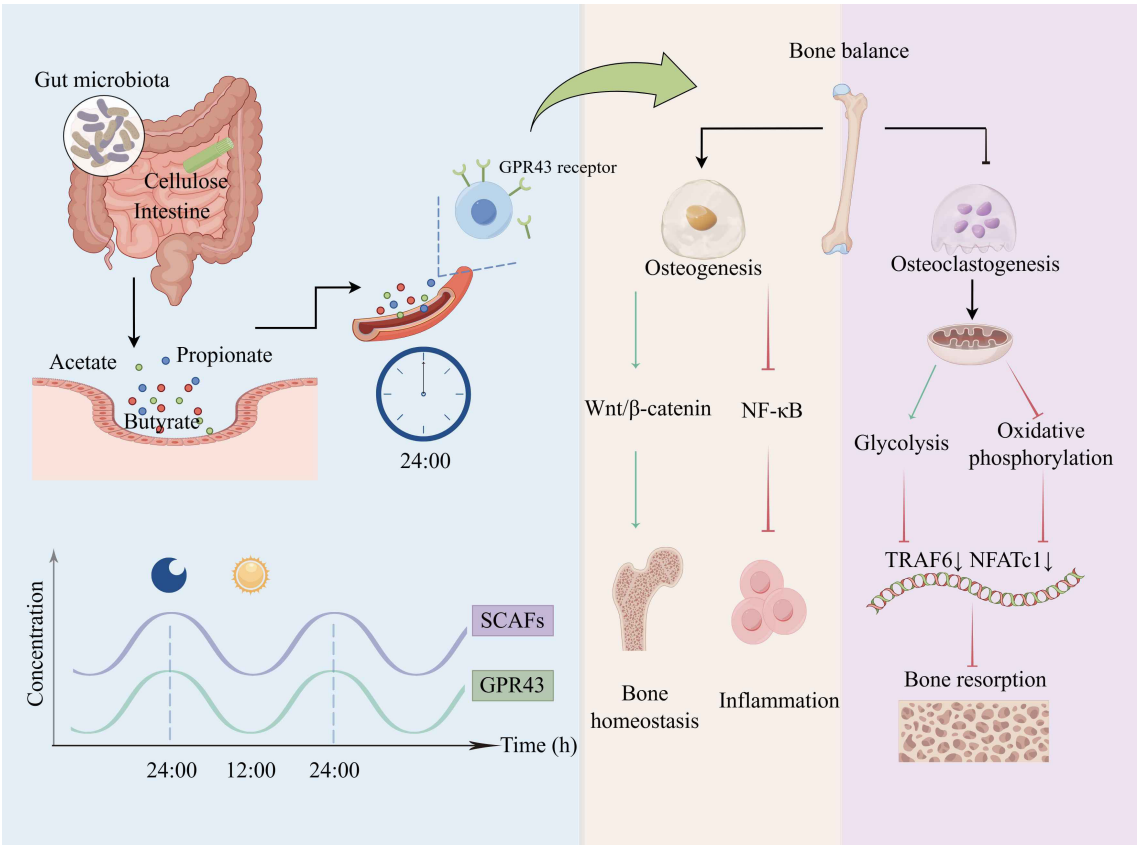



FIGURE 2
Circadian regulation of bone metabolism by gut microbiota-derived short-chain fatty acids (SCFAs) via GPR43 signaling. The gut microbiota ferments dietary cellulose to produce SCFAs (acetate, propionate, butyrate), which exhibit diurnal fluctuations in concentration. SCFAs activate the rhythmically expressed GPR43 (G protein-coupled receptor 43) in bone cells, peaking at midnight. This signaling cascade promotes osteogenesis by enhancing the Wnt/β-catenin pathway and suppressing NF-κB-mediated inflammation. Concurrently, SCFAs drive metabolic reprogramming in bone cells, balancing glycolysis and oxidative phosphorylation to favor bone mineralization over resorption. Ultimately, the gut microbiota–SCFA–GPR43 axis orchestrates bone homeostasis through integrated circadian, metabolic, and immune mechanisms. ⊥: Inhibits/decreases.

maintaining metabolic homeostasis. It was initially widely recognized for its key roles in regulating bile acid synthesis, lipid metabolism, and glucose homeostasis in the liver and intestine. Upon binding of bile acids to FXR, a series of downstream events are triggered, including the induction of small heterodimer partner (SHP) expression, which in turn inhibits bile acid synthesis and regulates their enterohepatic circulation. In recent years, studies have revealed that FXR also has important functions in bone metabolism. FXR is highly expressed in osteoblasts, and its activation has been shown to directly regulate the expression of osteogenesis-related genes and affect bone energy metabolism, thereby closely linking bile acid signaling to the process of bone formation. Studies have shown that geniposidic acid (GPA) can activate the FXR signaling pathway, upregulating the mRNA expression of osteogenic markers such as Runx2, Osx, and Alpl, increasing the levels of related proteins and Alkaline phosphatase (ALP) enzyme activity, and promoting osteoblast mineralization (50). In an ovariectomized mouse model, GPA was able to increase bone density and quality, enhance bone formation rates, and elevate serum bone formation marker levels, as well as increase the expression of factors such as FXR, SHP, and RUNX2. In FXR gene-knockout mice, the effects of GPA were diminished, confirming that it promotes bone formation through the FXR/RUNX2 signaling pathway, offering a new strategy for the treatment of OP (5, 6, 51, 52).

Furthermore, bile acids enhance osteoblast function through the Wnt/β-catenin signaling pathway, which is crucial for the differentiation of bone marrow mesenchymal stem cells (BM-MSCs), osteoblast formation and function, osteoclast differentiation, and bone resorption. Wnt signaling promotes the differentiation of BM-MSCs into osteoblasts and inhibits adipocyte differentiation, such as Wnt3a activating Transcriptional coactivator with PDZ-binding motif (TAZ) to promote osteogenic differentiation (8, 53). It also enhances osteogenic differentiation and inhibits adipogenic differentiation through the noncanonical JNK pathway. In osteoblasts, Wnt signaling activates Runx2 through β-catenin/TCF1, promoting differentiation and bone formation, while also increasing the expression of bone morphogenetic proteins, which promote osteoblast proliferation and differentiation (54–56). Wnt signaling also inhibits osteoclast differentiation by regulating the expression of RANKL and OPG, such as activating Wnt signaling in osteoblasts to suppress RANKL

expression and increase OPG expression, thereby inhibiting bone resorption (57, 58). The activation of the Wnt signaling pathway enhances the sensitivity of osteocytes to mechanical stimuli, promoting bone formation and inhibiting bone resorption. Abnormalities in the Wnt signaling pathway are associated with various bone diseases, such as the inhibition of Wnt signaling leading to bone loss in OP and abnormal activation leading to increased bone mass in osteomalacia (54, 59).The regulation of the Wnt signaling pathway provides new targets for the treatment of bone diseases (57).

Bile acids can inhibit the formation and activity of osteoclasts. For example, chenodeoxycholic acid (CDCA) inhibits osteoclast differentiation through the FXR signaling pathway (48, 60, 61). The mechanism may be related to the upregulation of osteoprotegerin and other osteoclastogenesis inhibitory factors in osteoblasts after FXR activation, thereby indirectly inhibiting osteoclast formation. In addition, studies have shown that the activation of FXR can change the intracellular metabolic environment, but this mainly occurs in osteoblasts and other stromal cells, where it dominates the bone microenvironment by promoting anabolic metabolism, ultimately exerting a net inhibitory effect on osteoclasts (62, 63). In summary, current evidence indicates that bile acids mainly exert an inhibitory effect on osteoclastogenesis in the bone microenvironment through the FXR signaling pathway (Figure 3).

### 3.2.3 Tryptophan metabolite-AhR axis

Tryptophan, a precursor to serotonin (5-hydroxytryptamine), melatonin, kynurenine, and niacin, has metabolic byproducts that influence bone remodeling. In diseases of bone metabolism, changes in tryptophan levels occur and play a role in osteoblast differentiation (64) Gut-derived serotonin, which accounts for approximately 95% of the body's total serotonin, is synthesized by enterochromaffin cells and does not cross the blood-brain barrier. It acts as a potent inhibitor of bone formation. Mechanistically, gut-derived serotonin binds to the 5-HT1B receptor on osteoblasts, inhibiting their proliferation by suppressing cyclic AMP (cAMP) production and cyclin expression. In contrast, brain-derived serotonin, synthesized in the raphe nuclei of the brainstem, functions as a positive regulator of bone mass by enhancing bone formation and reducing bone resorption through a sympathetic nervous system-independent pathway, primarily involving the 5-HT2C receptor in the hypothalamus. The dual origin and opposing actions of serotonin create a complex therapeutic landscape, especially in the context of the widely used selective serotonin reuptake inhibitors (SSRIs). SSRIs increase synaptic serotonin levels to treat depression but also elevate peripheral serotonin bioavailability. Epidemiological studies consistently report that long-term SSRI use is associated with reduced bone mineral density (BMD) and an increased risk of fractures, an effect

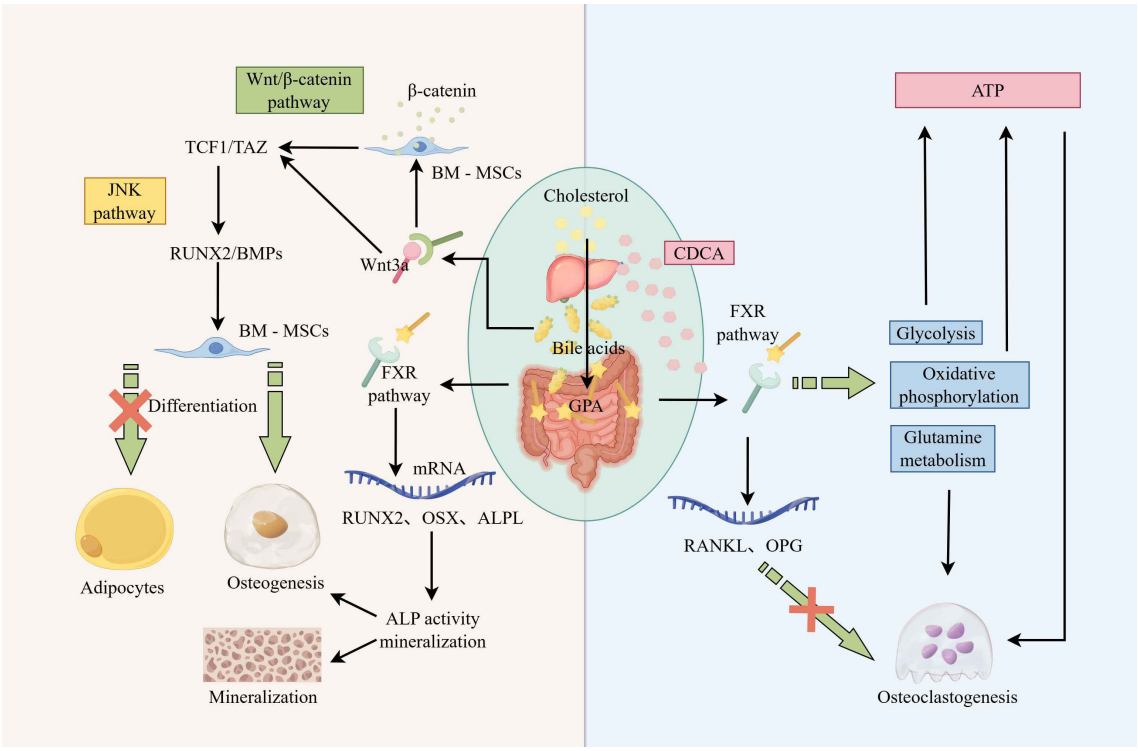



FIGURE 3
Regulation of bone metabolism by bile acids through FXR and Wnt/β-catenin signaling pathways. Bile acids (e.g., CDCA) and synthetic agonists like GPA activate the Farnesoid X receptor (FXR) pathway. This upregulates the expression of RUNX2/OSX/ALPL, enhancing alkaline phosphatase activity and mineralization. Concurrently, bile acids enhance Wnt/β-catenin signaling via TCF1/TAZ and the non-canonical JNK pathway, further driving BM-MSCs toward osteogenesis and inhibiting adipogenesis. Downstream effects include the inhibition of osteoclastogenesis through metabolic reprogramming (glycolysis, oxidative phosphorylation, and glutamine metabolism). Overall, the FXR and Wnt pathways integrate to coordinate bone formation and resorption.

attributed to the unopposed inhibitory action of gut-derived serotonin on osteoblasts. This presents a clinical dilemma: the neuropsychiatric benefits of SSRIs may come at the cost of skeletal health, particularly in vulnerable populations such as postmenopausal women. Consequently, selectively inhibiting gut-derived serotonin synthesis without affecting the central nervous system has emerged as a promising therapeutic avenue for bone anabolism. Additionally, microbial tryptophan metabolites indole-3-acetic acid (IAA) and indole-3-propionic acid can significantly improve bone loss by repairing intestinal barrier integrity (64–67). These metabolites activate the aryl hydrocarbon receptor (AhR) in the gut, stimulate the Wnt/β-catenin signaling pathway, repair the intestinal barrier, promote osteoblastogenesis, and inhibit osteoclasts. Tryptophan metabolites affect the senescence of musculoskeletal stem cells, with metabolites of the kynurenine pathway such as 3-hydroxykynurenine, kynurenic acid, and anthranilic acid promoting bone aging, reducing bone mineral density, and increasing fracture risk (68). In contrast, 3-hydroxyanthranilic acid, xanthuric acid, picolinic acid, quinolinic acid, and NAD+ increase bone mineral density and reduce fracture risk (69–72) (Figure 4).

### 3.2.4 Gaseous microbiota-derived metabolites

Emerging evidence indicates that gaseous metabolites derived from gut microbial metabolism of dietary fiber, sulfur-containing amino acids, and proteins—including hydrogen sulfide ($H_2S$), nitric oxide (NO), ammonia ($NH_3$), carbon monoxide (CO), and others—function not as waste products but as signaling molecules that regulate skeletal homeostasis via the gut–bone axis (GBA). Among these, $H_2S$ acts as a protective gaseous mediator, similar to NO and CO, and exerts significant effects on nervous, cardiovascular, and skeletal systems, as well as inflammatory processes. It supports skeletal development through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms, notably by rebalancing bone remodeling in OP, stimulating bone formation, and preserving skeletal integrity (73). Please note that CO is different from carbon dioxide and is a gaseous molecule that is often considered toxic due to its high affinity for hemoglobin at high concentrations. However, at low concentrations that are physiologically relevant, it exerts anti-inflammatory and reparative effects via the HO-1 pathway. Meanwhile, gut-derived serotonin, which is regulated by the microbiota, inhibits bone formation when upregulated, illustrating the complex gut-brain-bone crosstalk.

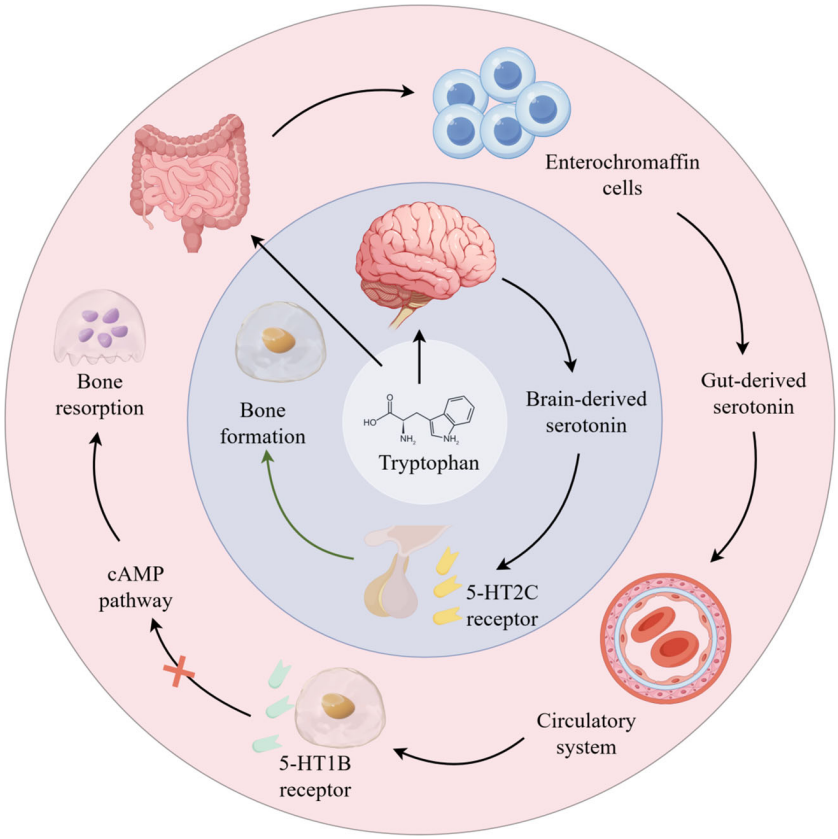



FIGURE 4
Opposing effects of gut- and brain-derived serotonin on bone remodeling. Tryptophan metabolism produces serotonin in two distinct compartments with opposing effects on bone. Gut-derived serotonin (95% of total) acts on osteoblasts via the 5-HT1B receptor, inhibiting bone formation by suppressing cAMP signaling. In contrast, brain-derived serotonin signals through hypothalamic 5-HT2C receptors to promote bone formation and reduce resorption. This duality explains the bone loss associated with drugs that increase peripheral serotonin (e.g., SSRIs), highlighting the gut-derived pathway as a therapeutic target.

Produced by sulfate-reducing bacteria such as Desulfovibrio spp., $H_2S$ promotes osteoblast differentiation via the Wnt/β-catenin pathway and inhibits inflammation-induced osteoclastogenesis through suppression of NF-κB signaling. Studies using the $H_2S$ donor sodium hydrosulfide (NaHS) have shown attenuation of dexamethasone (Dex)-induced reactive oxygen species (ROS) generation and ATP depletion, mediated via AMPK pathway activation (74, 75). Furthermore, $H_2S$ protects osteoblasts from Dex-induced apoptosis and necrosis through AMPK-dependent mechanisms, highlighting its cytoprotective and antioxidant roles (76). Notably, $H_2S$-releasing compounds have been shown to increase bone mineral density in murine OP models.

NO, generated via microbial nitrite reduction and host eNOS activity, bidirectionally modulates osteoclast–osteoblast coupling through the cGMP–PKG pathway. Bone-implant materials engineered for NO release have demonstrated efficacy in enhancing defect repair. Elevated $NH_3$, produced by proteolytic microbes, aggravates myeloma-related osteolysis by stimulating osteoclast activity, spurring interest in targeting ammoniagenic pathways therapeutically. CO, via the HO-1 pathway, exerts anti-inflammatory and reparative effects, while gut-derived serotonin—under microbiota control—inhibits bone formation when upregulated, illustrating complex gut–brain–bone crosstalk.

Capitalizing on these mechanisms, advanced micro- and nano-delivery systems enabling controlled release of $H_2S$, NO, and CO are being developed. These innovations hold translational promise for preventing implant-related infections, enhancing bone regeneration, and enabling combinatorial gas therapies for complex skeletal disorders (Figure 5).

## 3.3 Immune regulatory network

### 3.3.1 Microbiota-immune cell interactions

Modulating the gut microbiota has emerged as a potential therapeutic strategy for rheumatoid arthritis (RA), where probiotics (e.g., Lactobacillus) and prebiotics (e.g., dietary fiber) alleviate RA symptoms and slow disease progression by improving microbial composition (77). For instance, Lactobacillus species prevent bone loss and maintain bone density through modulating the Treg-Th17 cell ratio and inhibiting osteoclastogenesis (78, 79). Additionally, polyphenolic compounds such as ellagic acid exhibit anti-inflammatory effects by regulating the gut microbiota (80, 81). Beyond RA, the gut microbiota critically influences bone remodeling by balancing Th17/Treg dynamics (82). Probiotics (e.g., Lactobacillus reuteri) suppress osteoclast precursor (CD11b+/Gr1-) recruitment and

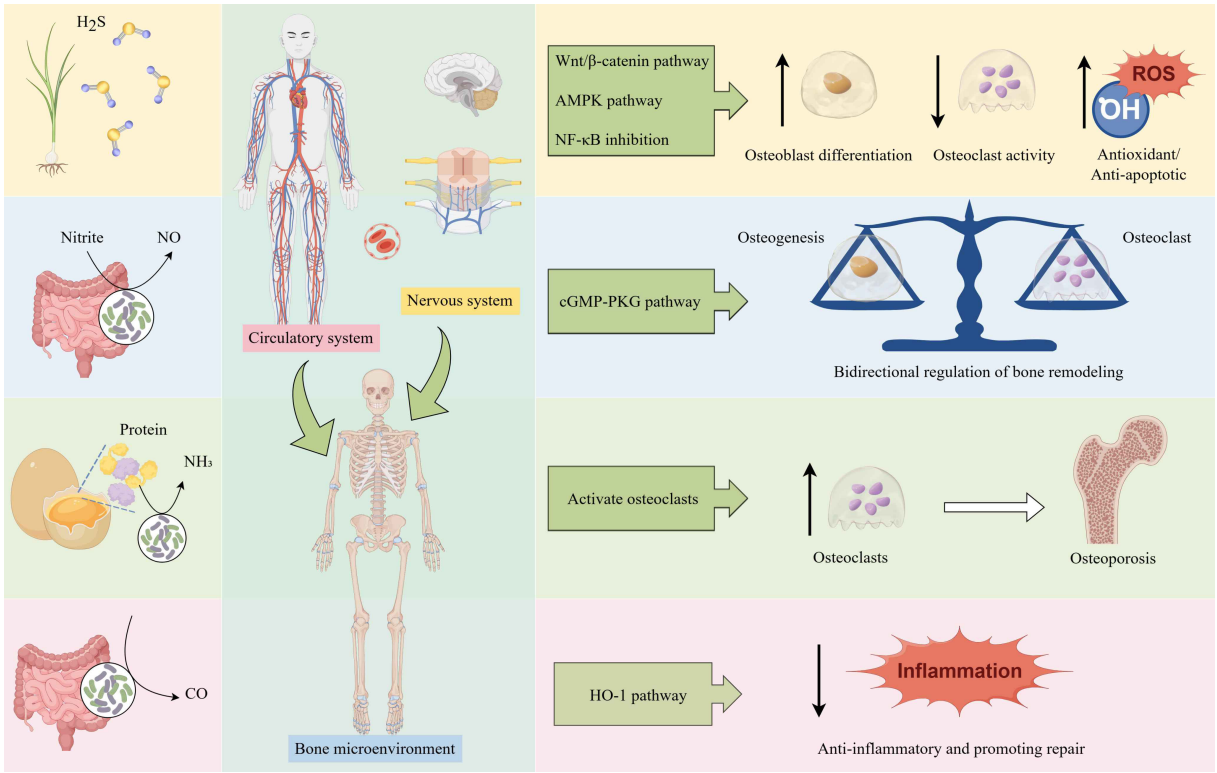



FIGURE 5
Mechanisms of gut microbiota-derived gaseous metabolites in bone remodeling. Gut microbial fermentation produces gaseous metabolites that enter circulation and regulate bone homeostasis. $H_2S$ (e.g., from Desulfovibrio) promotes osteogenesis via Wnt/β-catenin signaling and inhibits osteoclastogenesis via NF-κB suppression and AMPK-mediated antioxidant effects. NO bidirectionally modulates osteoclast-osteoblast coupling through the cGMP-PKG pathway. $NH_3$ (from proteolytic bacteria) activates osteoclasts and exacerbates bone resorption. CO exerts anti-inflammatory and pro-repair effects via the HO-1 pathway. These mechanisms inform targeted gas-delivery systems (e.g., nano-scaffolds) for treating bone defects and osteoporosis. Abbreviations: AMPK, AMP-activated protein kinase; HO-1, heme oxygenase-1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.↑: Promotes/increases; ↓: Inhibits/decreases.

reduce pro-inflammatory cytokines (TNF-α, IL-6) via Treg cell expansion (83, 84). Single-cell immunogenomics further reveal enhanced Th17 infiltration and metabolic reprogramming of GPR43 + macrophages in osteoporotic bone marrow, highlighting microbial metabolites' role in mediating "metabolite-immune dialogue" to regulate the bone microenvironment (85).

### 3.3.2 Bone loss mediated by inflammatory cytokines

Microbial products such as LPS induce chronic low-grade inflammation and promote RANKL-dependent osteoclastogenesis through the TLR4/NF-κB pathway (86, 87). Clinical cohort data show that serum TNF-α levels in OA patients are negatively correlated with gut microbiota diversity (88). Mechanistically, SCFAs produced by gut microbiota can reduce NLRP3 inflammasome activation by inhibiting histone deacetylase 6 (HDAC6), thereby attenuating inflammatory responses (89, 90). Additionally, FMT therapy has been shown to reduce IL-1β levels in a randomized controlled trial (RCT), verifying the clinical translational potential of the "immune-bone axis." These findings highlight the critical role of the gut microbiota in modulating systemic inflammation and its impact on bone health, suggesting that microbiota-targeted therapies may have therapeutic potential for inflammatory bone diseases (49, 90–92).

### 3.3.3 Microbiota-immune clock synergy

Disruption of circadian rhythms alters the structure and function of the gut microbiota, impacting host metabolism and immune function (93–95). Microbial metabolites (e.g., SCFAs) regulate host immune cell function and infiltration by activating receptors (e.g., GPR43) (96, 97) and modulate gene expression via epigenetic mechanisms like histone modifications (96, 98). These actions affect bone matrix cells, thereby regulating bone metabolism (18, 99, 100). Concurrently, circadian rhythms directly regulate immune cell function; their disruption impairs intestinal immune barrier function, increasing sensitivity to endotoxins like LPS (101–103). Therefore, the gut microbiota, through its metabolic signaling and in coordination with the host's immune circadian rhythms, constitutes the "metabolite-immune-bone triad model" to maintain bone homeostasis (5, 49, 104).

Experimental validation of the "metabolite-immune-bone triad" in animal models provides mechanistic credibility to this integrative framework: Probiotic-mediated Treg expansion ameliorates bone loss in ovariectomized (Ovx) mice, where Lactobacillus reuteri supplementation increased bone marrow Treg frequency by 2.1-fold, suppressed osteoclast precursor recruitment, and reduced trabecular bone loss by 32% compared to controls. This correlated with decreased serum TNF-α and IL-6 levels, confirming microbiota-immune crosstalk in skeletal protection. Germ-free (GF) mouse studies reveal direct immune-bone coupling: GF mice exhibit 40% higher trabecular bone volume alongside reduced CD4+T cells and osteoclast precursors (CD11b +/Gr1-) in bone marrow.Reconstitution with conventional microbiota normalized immune cell profiles and decreasing the bone mass to normal levels. SCFAs synchronize circadian bone remodeling through epigenetic-immune coordination. Butyrate gavage in mice elevated H3K27ac acetylation at GPR43 promoter regions in bone stromal cells, amplifying midnight receptor expression by 3.5-fold. This enhanced Treg differentiation and suppressed NLRP3 inflammasome activation, reducing osteoclastogenesis by 45% in circadian-misaligned models.These findings solidify the triad model: microbial metabolites (e.g., SCFAs) rhythmically engage immune cells (Treg/Th17) and epigenetic machinery (H3K27ac) to orchestrate bone remodeling, offering actionable targets for chronotherapeutic interventions.

## 3.4 Therapeutic implications: translating mechanistic insights into interventions

The comprehensive mechanistic studies reviewed above reveal that gut microbiota-derived metabolites (SCFAs, bile acids, tryptophan derivatives, and $H_2S$) precisely regulate bone homeostasis through multidimensional pathways: receptor signaling (e.g., GPR43, FXR, AhR), epigenetic reprogramming (HDAC inhibition/H3K27ac acetylation), metabolic remodeling (glycolysis-oxidative phosphorylation balance), immune modulation (Th17/Treg balance, inflammatory cytokine inhibition), and circadian coordination (receptor oscillatory expression). These findings lay a direct theoretical foundation and provide actionable targets for developing precision therapies targeting the gut-bone axis:

SCFAs pathway inhibits osteoclast differentiation and promotes osteoblast metabolic reprogramming through GPR43/HDAC signaling, directly supporting the use of SCFA-producing probiotics (such as Lactobacillus reuteri) and dietary fiber interventions, and providing a basis for developing GPR43 agonists or selective HDAC6 inhibitors (18); the circadian oscillation of its receptor further drives the clinical translation of chrono-nutritional strategies (such as timed fiber supplementation) (15). The bile acid/FXR/RUNX2-Wnt cascade validates the pharmacological effects of FXR agonists (such as geniposide) and microbiota-modulating agents on bone formation, opening up new avenues for OP drug development (11). The tryptophan metabolite-AhR axis promotes bone formation by repairing the intestinal barrier and activating the Wnt signal, supporting the application of AhR ligands, tryptophan-metabolizing probiotics, or functional nutrients to synergistically inhibit NLRP3 inflammasome activation (64). The bone-protective effects of $H_2S$ suggest that $H_2S$ donors (such as sodium hydrosulfide) or $H_2S$-producing microbial transplantation may reduce oxidative stress damage in osteoblasts (105).

The regulation of the immune-metabolic network provides scientific support for probiotics, FMT, and cytokine-targeted therapies (such as anti-TNF-α biologics) to reshape the Th17/Treg balance and inhibit inflammatory bone loss. Especially crucial is the "metabolite-immune-circadian" triad model, which integrates circadian rhythms into therapeutic design and advocates for time-resolved intervention strategies based on host-microbe metabolic rhythms. The precise correspondence between these mechanistic targets and therapeutic approaches marks a

substantive leap from basic discovery to clinical translation—offering a new paradigm for precision microbiota-based therapies for OP, OA, and other related diseases (Table 1).

## 3.5 Metabolite-bone temporal dynamics

The circadian oscillations of gut microbiota metabolites are a key bridge connecting the host circadian clock with bone metabolism. The production of SCFAs peaks during the active phase, in sync with the host's feeding patterns. Studies have shown that the concentrations of acetate, propionate, and butyrate in the cecum of mice are 30-50% higher during the dark (active) phase than during the light phase (115). This rhythmic variation directly regulates the diurnal fluctuations of bone metabolism: SCFAs inhibit osteoclast differentiation at night through GPR43 signaling, while bile acids promote osteoblast differentiation during the day via FXR signaling, forming a complementary temporal coordination (48). The specific mechanisms involve multi-level spatiotemporal control: time-restricted feeding can enhance the circadian amplitude of SCFAs and improve bone density. In mouse models, restricting the feeding window to 12 hours within the active phase can increase bone formation rates by 25% (115). The circadian rhythm of glucocorticoids regulates the temporal distribution of metabolites by modulating gut permeability and microbial composition. The morning peak of cortisol can enhance SCFA production, indirectly promoting daytime bone formation (14, 95, 116). Individual circadian pattern variations significantly impact the function of the gut-bone axis. Polymorphisms in the PER3 gene lead to differences in "morningness" and "eveningness" chronotypes, affecting the phase of metabolite rhythms. Individuals carrying the long allele of PER3 have a 2–3 hour phase advance in SCFA rhythms, which may explain their differential susceptibility to shift work-related bone loss (117, 118). Age-related attenuation of rhythmic amplitude also leads to flattened metabolite oscillations in the elderly, which is associated with an increased risk of osteoporosis (62, 119).

# 4 Biomarkers in the gut-bone axis: implications for pathogenesis and clinical management

GBA represents a dynamic, bidirectional communication network between the gastrointestinal and skeletal systems, orchestrated through immune, metabolic, and microbiome-derived signaling pathways. Inflammatory biomarkers such as TNF-α and IL-6 play pivotal roles in promoting osteoclastogenesis via RANKL/NF-κB activation in inflammatory conditions like inflammatory bowel disease (IBD) and

TABLE 1 Key mechanisms, effects, and therapeutic strategies of gut microbiota-derived metabolites in skeletal disorders.

| Metabolites | Signaling pathway | Key function/ mechanism | Effects on bone cells | Direction of effect | Related diseases | Intervention strategies | Ref. |
|---|---|---|---|---|---|---|---|
| SCFAs | GPR43/ FFAR2; HDAC inhibition | ↓ osteoclastogenesis; promote osteoblast glycolysis shift; epigenetic modulation (e.g., H3K27ac); circadian synchronization | ↓ osteoclastogenesis; promote osteoblast function | → | OP, OA | SCFA-producing probiotics (e.g., L. reuteri); dietary fiber; GPR43 agonists; HDAC6 inhibitors | (14, 17, 18, 40, 41) |
| Bile Acids | FXR; Wnt/β-catenin | ↑ osteoblast differentiation and mineralization via RUNX2/Osx; ↓osteoclastogenesis; ↑ bone formation | ↑ osteogenesis; ↓ osteoclast differentiation | → | OP, OA | FXR agonists (e.g., geniposidic acid - GPA); microbiota-modulating agents | (5, 48, 50, 51) |
| Tryptophan Derivatives | AhR; Wnt/β-catenin | Repair intestinal barrier; inhibit NLRP3 inflammasome; promote osteoblastogenesis; regulate osteoimmunity | ↑ osteogenesis; ↓ osteoclasts | → | Inflammatory bone loss, OP | AhR ligands; tryptophan-metabolizing probiotics; functional nutrients | (64, 65) |
| Gut-derived Serotonin | – | ↓ bone formation | ↓ osteoblast proliferation | ← | OP | – | (106, 107) |
| Brain-derivedSerotonin | – | ↑ bone formation and inhibit bone resorption | ↑ osteoblast activity; ↓ osteoclastogenesis | → | OP | – | (106, 107) |
| Melatonin | – | ↑ osteoblast differentiation; anabolic and anti-resorptive effects | ↑ osteogenesis; ↓ resorption | → | OP | – | (3, 108) |
| Kynurenine Pathway | – | ↓ BMSC proliferation and osteoblast differentiation; promote bone aging | ↓ bone formation | ← | OP | – | (68, 69, 109) |

*(Continued)*

TABLE 1 Continued

| Metabolites | Signaling pathway | Key function/ mechanism | Effects on bone cells | Direction of effect | Related diseases | Intervention strategies | Ref. |
|---|---|---|---|---|---|---|---|
| Tryptophan Metabolites (IAA, IPA) | AhR; Wnt/β-catenin | Repair intestinal barrier; activate AHR; stimulate Wnt/β-catenin; promote osteogenesis | Promote osteogenesis; improve bone density | → | OP | Probiotics; tryptophan supplementation | (64–67) |
| $H_2S$ | Wnt/β-catenin; NF-κB; AMPK | Antioxidant; anti-apoptotic; ↑ osteoblast differentiation; inhibits inflammation-induced osteoclastogenesis | ↑ osteogenesis; ↓ osteoclastogenesis | → | OP, OA | $H_2S$ donors (e.g., NaHS); transplantation of $H_2S$-producing bacteria (e.g., Desulfovibrio) | (73–75, 105) |
| NO | cGMP-PKG pathway | Regulation of osteoclast-osteoblast coupling; ↑ bone defect repair | Bidirectional regulation | ↔ | Bone defects, OP | NO-releasing bone implant materials | (3, 73) |
| CO | HO-1 pathway | Anti-inflammatory; ↑ bone repair | ↑ bone repair | → | BII, osteonecrosis | Targeting HO-1 pathway | (73) |
| $NH_3$ | – | ↑ osteoclast activity; aggravates osteolysis | ↑ osteoclast activity | ← | Multiple myeloma | Targeting ammoniagenic bacteria | (73) |
| Immune-Metabolic Network | Th17/Treg; TNF-α,IL-1β; RANKL/OPG | Modulate systemic inflammation; regulate osteoclastogenesis; maintain bone-immune homeostasis | Modulate bone remodeling | Variable | OP, OA, RA | Probiotics; Prebiotics; FMT; Anti-cytokine biologics (e.g., anti-TNF-α, anti-IL-1β) | (49, 82–84, 87) |
| Circadian Integration | GPR43, FXR; H3K27ac | Synchronize osteoblast-osteoclast dynamics through epigenetic-immune coordination; temporal regulation of bone remodeling | Synchronize bone remodeling | → | OP, OA | Chronotherapeutic interventions; time-based probiotic/fiber dosing | (10, 17, 24) |

↑, Promotes/increases; ↓, Inhibits/decreases; →, Positive; ←, Negative ↔, Bidirectional; AhR, Aryl Hydrocarbon Receptor; AMPK, AMP-activated Protein Kinase; BII, Bone implant infection; CO, Carbon Monoxide;FMT, Fecal Microbiota Transplantation; FXR, Farnesoid X Receptor; GPA, Geniposidic Acid; GPR, G Protein-Coupled Receptor; GPR43, G Protein-Coupled Receptor 43; HDAC, Histone Deacetylase; HO-1, Heme Oxygenase-1; $H_2S$, Hydrogen Sulfide; IAA, Indole-3-Acetic Acid; IL, Interleukin; IPA, Indole-3-Propionic Acid; NaHS, Sodium Hydrosulfide; NF-κB, Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; $NH_3$, Ammonia; NLRP3, NLR Family Pyrin Domain Containing 3; NO, Nitric Oxide; OA, Osteoarthritis; OP, Osteoporosis; OPG, Osteoprotegerin; RA, Rheumatoid Arthritis; RANKL, Receptor Activator of Nuclear Factor Kappa-B Ligand; RUNX2, Runt-Related Transcription Factor 2; SCFAs, Short-Chain Fatty Acids; TNF-α, Tumor Necrosis Factor Alpha; Treg, Regulatory T Cell; Wnt, Wingless-related Integration Site.

rheumatoid arthritis, while C-reactive protein (CRP) levels are inversely correlated with bone mineral density (87, 110). Meanwhile, bone turnover markers—including β-CTX (reflecting osteoclast activity) and PINP/osteocalcin (indicating osteoblast function)—are frequently dysregulated in metabolic bone diseases. Notably, gut microbiota influence osteocalcin's γ-carboxylation, further linking microbial ecology to bone metabolism (111, 112). Together, these biomarkers facilitate early detection of inflammatory bone loss and allow for monitoring therapeutic responses to biological agents such as anti-TNF-α antibodies.

Gut microbiota-derived metabolites are central regulators of bone homeostasis. SCFAs exert osteoprotective effects by promoting regulatory T cell (Treg) differentiation and inhibiting osteoclastogenesis, whereas lipopolysaccharide (LPS) exacerbates bone resorption through TLR4/NF-κB signaling (113). These mechanisms are exemplified in germ-free mice, which exhibit elevated bone mass accompanied by reduced osteoclast precursors (CD11b+/Gr1-), lowered TNF-α and IL-1 expression, and altered serotonin metabolism due to suppressed Tph1 activity (120, 121). Additionally, hormonal regulators—including vitamin D (25(OH) D3), parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23)—modulate bone mineralization. Deficiencies in these factors often lead to secondary hyperparathyroidism and accelerated bone loss, particularly in malabsorption syndromes.

In OA, the bone-cartilage interface offers critical biomarker insights: CTX-II levels correlate with osteophyte formation (122, 123),and IL-1β drives cross-tissue degradation via matrix metalloproteinase activation (124). Proteomic studies further indicate dysregulation of subchondral bone during early OA, though these findings await broader validation. The RANKL/OPG ratio and zonulin—a marker of intestinal permeability—provide additional mechanistic insight, with elevated RANKL observed in postmenopausal osteoporosis and zonulin predicting bone loss associated with microbial translocation (114). Collectively, these findings support the use of combined biomarker panels incorporating gut-immune signals (e.g., serotonin, TNF-α) and osteochondral markers (e.g., CTX-II, IL-1β) for a more comprehensive assessment of bone health.

Clinically, integration of GBA biomarkers enables mechanistic stratification of bone disorders (e.g., inflammatory vs. metabolic

origins) and guides personalized interventions such as microbiome modulation, nutritional support, and targeted biologics like anti-IL-1β agents in OA (125, 126). Future multi-omics approaches—including metagenomics and metabolomics—promise to unveil novel biomarkers, advancing precision medicine for osteoporosis, inflammatory osteopathies, and age-related skeletal decline. This integrative framework not only aids early risk detection in high-risk populations such as IBD patients but also enables monitoring of OA progression through osteochondral markers and supports the development of novel therapies targeting gut-microbiota-bone signaling pathways.

Recent single-cell transcriptomic studies have revealed circadian oscillations in metabolite receptors (e.g., GPR43, FXR) within bone marrow stromal cells. Disruption of these rhythms in osteoporosis patients underscores a "metabolite-immune clock" model, wherein microbial metabolites help synchronize osteoblast-osteoclast dynamics through epigenetic mechanisms such as H3K27ac acetylation (114) (Table 2) (142).

# 5 Clinical interventions of bone disorders based-GBA

## 5.1 Dietary interventions

The gut microbiota significantly influences bone health through nutrient-mediated mechanisms, particularly by enhancing monosaccharide absorption and energy harvesting (5, 23, 140,

TABLE 2 Biomarkers of bone diseases in the gut-bone axis.

| Category | Marker | Function | Key mechanism | Related diseases | Refs. |
|---|---|---|---|---|---|
| Inflammation | TNF-α | Pro-inflammatory; ↓OB; ↑OC | Activates NF-κB; ↑RANKL | RA, IBD, OP | (5, 20, 21, 87) |
| | IL-6 | ↑Osteoclastogenesis; ↓bone formation | Activates JAK/STAT | IBD, postmenopausal OP | (5, 20, 21, 87) |
| | IL-17 | ↑Bone resorption; ↓bone formation | Stimulates RANKL secretion | AS, PsA | (88) |
| | CRP | Systemic inflammation marker; ↓BMD | Negatively correlates with BMD | Chronic bone loss | (87, 110) |
| Bone Metabolism | β-CTX | Bone resorption marker | Collagen breakdown, OC activity↑ | OP, Paget's disease | (111) |
| | PINP | Bone formation marker | Collagen synthesis, OB activity ↑ | OP, osteomalacia | (111) |
| | OC | Regulates mineralization; bone formation | Secreted by osteoblasts; reflects bone formation; Gut microbiota modulates γ-carboxylation | OP, fracture risk | (111, 112) |
| | TRAP-5b | OC-specific enzyme; bone resorption | ↑Acid phosphatase activity | Multiple myeloma, OP | (111) |
| Gut Microbiota | SCFAs | Anti-inflammatory; ↓OC; ↑bone formation | Activate GPR43/41; ↑Treg differentiation | IBD, OP | (14, 17, 18, 113) |
| | LPS | Pro-inflammatory; ↑bone resorption | Activates TLR4/NF-κB; ↑RANKL | Metabolic bone disease | (25, 26, 37, 86) |
| Vitamins/ Hormones | 25(OH)D3 | ↑ $Ca^{2+}$ absorption; bone mineralization | Deficiency → secondary hyperparathyroidism | Rickets, OP | (5, 8, 14) |
| | PTH | Regulates $Ca^{2+}$/P metabolism; ↑bone resorption | ↑OC activation in hypocalcemia/VitD deficiency | Secondary OP, renal osteodystrophy | (5) |
| | FGF23 | ↓Phosphate reabsorption; modulates VitD | Gut dysbiosis may alter expression | CKD-MBD | (5) |
| Other Molecules | RANKL/OPG | RANKL: ↑OC; OPG: inhibitor | Gut inflammation ↑RANKL; disrupts balance | Postmenopausal OP, RA | (57, 58, 114) |
| | Zonulin | Gut permeability marker | Barrier breach → LPS translocation → bone loss | CD, IBD-related OP | (9, 114) |
| | IgA | Gut immune regulation | Mucosal immunity disruption effects bone metabolism | Autoimmune bone disorders | (5, 49) |

↑, Promotes/increases; ↓, Inhibits/decreases; AS, Ankylosing Spondylitis; BMD, Bone Mineral Density; CKD-MBD, Chronic Kidney Disease-Mineral and Bone Disorder; CD, Celiac disease; CRP, C-Reactive Protein; FGF23, Fibroblast Growth Factor 23; GPR, G Protein-Coupled Receptor; IBD, Inflammatory Bowel Disease; IgA, Immunoglobulin A; IL, Interleukin; LPS, Lipopolysaccharide; NF-κB, Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; OB, Osteoblast; OC, Osteocalcin; OP, Osteoporosis; OPG, Osteoprotegerin; PINP, Procollagen Type I N-Terminal Propeptide; PsA, Psoriatic Arthritis; PTH, Parathyroid Hormone; RA, Rheumatoid Arthritis; RANKL, Receptor Activator of Nuclear Factor Kappa-B Ligand; SCFAs, Short-Chain Fatty Acids; TLR4, Toll-Like Receptor 4; TNF-α, Tumor Necrosis Factor Alpha; TRAP-5b, Tartrate-Resistant Acid Phosphatase 5b; Treg, Regulatory T Cell; VitD, Vitamin D.

143). Specific strains, such as Lactobacillus plantarum, have been shown to stimulate skeletal development under malnutrition conditions, suggesting that microbiota modulation could optimize nutrient utilization for bone health enhancement. Dietary fiber bridges gut microbiota and bone health through microbial fermentation-derived SCFAs. This process not only acidifies the intestinal environment but also generates SCFAs that reinforce gut barrier integrity and suppress systemic inflammation, thereby indirectly supporting skeletal homeostasis (127, 128). Increased dietary fiber intake positively modulates microbial composition, establishing a beneficial dietary-microbiota-bone axis (144, 145).

## 5.2 Prebiotics

Prebiotics, including inulin and fructooligosaccharides (FOS/GOS), augment mineral bioavailability through gut microbiota remodeling. Fermentation of these fibers elevates SCFAs production, which acidifies intestinal pH to enhance calcium solubility and activates mineral transport pathways (129). Clinical trials demonstrate that daily intake of 8 g inulin-type fructans increases calcium absorption by 12% in adolescents and improves whole-body bone mineral density within one year, paralleled by Bifidobacterium enrichment (129, 130). Similarly, soluble corn fiber enhances calcium retention in postmenopausal women dose-dependently, correlating with increased Lachnospiraceae and Bacteroides abundance (130). Mechanistically, prebiotics reshape microbial composition (e.g., reduced Firmicutes/Bacteroidetes ratio) and modulate metabolic pathways for carbohydrate/lipid utilization, synergistically promoting calcium absorption and bone mechanical strength (130). These effects are further amplified by SCFA-mediated expansion of intestinal absorptive surface and permeability (130, 146). Collectively, prebiotic-driven microbiota modulation offers a dietary strategy to combat OP, particularly in calcium-deficient populations (129).

## 5.3 Probiotics

Probiotics demonstrate osteoprotective effects through microbiota-immune interactions. Lactobacillus reuteri enhances bone density in healthy male mice by suppressing intestinal inflammation and restructures gut microbial communities in ovariectomy (Ovx) models, effectively attenuating estrogen deficiency-induced bone loss (131, 147). Mechanistically, L. reuteri inhibits osteoclastogenesis via dual pathways: downregulating bone marrow CD4+ T cell recruitment (key osteoclast activators) and directly suppressing osteoclast differentiation *in vitro*. These actions correlate with reduced bone resorption markers and preserved trabecular architecture (131, 148). Similarly, L. paracasei and L. plantarum mitigate hormone deficiency-driven OP, highlighting genus-specific modulation of bone remodeling pathways (130, 148–150).

## 5.4 FMT

The efficacy of FMT in treating OP exhibits considerable heterogeneity, influenced by factors including donor selection (e.g., potential fecal pathogens affecting safety) (137, 138), recipient response, host gut microbiota composition/function, donor microbial diversity, immune status, and administration protocols (151, 152). Variations in donor microbiota, host immunity, and delivery methods further contribute to inconsistent outcomes (153). The composition and function of the donor microbiota are the "cornerstones" determining the success of FMT. The concept of "super donors" suggests that certain donors' microbiota have higher engraftment capabilities and stability. For example, the presence of Akkermansia muciniphila (which improves gut barrier function) (154), Faecalibacterium prausnitzii (anti-inflammatory) (155), and high SCFA-producing Lachnospiraceae and Ruminococcaceae in the donor microbiota are associated with better osteoprotective effects (23). Conversely, if potential pathogens (such as Enterobacteriaceae) in the donor microbiota are not adequately screened, it may lead to FMT failure or even exacerbate bone loss. Additionally, the recipient's intrinsic environment serves as the "soil" for microbial engraftment, and its condition is crucial. The recipient's low microbial diversity, high proportion of antibiotic-resistant bacteria, or specific microbial structure can resist the implantation of new microbiota, a phenomenon known as "colonization resistance." For instance, a high abundance of Bacteroides in the recipient's gut may hinder the engraftment of other strains. The host's immune status (such as Treg/Th17 balance), genetic background (such as polymorphisms in immune-related genes), and gut barrier integrity together form a unique microenvironment that determines the fate of the transplanted microbiota and the variability of immune modulation effects (156). Moreover, the type and preparation of the FMT formulation also affect the efficacy. Different methods, such as fresh versus frozen fecal preparations and oral capsules versus endoscopic infusion, have significant impacts on microbial viability and delivery efficiency. The freeze-thaw process may damage the viability of some strictly anaerobic bacteria, while the enteric coating technology of capsules affects the precise release of microbiota in the gut.

FMT restores gut microbiota diversity and shows osteoprotective effects in preclinical models. In ovariectomy-induced OP, FMT inhibits osteoclastogenesis and improves bone density via microbiota-metabolite interactions; in OA, it reduces inflammation through Th17/Treg modulation (52, 132). Clinical data indicate immunomodulatory shifts in IBD patients after FMT, suggesting bone benefits via the gut-bone axis (149, 157). Mechanisms include enrichment of anti-inflammatory taxa, regulation of bone remodeling via microbial metabolites (e.g., SCFAs), and modulation of osteoclast-related cytokines (149, 158).

Future directions involve elucidating microbiota-bone crosstalk, validating efficacy via RCTs, and exploring combination therapies with nutritional or pharmacological interventions. Preventive FMT applications in high-risk populations warrant further investigation (Supplementary Table 1).

## 5.5 Traditional Chinese medicine

TCM represents a holistic medical system with a history spanning thousands of years. For the purpose of this review, which focuses on the GBA and metabolic regulation, we specifically refer to the phytochemical or herbal medicine component of TCM, rather than practices such as acupuncture or Tai Chi. Numerous active compounds derived from TCM herbs have demonstrated potent osteogenic, angiogenic, and anti-inflammatory properties in preclinical studies, positioning them as promising candidates for bone tissue engineering and the management of metabolic bone diseases. Among the most extensively studied osteo-promotive TCM compounds are flavonoids (e.g., icariin and icaritin) (159), polyphenols (e.g., resveratrol) (160, 161). To enhance clarity for a global readership, we will elucidate the botanical origins and mechanisms of these specific compounds:

Icariin and Icaritin: These are prenylated flavonoids isolated from the herb *Epimedium* (commonly known as Horny Goat Weed). They are recognized as principal active constituents responsible for the bone-strengthening effects traditionally attributed to *Epimedium* in TCM theory. Mechanistically, icariin and icaritin significantly enhance the proliferation and differentiation of osteoblasts by upregulating key osteogenic genes, including RUNX2, ALP, Osteocalcin (OCN), and Collagen I (COLI) (159, 162). Furthermore, they promote intraosseous angiogenesis—a critical process for bone regeneration—via the VEGF and HIF-1α pathways (56, 133–136, 139, 141, 163–168).

Resveratrol: While also present in grapes and berries, resveratrol is a stilbenoid polyphenol abundantly found in *Polygonum cuspidatum* (Japanese Knotweed), a plant used in TCM. Similar to baicalin, resveratrol exhibits strong anti-inflammatory and antioxidant effects (160, 161). It helps protect bone cells from oxidative damage and suppresses pro-inflammatory pathways, thereby preserving bone mass and promoting a healthy remodeling balance.

Additionally, TCM components like baicalin and resveratrol exhibit potent anti-inflammatory and antioxidant effects, mitigating oxidative stress and creating a favorable microenvironment for bone regeneration (136, 167–169).

While the direct modulation of the microbiota-GBA by TCM remains underexplored, its systemic effects on bone metabolism and inflammation suggest potential interactions with gut microbiota. For example, TCM may enhance gut barrier function and reduce systemic inflammation by modulating microbial composition and metabolite production, such as SCFAs and bile acids, thereby indirectly supporting bone health (167, 170). Future research should focus on elucidating these mechanisms to optimize TCM-based strategies for bone regeneration and disease management (Figure 6) (Table 3).

TABLE 3 Systematic comparison of clinical interventions based on the gut-bone axis.

| Category | Dietary interventions | Probiotics | Prebiotics | FMT | TCM | Refs. |
|---|---|---|---|---|---|---|
| Mechanism of Action | Boost gut barrier; ↓inflammation | Modulate microbiota; ↓osteoclast differentiation | ↑SCFAs; ↑$Ca^{2+}$ absorption | Restore microbiota balance; ↓LPS/TLR4 → ↓inflammation | Multi-target (e.g., Icariin→Wnt/β-catenin); Antioxidant | (52, 78, 79, 127–136) |
| Clinical Efficacy | 12%↑BMD (teens, inulin); 12%↑$Ca^{2+}$ absorption | 5–10%↑BMD (postmenopausal); 25%↓IL-6 | 8%↑BMD (postmenopausal, inulin); 15%↑$Ca^{2+}$ retention | 15%↑BMD (OVX mice); 32%↓TNF-α (RCT) | 8–12%↑BMD; 20%↑osteocalcin | (52, 84, 129–132, 135, 136) |
| Type of Study and Level of Evidence | RCT (B) | RCT Phase II (B) | RCT + Animal (B) | Animal + RCT Phase II (B) | Meta-analysis + Cellular (C) | (52, 84, 129, 130, 132) (135, 136) |
| Advantages | Safe, low cost, good adherence | Strain-specific efficacy; combinable | Broad applicability (no live bacteria) | Potent (refractory cases); holistic | Multi-component synergy; high acceptance | (52, 78, 127, 129, 131–134) |
| Limitations | Slow effect, limited in severe cases | Strain variability; unstable long-term effects | High dose → GI discomfort | Unknown long-term safety; donor screening | Complex composition; lack of RCTs | (127, 130, 131, 137–139) |
| Target Population | Adolescents/poor $Ca^{2+}$ absorption; early OP prevention | Postmenopausal women; mild OP | Elderly/inadequate $Ca^{2+}$ intake; high-risk OP | Severe OP; arthritis w/ bone destruction | Chronic bone diseases; natural therapy preference | (52, 84, 129, 130, 132, 135, 136) |
| Ethics and Compliance | No ethics approval; low privacy risk | GRAS; strain safety assessment | GRAS; dose standardization | IRB approval; donor anonymization (GDPR) | Minimal ethical issues; GMP required | (78, 127, 129, 137, 139) |
| Future Research Directions | Personalized diets; long-term BMD tracking | Strain-metabolite combos; dose optimization | New prebiotic screening; multi-population validation | Freeze-dried capsules; 10-yr safety follow-up | Monomer mechanism (e.g., icariin); multicenter RCTs | (131, 135, 137, 140, 141) |

↑, Increase/improvement; ↓, Decrease/reduction; BMD, Bone Mineral Density; Ca, Calcium; FMT, Fecal Microbiota Transplantation; GDPR, General Data Protection Regulation; GI, Gastrointestinal; GMP, Good Manufacturing Practice; GRAS, Generally Recognized As Safe; IRB, Institutional Review Board; OP, Osteoporosis; OVX, Ovariectomized; RCT, Randomized Controlled Trial; SCFA, Short-Chain Fatty Acid; TCM, Traditional Chinese Medicine.

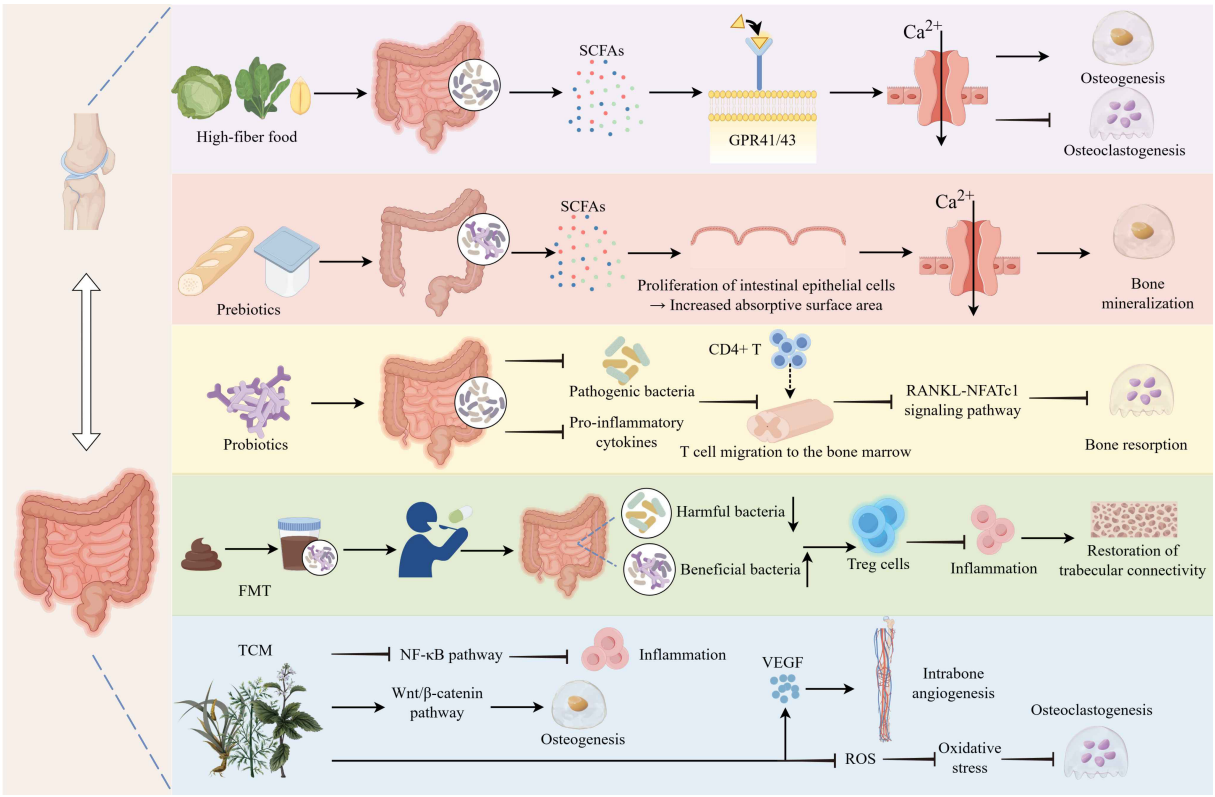



FIGURE 6
Five-pronged therapeutic strategies targeting the gut-bone axis to restore skeletal homeostasis. ① High-fiber diets yield microbiota-derived short-chain fatty acids (SCFAs) that activate G Protein-Coupled Receptor (GPR)41/43, enhancing calcium absorption and osteogenesis while suppressing osteoclastogenesis. ② Prebiotics (e.g., inulin) amplify SCFA production, promoting intestinal epithelial proliferation to increase absorptive surface area and expand beneficial bacteria.③ Probiotics (e.g., *Lactobacillus*) directly inhibit pathogenic bacteria, reduce pro-inflammatory cytokines (TNF-α/IL-6), and block RANKL-NFATc1 signaling to limit T-cell migration to bone marrow and bone resorption. ④ Fecal microbiota transplantation (FMT) restores microbial balance by elevating beneficial taxa and suppressing harmful bacteria, augmenting Treg cells to resolve inflammation-driven bone loss. ⑤ Traditional Chinese Medicine (TCM) activates the Wnt/β-catenin pathway and VEGF-mediated intra-bone angiogenesis to stimulate osteogenesis, while inhibiting NF-κB and reactive oxygen species (ROS) to mitigate oxidative stress and resorption. These interventions collectively remodel the bone microenvironment through immunometabolic crosstalk.↑: Promotes/increases; ⊥: Inhibits/decreases.

## 6 Challenges and translational prospects

Microbiota-bone axis research is still hampered by three major gaps: germ-free models fail to replicate the complexity of the human microbiota, individual metabolite receptor circadian peak phases differ by 4–6 hours, and FMT colonization is highly heterogeneous. Existing clinical evidence supports the immediate implementation of "chronotherapy" for skeletal health: taking bisphosphonates on an empty stomach in the morning increases bioavailability by 20–30%, calcium supplementation in the evening can additionally reduce nocturnal CTX by 15%, taking vitamin D with a fat-containing meal at noon is in phase with the solar rhythm, and orally administering SCFA-producing Lactobacillus reuteri in the morning increases lumbar bone density by 40% in postmenopausal women. Shift work and jet lag increase the risk of hip fractures by 32% through three pathways: reduced microbial rhythm amplitude with a phase delay of 4–6 hours, increased intestinal LPS translocation, and sustained elevation of IL-6 and TNF-α. Corresponding interventions include avoiding high-fat diets during night shifts and supplementing with resistant starch during the "biological morning" to partially restore SCFA rhythms and inhibit bone resorption. The next step is to develop CRISPR-engineered strains that continuously secrete butyrate and FXR agonists, and combine them with an AI-chronotherapy platform to analyze individual 24-hour single-cell transcriptome and plasma metabolome curves, generating a triphasic chronotherapy schedule for "drugs-nutrition-microbiota," which will be included in Phase II randomized controlled trials to further enhance bone density benefits by one effect size.

## 7 Conclusion

This review elucidates how gut microbiota-derived metabolites (e.g., SCFAs, bile acids, and tryptophan derivatives) regulate bone homeostasis through a novel "metabolite–immune–bone triad" model, incorporating circadian biology to reveal rhythmic expression of receptors (e.g., GPR43, FXR) in bone stromal cells and their synchronization of osteoblastic/osteoclastic activities via

epigenetic mechanisms such as H3K27ac acetylation. These insights deepen our understanding of the pathogenesis of OP and OA and provide a mechanistic foundation for targeting the GBA through precision interventions including chrononutrition, probiotics, and microbiota transplantation. Our work underscores the transformative potential of multi-omics-guided approaches and individualized temporal therapeutics for future research and clinical translation in skeletal disorders.

## Author contributions

ML: Writing – review & editing, Writing – original draft. JG: Writing – original draft, Writing – review & editing. YL: Writing – original draft, Writing – review & editing. JY: Writing – original draft, Writing – review & editing. ZH: Writing – original draft, Writing – review & editing. ZL: Writing – review & editing, Writing – original draft.

## Funding

The author(s) declared that financial support was received for this work and/or its publication. This work was funded by Shaoxing University Enterprise Important Horizontal Topic (No.2024USXH287).

## Conflict of interest

The authors declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. During the preparation of this work the authors used DeepSeek and Kimi in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1719445/full#supplementary-material

## References

1. Global, regional, and national burden of osteoarthritis, 1990–2020 and projections to 2050: a systematic analysis for the global burden of disease study 2021. *Lancet Rheumatol.* (2023) 5:e508–22. doi: 10.1016/S2665-9913(23)00163-7

2. Ma W, Chen H, Yuan Q, Chen X, Li H. Global, regional, and national epidemiology of osteoarthritis in working-age individuals: insights from the global burden of disease study 1990-2021. *Sci Rep.* (2025) 15:7907. doi: 10.1038/s41598-025-91783-6

3. Chen Y, Yang C, Deng Z, Xiang T, Ni Q, Xu J, et al. Gut microbially produced tryptophan metabolite melatonin ameliorates osteoporosis via modulating scfa and tmao metabolism. *J Pineal Res.* (2024) 76:e12954–79. doi: 10.1111/jpi.12954

4. Nshanian M, Gruber JJ, Geller BS, Chleilat F, Lancaster SM, White SM, et al. Short-chain fatty acid metabolites propionate and butyrate are unique epigenetic regulatory elements linking diet, metabolism and gene expression. *Nat Metab.* (2025) 7:196–211. doi: 10.1038/s42255-024-01191-9

5. Ticinesi A, Siniscalchi C, Meschi T, Nouvenne A. Gut microbiome and bone health: update on mechanisms, clinical correlations, and possible treatment strategies. *Osteoporos Int.* (2025) 36:167–91. doi: 10.1007/s00198-024-07320-0

6. Ma S, Wang N, Zhang P, Wu W, Fu L. Fecal microbiota transplantation mitigates bone loss by improving gut microbiome composition and gut barrier function in aged rats. *Peerj.* (2021) 9:e12293. doi: 10.7717/peerj.12293

7. Lu L, Tang M, Li J, Xie Y, Li Y, Xie J, et al. Gut microbiota and serum metabolic signatures of high-fat-induced bone loss in mice. *Front Cell Infect Microbiol.* (2021) 11:788576. doi: 10.3389/fcimb.2021.788576

8. Guo X, Zhong K, Zhang J, Hui L, Zou L, Xue H, et al. Gut microbiota can affect bone quality by regulating serum estrogen levels. *Am J Transl Res.* (2022) 14:6043–55.

9. Wang N, Ma S, Fu L. Gut microbiota dysbiosis as one cause of osteoporosis by impairing intestinal barrier function. *Calcif Tissue Int.* (2022) 110:225–35. doi: 10.1007/s00223-021-00911-7

10. Luo B, Zhou X, Tang Q, Yin Y, Feng G, Li S, et al. Circadian rhythms affect bone reconstruction by regulating bone energy metabolism. *J Transl Med.* (2021) 19:410. doi: 10.1186/s12967-021-03068-x

11. Fujimori K, Iguchi Y, Yamashita Y, Gohda K, Teno N. Fxr activation accelerates early phase of osteoblast differentiation through cox-2-pge(2)-ep4 axis in bmp-2-induced mouse mesenchymal stem cells. *Molecules.* (2024) 30:58–76. doi: 10.3390/molecules30010058

12. Greenbaum J, Lin X, Su K, Gong R, Shen H, Shen J, et al. Integration of the human gut microbiome and serum metabolome reveals novel biological factors involved in the regulation of bone mineral density. *Front Cell Infect Microbiol.* (2022) 12:853499. doi: 10.3389/fcimb.2022.853499

13. Yan J, Takakura A, Zandi-Nejad K, Charles JF. Mechanisms of gut microbiota-mediated bone remodeling. *Gut Microbes.* (2018) 9:84–92. doi: 10.1080/19490976.2017.1371893

14. Lucas S, Omata Y, Hofmann J, Bottcher M, Iljazovic A, Sarter K, et al. Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss. *Nat Commun.* (2018) 9:55. doi: 10.1038/s41467-017-02490-4

15. Yang KL, Mullins BJ, Lejeune A, Ivanova E, Shin J, Bajwa S, et al. Mitigation of osteoclast-mediated arthritic bone remodeling by short chain fatty acids. *Arthritis Rheumatol.* (2024) 76:647–59. doi: 10.1002/art.42765

16. Yang L, Fanok MH, Mediero-Munoz A, Fogli LK, Corciulo C, Abdollahi S, et al. Augmented th17 differentiation leads to cutaneous and synovio-entheseal

inflammation in a novel model of psoriatic arthritis. *Arthritis Rheumatol*. (2018) 70:855–67. doi: 10.1002/art.40447

17. Feng B, Lu J, Han Y, Han Y, Qiu X, Zeng Z. The role of short-chain fatty acids in the regulation of osteoporosis: new perspectives from gut microbiota to bone health: a review. *Med (Baltimore)*. (2024) 103:e39471. doi: 10.1097/MD.0000000000039471

18. Han D, Wang W, Gong J, Ma Y, Li Y. Microbiota metabolites in bone: shaping health and confronting disease. *Heliyon*. (2024) 10:e28435. doi: 10.1016/j.heliyon.2024.e28435

19. Wallimann A, Magrath W, Thompson K, Moriarty T, Richards RG, Akdis CA, et al. Gut microbial-derived short-chain fatty acids and bone: a potential role in fracture healing. *Eur Cell Mater*. (2021) 41:454–70. doi: 10.22203/eCM.v041a29

20. Ding K, Hua F, Ding W. Gut microbiome and osteoporosis. *Aging Dis*. (2020) 11:438–47. doi: 10.14336/AD.2019.0523

21. Ji J, Cai F, Yuan C, Huang C, Zhang H, Xu C, et al. Gut microbiota in primary osteoporosis: a systematic review. *Phenomics*. (2024) 4:293–7. doi: 10.1007/s43657-024-00164-y

22. Cao Y, Gao Y, Huang J. Perturbations in gut microbiota composition in osteoporosis: a systematic review and meta-analysis. *J Bone Miner Metab*. (2024) 42:551–63. doi: 10.1007/s00774-024-01517-3

23. Lin X, Xiao HM, Liu HM, Lv WQ, Greenbaum J, Gong R, et al. Gut microbiota impacts bone via bacteroides vulgatus-valeric acid-related pathways. *Nat Commun*. (2023) 14:6853. doi: 10.1038/s41467-023-42005-y

24. Yuille S, Reichardt N, Panda S, Dunbar H, Mulder IE. Human gut bacteria as potent class i histone deacetylase inhibitors *in vitro* through production of butyric acid and valeric acid. *PloS One*. (2018) 13:e201073. doi: 10.1371/journal.pone.0201073

25. Longo UG, Lalli A, Bandini B, de Sire R, Angeletti S, Lustig S, et al. Role of the gut microbiota in osteoarthritis, rheumatoid arthritis, and spondylarthritis: an update on the gut-joint axis. *Int J Mol Sci*. (2024) 25:3242–58. doi: 10.3390/ijms25063242

26. Yang P, Feng W, Li C, Kou Y, Li D, Liu S, et al. Lps induces fibroblast-like synoviocytes rsc-364 cells to pyroptosis through nf-kappab mediated dual signalling pathway. *J Mol Histol*. (2021) 52:661–9. doi: 10.1007/s10735-021-09988-8

27. Rahman SO, Bariguian F, Mobasheri A. The potential role of probiotics in the management of osteoarthritis pain: current status and future prospects. *Curr Rheumatol Rep*. (2023) 25:307–26. doi: 10.1007/s11926-023-01108-7

28. Jiang H, Li L, Bao Y, Cao X, Ma L. Microbiota in tumors: new factor influencing cancer development. *Cancer Gene Ther*. (2024), 1773–85. doi: 10.1038/s41417-024-00833-0

29. Jia W, Li Y, Cheung K, Zheng X. Bile acid signaling in the regulation of whole body metabolic and immunological homeostasis. *Sci China Life Sci*. (2024) 67:865–78. doi: 10.1007/s11427-023-2353-0

30. Chen S, Xu Y, Zhuo W, Zhang L. The emerging role of lactate in tumor microenvironment and its clinical relevance. *Cancer Lett*. (2024) 590:216837. doi: 10.1016/j.canlet.2024.216837

31. Baltazar F, Afonso J, Costa M, Granja S. Lactate beyond a waste metabolite: metabolic affairs and signaling in Malignancy. *Front Oncol*. (2020) 10:231. doi: 10.3389/fonc.2020.00231

32. Sevcikova A, Martiniakova M, Omelka R, Stevurkova V, Ciernikova S. The link between the gut microbiome and bone metastasis. *Int J Mol Sci*. (2024) 25:12086–113. doi: 10.3390/ijms252212086

33. Wu KC, Mccauley KE, Lynch SV, Nayak RR, King NJ, Patel S, et al. Alteration in the gut microbiome is associated with changes in bone metabolism after laparoscopic sleeve gastrectomy. *J Bone Miner Res*. (2024) 39:95–105. doi: 10.1093/jbmr/zjad017

34. Wang M, Zhang L, Chang W, Zhang Y. The crosstalk between the gut microbiota and tumor immunity: implications for cancer progression and treatment outcomes. *Front Immunol*. (2022) 13:1096551. doi: 10.3389/fimmu.2022.1096551

35. He Y, Huang J, Li Q, Xia W, Zhang C, Liu Z, et al. Gut microbiota and tumor immune escape: a new perspective for improving tumor immunotherapy. *Cancers (Basel)*. (2022) 14:5317–37. doi: 10.3390/cancers14215317

36. Kyriazi AA, Karaglani M, Agelaki S, Baritaki S. Intratumoral microbiome: foe or friend in reshaping the tumor microenvironment landscape? *Cells*. (2024) 13:1279–316. doi: 10.3390/cells13151279

37. Tominari T, Matsumoto C, Tanaka Y, Shimizu K, Takatoya M, Sugasaki M, et al. Roles of toll-like receptor signaling in inflammatory bone resorption. *Biol (Basel)*. (2024) 13:692–707. doi: 10.3390/biology13090692

38. Jin S, Jiang H, Sun Y, Li F, Xia J, Li Y, et al. Osteogenic differentiation of periodontal membrane stem cells in inflammatory environments. *Open Life Sci*. (2022) 17:1240–8. doi: 10.1515/biol-2022-0474

39. Goudarzi F, Kiani A, Nami Y, Shahmohammadi A, Mohammadalipour A, Karami A, et al. Potential probiotic lactobacillus delbrueckii subsp. Lactis kums-y33 suppresses adipogenesis and promotes osteogenesis in human adipose-derived mesenchymal stem cell. *Sci Rep*. (2024) 14:9689. doi: 10.1038/s41598-024-60061-2

40. Lai J, Gong L, Liu Y, Zhang X, Liu W, Han M, et al. Associations between gut microbiota and osteoporosis or osteopenia in a cohort of chinese han youth. *Sci Rep*. (2024) 14:20948. doi: 10.1038/s41598-024-71731-6

41. Kapoor S, Gupta M, Sapra L, Kaur T, Srivastava RK. Delineating the nexus between gut-intratumoral microbiome and osteo-immune system in bone metastases. *Bone Rep*. (2024) 23:101809. doi: 10.1016/j.bonr.2024.101809

42. Ramos MG, Samouda H, Bohn T. Short chain fatty acid metabolism in relation to gut microbiota and genetic variability. *Nutrients*. (2022) 14:5361–409. doi: 10.3390/nu14245361

43. Zietek M, Celewicz Z, Szczuko M. Short-chain fatty acids, maternal microbiota and metabolism in pregnancy. *Nutrients*. (2021) 13:1244–64. doi: 10.3390/nu13041244

44. Ikeda T, Nishida A, Yamano M, Kimura I. Short-chain fatty acid receptors and gut microbiota as therapeutic targets in metabolic, immune, and neurological diseases. *Pharmacol Ther*. (2022) 239:108273. doi: 10.1016/j.pharmthera.2022.108273

45. Li F, Tai L, Sun X, Lv Z, Tang W, Wang T, et al. Molecular recognition and activation mechanism of short-chain fatty acid receptors ffar2/3. *Cell Res*. (2024) 34:323–6. doi: 10.1038/s41422-023-00914-z

46. Schroeder TM, Westendorf JJ. Histone deacetylase inhibitors promote osteoblast maturation. *J Bone Miner Res*. (2005) 20:2254–63. doi: 10.1359/JBMR.050813

47. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory t cells by suppression of histone deacetylases and regulation of the mtor-s6k pathway. *Mucosal Immunol*. (2015) 8:80–93. doi: 10.1038/mi.2014.44

48. Dong Q, Fu H, Li W, Ji X, Yin Y, Zhang Y, et al. Nuclear farnesoid x receptor protects against bone loss by driving osteoblast differentiation through stabilizing runx2. *Bone Res*. (2025) 13:20. doi: 10.1038/s41413-024-00394-w

49. Tu Y, Yang R, Xu X, Zhou X. The microbiota-gut-bone axis and bone health. *J Leukoc Biol*. (2021) 110:525–37. doi: 10.1002/JLB.3MR0321-755R

50. Fan M, Xu Y, Wu B, Long J, Liu C, Liang Z, et al. Geniposidic acid targeting fxr "s332 and h447" mediated conformational change to upregulate cyps and mir-19a-3p to ameliorate drug-induced liver injury. *Adv Sci (Weinh)*. (2025), e2409107-26. doi: 10.1002/advs.202409107

51. Liu M, Jin F, Zhang S, Li S, Zhu D, Cui Y, et al. Activation of farnesoid x receptor signaling by geniposidic acid promotes osteogenesis. *Phytomedicine*. (2022) 103:154258. doi: 10.1016/j.phymed.2022.154258

52. Zhang Y, Cao M, Li Y, Lu P, Dai G, Zhang M, et al. Fecal microbiota transplantation ameliorates bone loss in mice with ovariectomy-induced osteoporosis via modulating gut microbiota and metabolic function. *J Orthop Translat*. (2022) 37:46–60. doi: 10.1016/j.jot.2022.08.003

53. Li J, Yang M, Lu C, Han J, Tang S, Zhou J, et al. Tuna bone powder alleviates glucocorticoid-induced osteoporosis via coregulation of the nf-kappab and wnt/beta-catenin signaling pathways and modulation of gut microbiota composition and metabolism. *Mol Nutr Food Res*. (2020) 64:e1900861. doi: 10.1002/mnfr.201900861

54. Wang X, Qu Z, Zhao S, Luo L, Yan L. Wnt/beta-catenin signaling pathway: proteins' roles in osteoporosis and cancer diseases and the regulatory effects of natural compounds on osteoporosis. *Mol Med*. (2024) 30:193. doi: 10.1186/s10020-024-00957-x

55. Zeng D, Umar M, Zhu Z, Pan H, Lu WW, Xiao G, et al. Development of novel osteoarthritis therapy by targeting ampk-beta-catenin-runx2 signaling. *Genes Dis*. (2025) 12:101247. doi: 10.1016/j.gendis.2024.101247

56. Arya PN, Saranya I, Selvamurugan N. Runx2 regulation in osteoblast differentiation: a possible therapeutic function of the lncrna and mirna-mediated network. *Differentiation*. (2024) 140:100803. doi: 10.1016/j.diff.2024.100803

57. Hu L, Chen W, Qian A, Li YP. Wnt/beta-catenin signaling components and mechanisms in bone formation. *Homeost Dis Bone Res*. (2024) 12:39. doi: 10.1038/s41413-024-00342-8

58. Kobayashi Y, Iwamoto R, He Z, Udagawa N. Wnt family members regulating osteogenesis and their origins. *J Bone Miner Metab*. (2025) 43:39–45. doi: 10.1007/s00774-024-01554-y

59. Song J, Chang W, Wang Y, Gao P, Zhang J, Xiao Z, et al. Inhibitors of the wnt pathway in osteoporosis: a review of mechanisms of action and potential as therapeutic targets. *Biomol BioMed*. (2025) 25:511–24. doi: 10.17305/bb.2024.11200

60. Srivastava RK, Sapra L, Mishra PK. Osteometabolism: metabolic alterations in bone pathologies. *Cells (Basel Switzerland)*. (2022) 11:3943. doi: 10.3390/cells11233943

61. Zhang P, Li X, Liang J, Zheng Y, Tong Y, Shen J, et al. Chenodeoxycholic acid modulates cholestatic niche through fxr/myc/p-selectin axis in liver endothelial cells. *Nat Commun*. (2025) 16:2093. doi: 10.1038/s41467-025-57351-2

62. Alunno A, Manetti M, Caterbi S, Ibba-Manneschi L, Bistoni O, Bartoloni E, et al. Altered immunoregulation in rheumatoid arthritis: the role of regulatory t cells and proinflammatory th17 cells and therapeutic implications. *Mediators Inflammation*. (2015) 2015:751793. doi: 10.1155/2015/751793

63. Lochmann D, Richardson T. Selective serotonin reuptake inhibitors. *Handb Exp Pharmacol*. (2019) 250:135–44. doi: 10.1007/164_2018_172

64. Chen C, Cao Z, Lei H, Zhang C, Wu M, Huang S, et al. Microbial tryptophan metabolites ameliorate ovariectomy-induced bone loss by repairing intestinal ahr-mediated gut-bone signaling pathway. *Adv Sci (Weinh)*. (2024) 11:e2404545. doi: 10.1002/advs.202404545

65. Jing W, Dong S, Xu Y, Liu J, Ren J, Liu X, et al. Gut microbiota-derived tryptophan metabolites regulated by wuji wan to attenuate colitis through ahr signaling activation. *Acta Pharm Sin B*. (2025) 15:205–23. doi: 10.1016/j.apsb.2024.11.009

66. Hou Y, Li J, Ying S. Tryptophan metabolism and gut microbiota: a novel regulatory axis integrating the microbiome, immunity, and cancer. *Metabolites*. (2023) 13:1166–84. doi: 10.3390/metabo13111166

67. Konopelski P, Mogilnicka I. Biological effects of indole-3-propionic acid, a gut microbiota-derived metabolite, and its precursor tryptophan in mammals' health and disease. *Int J Mol Sci*. (2022) 23:1222–41. doi: 10.3390/ijms23031222

68. Kim J, Che X, Kim S, Choi J, Lee JH, Lee J, et al. Sericin's potential in osteoporosis management: the roles of l-serine and d-serine in bone metabolism regulation. *Nutrients*. (2025) 17:574–91. doi: 10.3390/nu17030574

69. Anaya JM, Bollag WB, Hamrick MW, Isales CM. The role of tryptophan metabolites in musculoskeletal stem cell aging. *Int J Mol Sci*. (2020) 21:6670–84. doi: 10.3390/ijms21186670

70. Liu Z, Xu X, Shen Y, Hao Y, Cui W, Li W, et al. Altered gut microbiota and metabolites profile are associated with reduced bone metabolism in ethanol-induced osteoporosis. *Cell Prolif*. (2022) 55:e13245. doi: 10.1111/cpr.13245

71. Gomez-De-Tejada-Romero M, Murias-Henriquez C, Saavedra-Santana P, Sablon-Gonzalez N, Abreu DR, Sosa-Henriquez M. Influence of serum uric acid on bone and fracture risk in postmenopausal women. *Aging Clin Exp Res*. (2024) 36:156. doi: 10.1007/s40520-024-02819-2

72. Song S, Cai X, Hu J, Zhu Q, Shen D, Ma H, et al. Serum uric acid and bone health in middle-aged and elderly hypertensive patients: a potential u-shaped association and implications for future fracture risk. *Metabolites*. (2025) 15:15–32. doi: 10.3390/metabo15010015

73. Zaichko NV, Melnik AV, Yoltukhivskyy MM, Olhovskiy AS, Palamarchuk IV. Hydrogen sulfide: metabolism, biological and medical role. *Ukr Biochem J*. (2014) 86:5–25. doi: 10.15407/ubj86.05.005

74. Zhao Y, Cao G, Guo L, Cheng Q. Hydrogen sulfide ameliorates myocardial injury caused by sepsis through suppressing ros-mediated endoplasmic reticulum stress. *Sichuan Da Xue Xue Bao Yi Xue Ban*. (2022) 53:798–804. doi: 10.12182/20220960106

75. Powell CR, Dillon KM, Matson JB. A review of hydrogen sulfide (h(2)s) donors: chemistry and potential therapeutic applications. *Biochem Pharmacol*. (2018) 149:110–23. doi: 10.1016/j.bcp.2017.11.014

76. Zhu Z, Lian X, Bhatia M. Hydrogen sulfide: a gaseous mediator and its key role in programmed cell death, oxidative stress, inflammation and pulmonary disease. *Antioxid (Basel)*. (2022) 11:2162–83. doi: 10.3390/antiox11112162

77. Xu X, Fu H, Quan H, Li Y, Chen Q, Qu D, et al. Effects of fructooligosaccharides and lactobacillus reuteri on the composition and metabolism of gut microbiota in students. *Food Funct*. (2025) 16:1562–75. doi: 10.1039/D4FO03763D

78. Sapra L, Dar HY, Bhardwaj A, Pandey A, Kumari S, Azam Z, et al. Lactobacillus rhamnosus attenuates bone loss and maintains bone health by skewing treg-th17 cell balance in ovx mice. *Sci Rep*. (2021) 11:1807. doi: 10.1038/s41598-020-80536-2

79. Guo M, Liu H, Yu Y, Zhu X, Xie H, Wei C, et al. Lactobacillus rhamnosus gg ameliorates osteoporosis in ovariectomized rats by regulating the th17/treg balance and gut microbiota structure. *Gut Microbes*. (2023) 15:2190304. doi: 10.1080/19490976.2023.2190304

80. Chasov V, Gilyazova E, Ganeeva I, Zmievskaya E, Davletshin D, Valiullina A, et al. Gut microbiota modulation: a novel strategy for rheumatoid arthritis therapy. *Biomolecules*. (2024) 14:1653–76. doi: 10.3390/biom14121653

81. Li J, Fan R, Zhang Z, Zhao L, Han Y, Zhu Y, et al. Role of gut microbiota in rheumatoid arthritis: potential cellular mechanisms regulated by prebiotic, probiotic, and pharmacological interventions. *Microbiol Res*. (2025) 290:127973. doi: 10.1016/j.micres.2024.127973

82. Qi P, Xie R, Liu H, Zhang Z, Cheng Y, Ma J, et al. Mechanisms of gut homeostasis regulating th17/treg cell balance in pmop. *Front Immunol*. (2024) 15:1497311. doi: 10.3389/fimmu.2024.1497311

83. Britton RA, Irwin R, Quach D, Schaefer L, Zhang J, Lee T, et al. Probiotic l. Reuteri treatment prevents bone loss in a menopausal ovariectomized mouse model. *J Cell Physiol*. (2014) 229:1822–30. doi: 10.1002/jcp.24636

84. Nilsson AG, Sundh D, Backhed F, Lorentzon M. Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J Intern Med*. (2018) 284:307–17. doi: 10.1111/joim.12805

85. Yang W, Wang Y, Mo K, Chen W, Xie X. Single-cell rna sequencing reveals multiple immune cell subpopulations promote the formation of abnormal bone microenvironment in osteoporosis. *Sci Rep*. (2024) 14:29493. doi: 10.1038/s41598-024-80993-z

86. Amarasekara DS, Yu J, Rho J. Bone loss triggered by the cytokine network in inflammatory autoimmune diseases. *J Immunol Res*. (2015) 2015:832127. doi: 10.1155/2015/832127

87. Zhou P, Zheng T, Zhao B. Cytokine-mediated immunomodulation of osteoclastogenesis. *Bone*. (2022) 164:116540. doi: 10.1016/j.bone.2022.116540

88. Le Goff B, Bouvard B, Lequerre T, Lespessailles E, Marotte H, Pers Y, et al. Implication of il-17 in bone loss and structural damage in inflammatory rheumatic diseases. *Mediators Inflammation*. (2019) 2019:8659302. doi: 10.1155/2019/8659302

89. Yu W, Sun S, Fu Q. The role of short-chain fatty acid in metabolic syndrome and its complications: focusing on immunity and inflammation. *Front Immunol*. (2025) 16:1519925. doi: 10.3389/fimmu.2025.1519925

90. Chen J, Jia S, Xue X, Guo C, Dong K. Gut microbiota: a novel target for exercise-mediated regulation of nlrp3 inflammasome activation. *Front Microbiol*. (2024) 15:1476908. doi: 10.3389/fmicb.2024.1476908

91. Karimi M, Shirsalimi N, Hashempour Z, Salehi Omran H, Sedighi E, Beigi F, et al. Safety and efficacy of fecal microbiota transplantation (fmt) as a modern adjuvant therapy in various diseases and disorders: a comprehensive literature review. *Front Immunol*. (2024) 15:1439176. doi: 10.3389/fimmu.2024.1439176

92. Fehily SR, Basnayake C, Wright EK, Kamm MA. Fecal microbiota transplantation therapy in crohn's disease: systematic review. *J Gastroenterol Hepatol*. (2021) 36:2672–86. doi: 10.1111/jgh.15598

93. Guo M, Tan L, Nie X, Zhu X, Pan Y, Gao Z. The pmt2p-mediated protein o-mannosylation is required for morphogenesis, adhesive properties, cell wall integrity and full virulence of magnaporthe oryzae. *Front Microbiol*. (2016) 7:630. doi: 10.3389/fmicb.2016.00630

94. Wollmuth EM, Angert ER. Microbial circadian clocks: host-microbe interplay in diel cycles. *BMC Microbiol*. (2023) 23:124. doi: 10.1186/s12866-023-02839-4

95. Brooks JFN, Behrendt CL, Ruhn KA, Lee S, Raj P, Takahashi JS, et al. The microbiota coordinates diurnal rhythms in innate immunity with the circadian clock. *Cell*. (2021) 184:4154–67. doi: 10.1016/j.cell.2021.07.001

96. Lee D, Kim M, Han J. Gpr41 and gpr43: from development to metabolic regulation. *BioMed Pharmacother*. (2024) 175:116735. doi: 10.1016/j.biopha.2024.116735

97. Cai X, Ren F, Yao Y. Gut microbiota and their metabolites in the immune response of rheumatoid arthritis: therapeutic potential and future directions. *Int Immunopharmacol*. (2025) 147:114034. doi: 10.1016/j.intimp.2025.114034

98. Zhao Y, Chen F, Wu W, Sun M, Bilotta AJ, Yao S, et al. Gpr43 mediates microbiota metabolite scfa regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mtor and stat3. *Mucosal Immunol*. (2018) 11:752–62. doi: 10.1038/mi.2017.118

99. Behera J, Ison J, Tyagi SC, Tyagi N. The role of gut microbiota in bone homeostasis. *Bone*. (2020) 135:115317. doi: 10.1016/j.bone.2020.115317

100. Mann ER, Lam YK, Uhlig HH. Short-chain fatty acids: linking diet. *Microb Immun Nat Rev Immunol*. (2024) 24:577–95. doi: 10.1038/s41577-024-01014-8

101. Voigt RM, Forsyth CB, Keshavarzian A. Circadian rhythms: a regulator of gastrointestinal health and dysfunction. *Expert Rev Gastroenterol Hepatol*. (2019) 13:411–24. doi: 10.1080/17474124.2019.1595588

102. Zeng Y, Guo Z, Wu M, Chen F, Chen L. Circadian rhythm regulates the function of immune cells and participates in the development of tumors. *Cell Death Discov*. (2024) 10:199. doi: 10.1038/s41420-024-01960-1

103. Barrios BE, Jaime CE, Sena AA, de Paula-Silva M, Gil CD, Oliani SM, et al. Brief disruption of circadian rhythms alters intestinal barrier integrity and modulates dss-induced colitis severity in mice. *Inflammation*. (2024), 1900–11. doi: 10.1007/s10753-024-02162-8

104. Hu T, Liu C, Lei M, Zeng Q, Li L, Tang H, et al. Metabolic regulation of the immune system in health and diseases: mechanisms and interventions. *Signal Transduct Target Ther*. (2024) 9:268. doi: 10.1038/s41392-024-01954-6

105. Xu Z, Wang X, Xiao D, Hu L, Lu M, Wu Z, et al. Hydrogen sulfide protects mc3t3-e1 osteoblastic cells against h2o2-induced oxidative damage-implications for the treatment of osteoporosis. *Free Radic Biol Med*. (2011) 50:1314–23. doi: 10.1016/j.freeradbiomed.2011.02.016

106. Wadhwa R, Kumar M, Talegaonkar S, Vohora D. Serotonin reuptake inhibitors and bone health: a review of clinical studies and plausible mechanisms. *Osteoporos Sarcopenia*. (2017) 3:75–81. doi: 10.1016/j.afos.2017.05.002

107. Yadav VK, Balaji S, Suresh PS, Liu XS, Lu X, Li Z, et al. Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. *Nat Med*. (2010) 16:308–12. doi: 10.1038/nm.2098

108. Sun Q, Xu L, Hu Z, Liu J, Yu T, Li M, et al. Melatonin regulates osteoblast differentiation through the m6a reader hnrnpa2b1 under simulated microgravity. *Curr Issues Mol Biol*. (2024) 46:9624–38. doi: 10.3390/cimb46090572

109. Yang W, Jin M, Gu Y, Zhao X, Zhu L, He S, et al. Intracellular osteopontin potentiates the immunosuppressive activity of mesenchymal stromal cells. *Stem Cell Res Ther*. (2024) 15:366. doi: 10.1186/s13287-024-03979-8

110. Braun T, Zwerina J. Positive regulators of osteoclastogenesis and bone resorption in rheumatoid arthritis. *Arthritis Res Ther*. (2011) 13:235. doi: 10.1186/ar3380

111. Greenblatt MB, Tsai JN, Wein MN. Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. *Clin Chem*. (2017) 63:464–74. doi: 10.1373/clinchem.2016.259085

112. Castaneda M, Strong JM, Alabi DA, Hernandez CJ. The gut microbiome and bone strength. *Curr Osteoporos Rep*. (2020) 18:677–83. doi: 10.1007/s11914-020-00627-x

113. Hansdah K, Lui JC. Emerging insights into the endocrine regulation of bone homeostasis by gut microbiome. *J Endocr Soc*. (2024) 8:bvae117. doi: 10.1210/jendso/bvae117

114. Azizieh FY, Shehab D, Jarallah KA, Gupta R, Raghupathy R. Circulatory levels of rankl, opg, and oxidative stress markers in postmenopausal women with normal or

low bone mineral density. *biomark Insights*. (2019) 14:90826255. doi: 10.1177/1177271919843825

115. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell*. (2014) 159:514–29. doi: 10.1016/j.cell.2014.09.048

116. Thaiss CA, Levy M, Korem T, Dohnalova L, Shapiro H, Jaitin DA, et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell*. (2016) 167:1495–510. doi: 10.1016/j.cell.2016.11.003

117. Feskanich D, Hankinson SE, Schernhammer ES. Nightshift work and fracture risk: the nurses' health study. *Osteoporos Int*. (2009) 20:537–42. doi: 10.1007/s00198-008-0729-5

118. Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J, et al. A length polymorphism in the circadian clock gene per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep*. (2003) 26:413–5. doi: 10.1093/sleep/26.4.413

119. Yamazaki S, Straume M, Tei H, Sakaki Y, Menaker M, Block GD. Effects of aging on central and peripheral mammalian clocks. *Proc Natl Acad Sci U.S.A*. (2002) 99:10801–6. doi: 10.1073/pnas.152318499

120. Wang H, Tian G, Pei Z, Yu X, Wang Y, Xu F, et al. Bifidobacterium longum increases serum vitamin d metabolite levels and modulates intestinal flora to alleviate osteoporosis in mice. *Msphere*. (2025) 10:e103924. doi: 10.1128/msphere.01039-24

121. Sanchez-Lanzas R, Jimenez-Pompa A, Ganuza M. The evolving hematopoietic niche during development. *Front Mol Biosci*. (2024) 11:1488199. doi: 10.3389/fmolb.2024.1488199

122. Bihlet AR, Byrjalsen I, Bay-Jensen A, Andersen JR, Christiansen C, Riis BJ, et al. Associations between biomarkers of bone and cartilage turnover, gender, pain categories and radiographic severity in knee osteoarthritis. *Arthritis Res Ther*. (2019) 21:203. doi: 10.1186/s13075-019-1987-7

123. Ishijima M, Kaneko H, Kaneko K. The evolving role of biomarkers for osteoarthritis. *Ther Adv Musculoskelet Dis*. (2014) 6:144–53. doi: 10.1177/1759720X14541175

124. Choi Y, Lee D, Lim H, Jeong J, Sonn J, Kang S, et al. Interleukin-1beta stimulates matrix metalloproteinase-2 expression via a prostaglandin e2-dependent mechanism in human chondrocytes. *Exp Mol Med*. (2004) 36:226–32. doi: 10.1038/emm.2004.31

125. Sun C, Zhou X, Guo T, Meng J. The immune role of the intestinal microbiome in knee osteoarthritis: a review of the possible mechanisms and therapies. *Front Immunol*. (2023) 14:1168818. doi: 10.3389/fimmu.2023.1168818

126. Liu S, Xu H, Liu L, Ma W, Fan H, Liu F, et al. Gut microbiome dysbiosis accelerates osteoarthritis progression by inducing ifp-sm inflammation in "double-hit" mice. *Arthritis Res Ther*. (2025) 27:137. doi: 10.1186/s13075-025-03602-y

127. Lv X, Deng X, Lai R, Liu S, Zou Z, Dai X, et al. The association between dietary fiber intake and osteoarthritis: a cross-sectional study from the 1999–2018 u.s. National health and nutrition examination survey. *J Orthop Surg Res*. (2025) 20:209. doi: 10.1186/s13018-025-05625-2

128. Yi C, Huang S, Zhang W, Guo L, Xia T, Huang F, et al. Synergistic interactions between gut microbiota and short chain fatty acids: pioneering therapeutic frontiers in chronic disease management. *Microb Pathog*. (2025) 199:107231. doi: 10.1016/j.micpath.2024.107231

129. Ibrahim I, Syamala S, Ayariga JA, Xu J, Robertson BK, Meenakshisundaram S, et al. Modulatory effect of gut microbiota on the gut-brain, gut-bone axes, and the impact of cannabinoids. *Metabolites*. (2022) 12:1247–77. doi: 10.3390/metabo12121247

130. Jakeman SA, Henry CN, Martin BR, Mccabe GP, Mccabe LD, Jackson GS, et al. Soluble corn fiber increases bone calcium retention in postmenopausal women in a dose-dependent manner: a randomized crossover trial. *Am J Clin Nutr*. (2016) 104:837–43. doi: 10.3945/ajcn.116.132761

131. Fernández-Murga ML, Olivares M, Sanz Y. Bifidobacterium pseudocatenulatum cect 7765 reverses the adverse effects of diet-induced obesity through the gut-bone axis. *Bone*. (2020) 141:115580. doi: 10.1016/j.bone.2020.115580

132. Minkoff NZ, Aslam S, Medina M, Tanner-Smith EE, Zackular JP, Acra S, et al. Fecal microbiota transplantation for the treatment of recurrent clostridioides difficile (clostridium difficile). *Cochrane Database Syst Rev*. (2023) 4:CD13871. doi: 10.1002/14651858.CD013871.pub2

133. Wu T, Shu T, Kang L, Wu J, Xing J, Lu Z, et al. Icaritin, a novel plant-derived osteoinductive agent, enhances the osteogenic differentiation of human bone marrow- and human adipose tissue-derived mesenchymal stem cells. *Int J Mol Med*. (2017) 39:984–92. doi: 10.3892/ijmm.2017.2906

134. Luo J, Zhu J, Wang L, Kang J, Wang X, Xiong J. Co-electrospun nano-/microfibrous composite scaffolds with structural and chemical gradients for bone tissue engineering. *Mater Sci Eng C Mater Biol Appl*. (2021) 119:111622. doi: 10.1016/j.msec.2020.111622

135. Ge YW, Lu JW, Sun ZY, Liu ZQ, Zhou J, Ke QF, et al. Ursolic acid loaded-mesoporous bioglass/chitosan porous scaffolds as drug delivery system for bone regeneration. *Nanomed (Lond)*. (2019) 18:336–46. doi: 10.1016/j.nano.2018.10.010

136. Wu Y, Cao L, Xia L, Wu Q, Wang J, Wang X, et al. Evaluation of osteogenesis and angiogenesis of icariin in local controlled release and systemic delivery for calvarial defect in ovariectomized rats. *Sci Rep*. (2017) 7:5077. doi: 10.1038/s41598-017-05392-z

137. Zhang Y, Cao M, Li Y, Zhang R, Wu M, Yu Q, et al. Fecal microbiota transplantation as a promising treatment option for osteoporosis. *J Bone Miner Metab*. (2022) 40:874–89. doi: 10.1007/s00774-022-01375-x

138. Ma P, Wang R, Chen H, Zheng J, Yang W, Meng B, et al. Fecal microbiota transplantation alleviates lipopolysaccharide-induced osteoporosis by modulating gut microbiota and long non-coding rna tug1 expression. *Front Cell Infect Microbiol*. (2025) 15:1535666. doi: 10.3389/fcimb.2025.1535666

139. Arpornmaeklong P, Sareethammanuwat M, Apinyauppatham K, Boonyuen S. Characteristics and biologic effects of thermosensitive quercetin-chitosan/collagen hydrogel on human periodontal ligament stem cells. *J BioMed Mater Res B Appl Biomater*. (2021) 109:1656–70. doi: 10.1002/jbm.b.34823

140. Zhou T, Wang M, Ma H, Li X, Heianza Y, Qi L. Dietary fiber, genetic variations of gut microbiota-derived short-chain fatty acids, and bone health in uk biobank. *J Clin Endocrinol Metab*. (2021) 106:201–10. doi: 10.1210/clinem/dgaa740

141. Cyranoski D. China to roll back regulations for traditional medicine despite safety concerns. *Nature*. (2017) 551:552–3. doi: 10.1038/nature.2017.23038

142. Dopp J, Ortega A, Davie K, Poovathingal S, Baz E, Liu S. Single-cell transcriptomics reveals that glial cells integrate homeostatic and circadian processes to drive sleep-wake cycles. *Nat Neurosci*. (2024) 27:359–72. doi: 10.1038/s41593-023-01549-4

143. Zhu M, Wang Q, Yang Y, Liu X, Zhang J, Li G, et al. Multiomics approach reveals the comprehensive interactions between nutrition and children's gut microbiota, and microbial and host metabolomes. *Nutr J*. (2025) 24:50. doi: 10.1186/s12937-025-01116-6

144. Yan J, Charles JF. Gut microbiome and bone: to build, destroy, or both? *Curr Osteoporos Rep*. (2017) 15:376–84. doi: 10.1007/s11914-017-0382-z

145. Yan J, Herzog JW, Tsang K, Brennan CA, Bower MA, Garrett WS, et al. Gut microbiota induce igf-1 and promote bone formation and growth. *Proc Natl Acad Sci U.S.A*. (2016) 113:E7554–63. doi: 10.1073/pnas.1607235113

146. Sun M, Wu W, Liu Z, Cong Y. Microbiota metabolite short chain fatty acids, gpcr, and inflammatory bowel diseases. *J Gastroenterol*. (2017) 52:1–8. doi: 10.1007/s00535-016-1242-9

147. Meyer C, Brockmueller A, Ruiz DPV, Shakibaei M. Microbiota and resveratrol: how are they linked to osteoporosis? *Cells*. (2024) 13:1145–89. doi: 10.3390/cells13131145

148. Wu Y, Zhang X, Liu X, Zhao Z, Tao S, Xu Q, et al. Galactooligosaccharides and limosilactobacillus reuteri synergistically alleviate gut inflammation and barrier dysfunction by enriching bacteroides acidifaciens for pentadecanoic acid biosynthesis. *Nat Commun*. (2024) 15:9291. doi: 10.1038/s41467-024-53144-1

149. Lyu Z, Hu Y, Guo Y, Liu D. Modulation of bone remodeling by the gut microbiota: a new therapy for osteoporosis. *Bone Res*. (2023) 11:31. doi: 10.1038/s41413-023-00264-x

150. Kim I, Park S, Kim J, Park SY, Seo J, Roh S. Treatment with lactobacillus paracasei l30 extract induces osteogenic differentiation of human bone marrow mesenchymal stem cells *in vitro*. *BioMed Pharmacother*. (2025) 184:117913. doi: 10.1016/j.biopha.2025.117913

151. Zhang Z, Mocanu V, Deehan EC, Hotte N, Zhu Y, Wei S, et al. Recipient microbiome-related features predicting metabolic improvement following fecal microbiota transplantation in adults with severe obesity and metabolic syndrome: a secondary analysis of a phase 2 clinical trial. *Gut Microbes*. (2024) 16:2345134. doi: 10.1080/19490976.2024.2345134

152. Fang H, Fu L, Wang J. Protocol for fecal microbiota transplantation in inflammatory bowel disease: a systematic review and meta-analysis. *BioMed Res Int*. (2018) 2018:8941340. doi: 10.1155/2018/8941340

153. Vermeire S, Joossens M, Verbeke K, Wang J, Machiels K, Sabino J, et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J Crohns Colitis*. (2016) 10:387–94. doi: 10.1093/ecco-jcc/jjv203

154. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, et al. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut*. (2016) 65:426–36. doi: 10.1136/gutjnl-2014-308778

155. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux J, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of crohn disease patients. *Proc Natl Acad Sci U.S.A*. (2008) 105:16731–6. doi: 10.1073/pnas.0804812105

156. Stein RR, Bucci V, Toussaint NC, Buffie CG, Ratsch G, Pamer EG, et al. Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota. *PloS Comput Biol*. (2013) 9:e1003388. doi: 10.1371/journal.pcbi.1003388

157. Yadegar A, Bar-Yoseph H, Monaghan TM, Pakpour S, Severino A, Kuijper EJ, et al. Fecal microbiota transplantation: current challenges and future landscapes. *Clin Microbiol Rev*. (2024) 37:e6022. doi: 10.1128/cmr.00060-22

158. Hwang D, Chong E, Li Y, Li Y, Roh K. Deciphering the gut microbiome's metabolic code: pathways to bone health and novel therapeutic avenues. *Front Endocrinol (Lausanne)*. (2025) 16:1553655. doi: 10.3389/fendo.2025.1553655

159. Peng S, Zhang G, He Y, Wang X, Leung P, Leung K, et al. Epimedium-derived flavonoids promote osteoblastogenesis and suppress adipogenesis in bone marrow

stromal cells while exerting an anabolic effect on osteoporotic bone. *Bone*. (2009) 45:534–44. doi: 10.1016/j.bone.2009.05.022

160. Tseng P, Hou S, Chen R, Peng H, Hsieh C, Kuo M, et al. Resveratrol promotes osteogenesis of human mesenchymal stem cells by upregulating runx2 gene expression via the sirt1/foxo3a axis. *J Bone Miner Res*. (2011) 26:2552–63. doi: 10.1002/jbmr.460

161. Mohammadzadeh M, Zarei M, Abbasi H, Webster TJ, Beheshtizadeh N. Promoting osteogenesis and bone regeneration employing icariin-loaded nanoplatforms. *J Biol Eng*. (2024) 18:29. doi: 10.1186/s13036-024-00425-4

162. Su Y, Chen K, Hassanshahi M, Tang Q, Howe PR, Xian CJ. Childhood cancer chemotherapy-induced bone damage: pathobiology and protective effects of resveratrol and other nutraceuticals. *Ann N Y Acad Sci*. (2017) 1403:109–17. doi: 10.1111/nyas.13380

163. Zhu B, Xu W, Liu J, Ding J, Chen X. Osteoinductive agents-incorporated three-dimensional biphasic polymer scaffold for synergistic bone regeneration. *ACS Biomater Sci Eng*. (2019) 5:986–95. doi: 10.1021/acsbiomaterials.8b01371

164. Shi GS, Li YY, Luo YP, Jin JF, Sun YX, Zheng LZ, et al. Bioactive plga/tricalcium phosphate scaffolds incorporating phytomolecule icaritin developed for calvarial defect repair in rat model. *J Orthop Translat*. (2020) 24:112–20. doi: 10.1016/j.jot.2020.05.008

165. Feng Q, Xu J, Zhang K, Yao H, Zheng N, Zheng L, et al. Dynamic and cell-infiltratable hydrogels as injectable carrier of therapeutic cells and drugs for treating challenging bone defects. *ACS Cent Sci*. (2019) 5:440–50. doi: 10.1021/acscentsci.8b00764

166. Guo YH, Zhao S, Du YX QJ, Chen BL, Yu CQ. Effects of ginsenoside rg1-loaded alginate-chitosan microspheres on human bone marrow stromal cells. *Biosci Rep*. (2017) 37:BSR20160566. doi: 10.1042/BSR20160566

167. Wan J, Ma T, Jin Y, Qiu S. The effects of morin on bone regeneration to accelerate healing in bone defects in mice. *Int J Immunopathol Pharmacol*. (2020) 34:1682200157. doi: 10.1177/2058738420962909

168. Liu X, Chen Y, Chen X, Su J, Huang C. Enhanced efficacy of baicalin-loaded tpgs polymeric micelles against periodontitis. *Mater Sci Eng C Mater Biol Appl*. (2019) 101:387–95. doi: 10.1016/j.msec.2019.03.103

169. Wang P, Guo W, Huang G, Zhen J, Li Y, Li T, et al. Berberine-based heterogeneous linear supramolecules neutralized the acute nephrotoxicity of aristolochic acid by the self-assembly strategy. *ACS Appl Mater Interf*. (2021) 13:32729–42. doi: 10.1021/acsami.1c06968

170. Zhou Y, Zhang D, Cheng H, Wu J, Liu J, Feng W, et al. Repairing gut barrier by traditional chinese medicine: roles of gut microbiota. *Front Cell Infect Microbiol*. (2024) 14:1389925. doi: 10.3389/fcimb.2024.1389925