Gut-bone metabolites: SCFAs, polyamines and microbial metabolomics in osteoporosis risk and therapy (Review)

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Abstract. Osteoporosis (OP), a major global skeletal disorder characterized by reduced bone mass and increased fracture risk, is increasingly understood through the lens of the gut-bone axis. The present review critically synthesizes mechanistic and translational evidence for two classes of gut microbial metabolites short-chain fatty acids (SCFAs) and polyamines, as active regulators of bone remodeling. SCFAs (acetate, propionate and butyrate) suppress osteoclastogenesis via the TRAF6/NFATc1 pathway, promote osteoblast differentiation through GPR43/GPR109A signaling and histone deacetylase inhibition, and expand immuno-protective regulatory T cells in the bone marrow. Microbial polyamines (spermidine, spermine and putrescine) promote osteogenesis by upregulating Runx2 and alkaline phosphatase and inhibit osteoclastogenesis through suppression of the Ca^{2+}-PYK2-Src-NFATc1 axis. Integrative multi-omics analyses and Mendelian randomization studies provide causal evidence linking specific gut taxa, their metabolic outputs and OP risk. Distinct from prior reviews of the gut-bone axis, the present review uniquely integrates microbial metabolomics platforms, eIF5A hypusination as a novel polyamine-dependent translational mechanism, and a hierarchical framework of translational interventions from prebiotics and probiotics to postbiotics and next-generation receptor agonists. Collectively, these insights support a hierarchical translational framework from dietary prebiotics and probiotics to standardized postbiotics and next-generation G-protein-coupled receptors agonists as a complement to existing anti-OP pharmacotherapy. Key knowledge gaps include metabolite identification challenges ('metabolic dark matter') and the need for adequately powered clinical trials with bone-specific endpoints.

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1. Introduction

Osteoporosis (OP) is a common skeletal disorder involving reduction in bone strength, posing high risk of fracture. Worldwide, it is estimated to affect more than 200 million individuals, making it a major public health issue (1). The burden of OP is especially in older individuals, and women are particularly affected by the disease, with a higher rate of affected patients and a higher number of disabilities-adjusted life years (DALYs) than men. Analyses from the Global Burden of Study found that there were 17.2 million DALYs associated with low bone mineral density (BMD), the main determinant of OP, in 2020 (2). This increasing prevalence equates to a staggering number of fractures, with more than 8.9 million osteoporotic fractures occurring every year, with epidemiological estimates indicating that an osteoporotic fracture occurs approximately every three seconds globally (1). These fractures not only affect quality of life, they also have a major economic impact; for example, a recent multinational real world study reported significant healthcare costs occurring with fracture management (3). Furthermore, OP is often accompanied by other chronic diseases, such as chronic kidney disease, rheumatoid arthritis and Parkinson's disease, complicating its management even further (4-6). Despite the improvements in the technology used for assessments and in the pharmacological treatments available, there are still major

gaps in clinical care. Notably, the recommendations for OP management are somewhat different in different parts of the world, especially in the Asia-Pacific where systematic reviews have shown a wide variation in treatment and fracture risk management (7). Even if effective therapies are available, problems still exist, including poor patient adherence and rare but serious adverse effects, as well as pronounced inter-individual variability in treatment response. These limitations have fueled increasing interest in a 'treat-to-target' approach, which seeks not only to prevent fracture but to restore pre-fracture function and minimize future fracture risk. Achieving such approach requires more personalized and metabolically informed interventions. In recent years, the gut microbiota has become a key regulator of the bone and is considered to constitute a 'metabolic organ' and communicate bi-directional with the skeleton. The gut-bone axis acts through various mechanisms such as immune modulation (including modulation of T-cell differentiation and systemic inflammation), endocrine signaling (nutrient absorption and hormone production), neural pathways, and metabolic crosstalk, in which metabolites produced by microbes directly influence bone cells (8). Long-term dietary behaviors and nutritional status influence the gut microbiome and in turn, bone health and disease indirectly. Animal models, including germ-free mice and models exposed to antibiotic treatment, present strong evidence that changes in, or loss of, the gut microbiota greatly affect BMD and micro-architecture, demonstrating causality (9). Within this regulatory network, metabolites produced by the microbes play key roles. Short-chain fatty acids (SCFAs), specifically acetate, propionate and butyrate, are formed through by bacterial fermentation of dietary fibers and have been shown to regulate bone metabolism. Preclinical studies suggested that SCFAs are able to inhibit osteoclastogenesis, enhance osteoblast activity, and modulate immune responses. Emerging clinical data also suggests beneficial effects on bone turnover markers in post-menopausal women (10). In addition, recent studies have pointed out the importance of polyamines (for example, spermidine and spermine) produced by gut microbes, which can directly improve osteoblast differentiation and suppress osteoclast activities. To elucidate the exact role of all these metabolites, the use of advanced metabolomics is indispensable. Comprehensive profiling of gut-derived compounds, instead of the identification of microbial taxa alone, uncovers a deeper mechanistic insight into how specific metabolites impact bone biology. This metabolite-centric approach holds promise for discovering novel therapeutic opportunities as well as targeted interventions within the gut-bone axis for improving skeletal health and complementing the existing treatments for OP. While existing reviews of the gut-bone axis have addressed microbiota composition, estrogen metabolism and broad immune mechanisms, the present review uniquely focuses on the metabolite-centric perspective, providing an integrated mechanistic and translational synthesis. Specifically, (i) the receptor-mediated (GPR43 and GPR109A) and epigenetic (HDAC inhibition) mechanisms by which SCFAs regulate osteoblast and osteoclast function were delineated; (ii) polyamine biosynthesis, catabolism and direct bone-cell effects including the novel eIF5A hypusination pathway were characterized; (iii) microbial metabolomics platforms and their annotation challenges in the bone context were evaluated; and (iv) a hierarchical translational framework was proposed spanning dietary prebiotics, probiotics, postbiotics and next-generation pharmacological strategies. This metabolite-centric approach offers actionable insights for developing precision interventions targeting the gut-bone axis.

2. Gut-bone axis from compositional dysbiosis to functional signaling

Dysbiosis in OP: Compositional and functional shifts. The pathogenesis of OP is increasingly recognized to involve alterations in gut microbiota composition and function, this phenomenon called as 'dysbiosis'. Two-sample Mendelian randomization (MR) studies have provided genetic evidence that specific gut taxa are causally linked to BMD, rather than merely being correlated. For example, one MR study identified multiple microbial taxa whose genetically predicted abundance is associated with lumbar spine, forearm and femoral neck BMD (11-13). Moreover, reverse causality was excluded in these analyses, strengthening the case for a direct gut bone effect (12).

In osteoporotic individuals, dysbiosis often manifests as reduced diversity (alpha- and beta-diversity) and loss of beneficial SCFA-producing bacteria such as those belonging to *Clostridium* clusters IV and XIVa, as well as *Faecalibacterium prausnitzii* (*F. prausnitzii*) (14,15). Observational and mechanistic studies suggest that such losses impair butyrate production, shifting the microbial milieu toward pro-inflammatory species (for example, Proteobacteria), which may increase circulating lipopolysaccharide (LPS) and systemic endotoxemia thereby creating a pro-resorptive environment (10,15). The contrasting physiological landscapes of the healthy (eubiotic) vs. osteoporotic (dysbiotic) gut-bone axis, highlighting these immunological and metabolic shifts, are illustrated in Fig. 1.

Microbial metabolite pathways: From substrates to systemic mediators. One of the central routes by which dysbiosis contributes to bone loss is via microbial metabolites. SCFAs particularly acetate, propionate and butyrate are generated by bacterial fermentation of dietary fibers [such as inulin, pectin and resistant starch (RS)] via pathways including the acetyl-CoA route. The efficiency of SCFA production depends on the abundance and metabolic capacity of SCFA-producing taxa; when these taxa decline, SCFA output and their systemic bioavailability fall.

Beyond carbohydrate fermentation, gut bacteria also metabolize amino acids into bioactive compounds. For instance, arginine and glutamate metabolism can lead to the synthesis of polyamines (for example, spermidine and putrescine), which have been shown in animal models to enhance osteoblast differentiation and suppress osteoclast activity, thereby supporting bone formation (16).

Other important pathways include the microbial conversion of primary bile acids into secondary bile acids, which signal via receptors such as the farnesoid X receptor (FXR) and TGR5. Activation of these receptors can modulate immune responses and bone remodeling (17,18). Similarly, tryptophan metabolism by the microbiota gives rise to indole derivatives that act as ligands for the aryl hydrocarbon receptor (AhR).

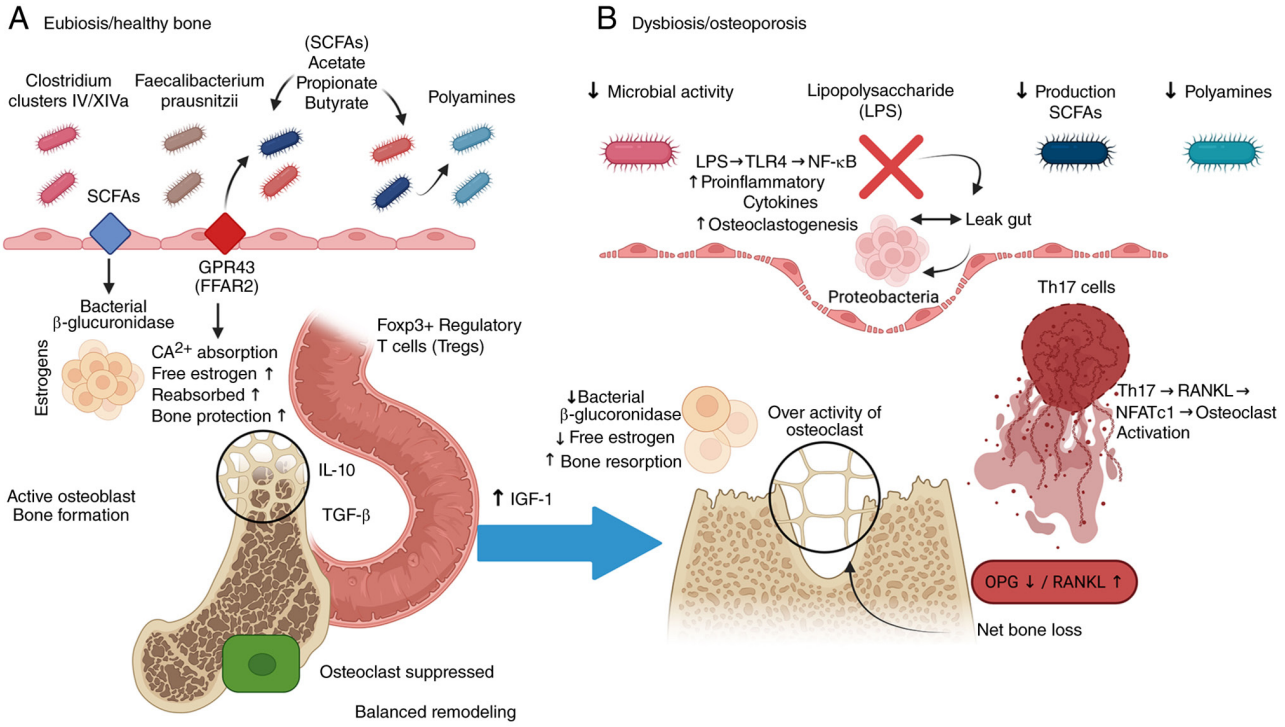

Figure 1. Gut-bone axis crosstalk in eubiosis and dysbiosis. (A) In eubiosis, fermentation of dietary fiber by *Clostridium* clusters IV/XIVa and *Faecalibacterium prausnitzii* produces SCFAs (acetate, propionate and butyrate) and polyamines. SCFAs activate GPR43 (FFAR2) on intestinal epithelial cells, enhancing Ca^{2+} absorption and barrier integrity. Bacterial β-glucuronidase deconjugates estrogens, promoting enterohepatic recirculation and bone protection. SCFA-driven $Foxp3^+$ Treg expansion sustains IL-10/TGF-β secretion, suppressing osteoclastogenesis and supporting balanced bone remodeling. (B) Dysbiosis depletes SCFA-producing taxa and expands pro-inflammatory Proteobacteria, compromising epithelial barrier integrity. LPS translocation activates TLR4 → NF-κB signaling, elevating pro-inflammatory cytokines and driving osteoclastogenesis. Reduced β-glucuronidase activity impairs estrogen recirculation. Th17 expansion upregulates RANKL → NFATc1-mediated osteoclast activation. The resulting OPG↓/RANKL↑ imbalance shifts bone remodeling toward net bone loss. SCFAs, short-chain fatty acids; LPS, lipopolysaccharide; Tregs, regulatory T cells; OPG, osteoprotegerin.

Through AhR signaling, these molecules regulate differentiation of Th17 cells and influence osteoclastogenesis and bone turnover (19).

Metabolite bioavailability depends on efficient absorption in the colon. SCFAs are taken up by colonocytes via monocarboxylate transporters (for example, MCT1 and MCT4) and paracellular routes, and their absorption is sensitive to changes in colonic pH. Dysbiosis-induced alterations in fermentation and pH may impair SCFA uptake, exacerbating the deficit.

RANKL/OPG axis and Th17 dysregulation: Osteoimmunology link. A key immunological mechanism by which dysbiosis affects bone is through an imbalance in the RANKL/OPG axis, mediated by T-cell subsets. In post-menopausal OP, estrogen deficiency is associated with gut dysbiosis, which in turn promotes expansion of Th17 cells. These Th17 cells produce pro-osteoclastogenic cytokines including RANKL and TNF-α driving bone resorption. Conversely, SCFA-producing bacteria (especially butyrate producers) foster the differentiation and expansion of $Foxp3^+$ regulatory T cells (Tregs). Butyrate acts via inhibition of histone deacetylases (HDACs), promoting Foxp3 acetylation and stable Treg lineage commitment. These Tregs secrete anti-inflammatory cytokines such as IL-10 and TGF-β, which suppress Th17 differentiation and blunt RANKL expression, thus countering bone loss (20,21).

IGF-1 and calcium bioavailability: Nutrient-level consequences of dysbiosis. Beyond immune regulation, dysbiosis impairs bone health through effects on nutrient absorption and anabolic bone-hormone signaling. Insulin-like growth factor-1 (IGF-1) is a central anabolic hormone that promotes osteoblast differentiation and bone formation, while suppressing osteoclastogenesis. SCFA-mediated signaling, particularly via GPR43 and GPR109A on hepatocytes and intestinal epithelial cells, has been shown to upregulate serum IGF-1; therefore, a dysbiosis-associated reduction in SCFA production can lead to diminished circulating IGF-1 and impaired bone anabolism. Recent reviews highlight the critical role of microbiota-derived SCFAs in modulating IGF-1 levels and consequently supporting bone formation (20,22).

Furthermore, fermentation of dietary fiber by SCFA-producing bacteria generates organic acids that acidify the colonic lumen, decreasing pH and thereby increasing the solubility and bioavailability of dietary calcium. This acidification, driven by acetate, propionate and butyrate, enhances passive paracellular calcium absorption. Dysbiosis-associated depletion of these SCFA-producing bacteria causes colonic pH to rise, promoting calcium precipitation and reducing its bioavailability, which impairs the mineral substrate necessary for bone formation (21).

Estrobolome: Estrogen metabolism and dysbiosis. The estrobolome, the aggregate genetic and metabolic capacity of the gut microbiota to metabolize estrogens, represents a key mechanism linking dysbiosis to post-menopausal bone loss. Estrogens, after liver conjugation (to glucuronides/sulfates),

are excreted into the bile and delivered to the gut. There, bacterial β-glucuronidase enzymes deconjugate these metabolites, regenerating free, active estrogens that can be reabsorbed across the intestinal epithelium via enterohepatic circulation (20,23).

Dysbiosis, characterized by a reduction in the abundance or activity of β-glucuronidase-expressing bacteria, impairs estrogen deconjugation and consequently decreases systemic estrogen levels. This phenomenon exacerbates the estrogen deficiency inherent to the post-menopausal state, thereby accelerating bone loss. Notably, estrogen deficiency itself can further aggravate dysbiosis by compromising intestinal barrier integrity and altering immune homeostasis, and establishing a bidirectional feedback loop that perpetuates both dysbiosis and bone loss. Recent studies in rodent models have confirmed that estrogen deficiency induces gut barrier disruption and dysbiosis, which in turn promote low-grade inflammation and bone loss (24).

Barrier integrity, serotonin and leptin: Systemic consequences of dysbiosis. Dysbiosis also impacts bone health by compromising intestinal barrier function and dysregulating key microbiota-derived signaling molecules. The enrichment of pro-inflammatory taxa, particularly Proteobacteria, and promotes intestinal inflammation which increased permeability (often termed 'leaky gut'), facilitating the translocation of LPS and other pathogen-associated molecular patterns into systemic circulation. This low-grade endotoxemia drives chronic systemic inflammation, which favors osteoclastogenesis and inhibits osteoblast activity. Recent investigations in post-menopausal bone-loss models have linked elevated circulating LPS to increased bone resorption in estrogen-deficient states (25). The gut microbiota further regulates peripheral serotonin (5-HT) production, with spore-forming bacteria (for example, Clostridium genera) influencing enterochromaffin cells to synthesize serotonin. Dysbiosis-associated loss of these bacteria may reduce gut-derived serotonin, with downstream effects on gut barrier function and systemic immune signaling, which ultimately impacting bone metabolism. Reviews of aging-related bone disease underscore the role of the microbiota in modulating 5-HT levels and, consequently, bone health via immune and endocrine pathways (26). Finally, commensal microbes, particularly *Lactobacillus* and *Bifidobacterium* species, modulate systemic leptin levels. Leptin which is an adipokine with bone-protective properties, supports osteoblast differentiation both centrally (via hypothalamic pathways) and peripherally inhibiting osteoclastogenesis. Dysbiosis-associated depletion of leptin-regulating bacteria may reduce circulating leptin, thereby impairing its anabolic effects on bone. This mechanism is increasingly recognized in gut-bone axis research, especially in the context of aging (20,26).

3. SCFAs and bone metabolism - molecular mechanisms and clinical evidence

SCFA biosynthesis and microbiota production. SCFAs are important metabolites produced mainly by the microbial breakdown and fermentation of complex carbohydrates, such as dietary fiber, in the gut microbiota (27). This biochemical phenomenon represents a cornerstone of the interaction between microbes and their hosts and has extensive influences on host physiology. The fermentability of dietary fibers is extremely heterogeneous, for example cellulose shows a low fermentability when used *in vitro* and *in vivo*, while pectin fermentability is high, thus resulting in high production of SCFA (28). Dietary fiber intake can have a significant impact on endogenous sterol synthesis and enhancing colonic SCFA production, which is of special interest in the pathophysiology of inflammatory bowel diseases (IBD) (28,29). The biosynthesis pathways of SCFAs involve various metabolic pathways within the gut microbiota. Although the supplied contexts do not specifically delineate mechanisms of bacterial synthesis under distinct CoA compounds in the formation of SCFAs, they highlight a critical role of such metabolic precursors as acetyl-CoA and malonyl-CoA. These cofactors are key intermediates for both polyketide and fatty acid biosynthesis and are partitioned in several subcellular locations in eukaryotes (30,31). A recent study has also investigated non-carboxylative routes for the efficient synthesis of malonyl-CoA, highlighting its central metabolic role (32). Several bacterial taxa are recognized for their substantial contributions to SCFA production. Whereas, the butyrate, a particularly significant SCFA, is synthesized by species such as *Roseburia hominis* and *F. prausnitzii*, both being members of the Firmicutes phylum. A reduction in these butyrate-producing species is associated with dysbiosis in patients with ulcerative colitis (UC) (33). Additionally, the anaerobic butyrate-producing bacterium *Coprococcus eutactus* has been correlated with development in rural Ugandan children, suggesting a role for butyrate in gut-brain axis communication (34). Depletion of SCFA-producing taxa, including Clostridia and Lachnospiraceae, has also been linked to autism spectrum disorder-like phenotypes in rat models, and underscoring their importance in neurodevelopmental health (35). Cross-feeding interactions among gut microbiota members are vital for optimizing SCFA production. For instance, xylitol, a polyol, can promote the proliferation of beneficial bacteria and enhance propionate synthesis in the colon through microbial cross-feeding (36). Similarly, 2'-fucosyllactose (2'-FL), a human milk oligosaccharide, in combination with cross-feeding bifidobacteria, synergistically increases the production of SCFAs, particularly acetate and propionate, while reshaping the gut microbiota in infants with atopic dermatitis (37). Microbiota-derived indoles have been shown to mediate inter-microbial communication and influence the gut microbiome and host physiology (27,38,39). Dietary interventions can modulate SCFA profiles; for example, increasing dietary concentrate levels in Holstein heifers has been shown to result in a linear decrease in the proportion of acetate in feces (40). Dysbiosis, or an imbalance in the microbial community of the gut is often accompanied by a significant decrease in the concentrations of beneficial metabolites such as SCFAs. This reduction is connected to increased chronic inflammatory processes, that are known to be the main cause of numerous chronic diseases, such as OP (41). The gut microbiota therefore plays an integral role in bone health, with reduction of SCFAs in dysbiosis being closely linked to OP progression (41,42). Notably, melatonin, a tryptophan metabolite produced by the gut microbiota, has been shown to improve OP through the modulation of SCFAs

and trimethylamine-N-oxide metabolism, further reflecting the close link between gut microbiota, SCFAs and bone health (41,42).

Free fatty acid receptor (FFAR) 2 (GPR43) and FFAR3 (GPR41) signaling. Differential coupling to G-protein and immune regulation SCFAs have varied physiological effects, such as immune modulation via activation of certain G-protein-coupled receptors (GPCRs), notably FFAR2 (also referred to as GPR43) and FFAR3 (also referred to as GPR41) (43). These receptors mediate a variety of cellular processes via distinct signaling pathways. The FFAR3 (GPR41) is characterized by its coupling to Gi/o proteins. Upon activation by SCFAs such as propionate, FFAR3 signaling leads to a reduction in cyclic 3',5'-adenosine monophosphate (cAMP) levels and activation of mitogen-activated protein kinases (MAPKs), including p38 MAPK (43,44). This Gi/o protein coupling is critical for mediating several actions of propionate, including effects on inflammation and fibrosis (44). The FFAR3 is highly expressed in peripheral sympathetic ganglia, where it enhances sympathetic nervous system outflow (43). The regulator of G protein signaling (RGS)-4, a member of the RGS superfamily, is essential for inactivating Gi/o-protein signaling. RGS4 dampens propionate FFAR3-dependent signaling in cardiomyocytes, and its depletion significantly amplifies the effects of propionic acid on cAMP reduction, Gi/o activation and p38 MAPK activation (43,44). Furthermore, RGS4 depletion augments pro-inflammatory interleukin (IL)-1β and IL-6 production, as well as pro-fibrotic transforming growth factor (TGF)-β synthesis, indicating that RGS4-mediated attenuation of FFAR3 signaling can mitigate inflammatory and fibrotic responses (44). Unlike FFAR3's exclusive Gαi/o coupling, FFAR2 (GPR43) exhibits dual G-protein coupling: It signals through both Gαi/o reducing intracellular cAMP and Gαq proteins, which activate phospholipase C to generate inositol triphosphate and diacylglycerol, triggering downstream ERK1/2 activation (43). This dual coupling mechanism confers a broader signaling repertoire on FFAR2 relative to FFAR3. In terms of tissue distribution, FFAR2 is highly expressed in hematopoietic cells including monocytes, neutrophils and dendritic cells as well as in intestinal L cells and Foxp3$^+$ Tregs. This expression pattern is of direct relevance to bone homeostasis; osteoclast precursors belong to the monocyte/macrophage lineage, and FFAR2 activation by acetate (its preferred short-chain ligand) has been shown to suppress RANKL-induced osteoclastogenesis and reduce bone resorption markers. Regarding ligand selectivity, acetate preferentially activates FFAR2, propionate activates both FFAR2 and FFAR3, and butyrate preferentially signals via GPR109A over FFAR3 (43). Although the precise extent of FFAR2 expression in mature osteoblasts and cortical bone tissue has not yet been fully characterized, current evidence positions FFAR2 as the more bone-relevant receptor of this pair given its direct expression on osteoclast precursor lineages.

The anti-inflammatory effects of SCFAs are also partially mediated through the endocannabinoid system, suggesting additional, receptor-independent pathways of immune modulation operating via the gut microbiome (45). Overall, the principal signaling cascade for FFAR3 involves coupling to Gαi/o proteins, resulting in decreased intracellular cAMP levels and activation of MAPKs such as p38 MAPK, negatively regulated by RGS4, which attenuates FFAR3 signaling and thereby modulates inflammatory and fibrotic responses (43,44).

GPR109A [hydroxycarboxylic acid receptor 2 (HCAR2)]: Butyrate specificity and epigenetic HDAC inhibition. HCAR2, which is also known as 'GPR109A', functions as a metabolite-sensing GPCR involved in mediating the physiological effects of butyrate (46-48). Its expression has been reported in a variety of cell types and tissues. Specifically, GPR109A expression is detected in medullary thymic epithelial cells (mTECs) in both murine and human systems, based on single-cell RNA sequencing and flow cytometric analyses (49). Elevated GPR109A expression has also been found on renal podocytes (46). In addition, GPR109A is detected on myeloid cells in the context of hepatocellular carcinoma, suggesting the presence of GPR109A on certain immune cell populations (50). Although not universally established for all intestinal epithelial cells, butyric acid is a natural ligand for GPR109A and also plays a part in the energetic requirements of epithelial cells and is therefore suggestive of a functional interaction in the gastrointestinal tract (47). Butyrate's effect on the glucose metabolism of cancerous colonocytes (intestinal epithelial cells) is additionally associated with the GPR109A-AKT signaling pathway (48). GPR109A expression and signaling are also involved in maintaining an immunoinhibitory microenvironment in the retina, modulating the interaction between resident retinal cells and immune cells from other sites (51). Butyrate exerts its actions through GPR109A, and these interactions are critical for different physiological processes. For example, SCFAs, including butyrate, can induce peripheral Treg differentiation through the activation of GPCRs such as GPR109A (49). Notably, recent investigations have helped to elucidate a novel role of GPR109A in thymic Treg development, in which Gpr109a-deficient mice have an increased number of Tregs in multiple organs under basal conditions. This observation suggests that GPR109A may have a negative regulatory influence on thymic Treg development, which is mediated by GPR109A expression on mTECs rather than by GPR109A expression on T cells (49).

HDAC inhibition increases Histone H3/H4 acetylation. Beyond receptor-mediated signaling at GPR109A, butyrate is known as a potent HDAC inhibitor (46-48). By blocking the functions of HDACs, butyrate induces histone acetylation, which in turn causes changes in chromatin structure and gene expression. This intranuclear epigenetic mechanism operates independently of surface receptor engagement and represents a second, distinct pathway through which butyrate exerts its bone-relevant effects. SCFAs, especially butyrate, are strong inhibitors of HDACs. HDAC inhibition triggers a strong increase in histone H3 and H4 tail acetylation, which results in a more permissive chromatin structure and increased transcriptional accessibility of specific genomic loci. Indicatively, sodium butyrate, a well-characterized SCFA, has been demonstrated to increase histone acetylation in the hippocampus, a modification that is associated with improved learning and memory in rodent models (52). In the osteogenic and immune regulation contexts, butyrate-induced HDAC inhibition or pharmacological inhibition by romidepsin increases histone

acetylation of promoters of genes required to differentiate osteoblasts and regulate T-cells, leading to their transcriptional activation (52,53).

Foxp3 expression in Tregs; Treg frequency increased in bone marrow. Butyrate and other SCFAs stimulate differentiation and expansion of $Foxp3^+$ Tregs in both receptor-mediated and epigenetic ways. Specifically, HDAC inhibition by butyrate results in the enhanced acetylation of Foxp3 promoter and enhancer regions, leading to enhanced Foxp3 transcription and Treg lineage stability (54). In the bone marrow, this mechanism leads to an upregulation of Tregs, which are key to maintaining immune homeostasis and the hematopoietic and bone-forming niches (55). Furthermore, studies have shown that bone marrow grafts containing higher numbers of Tregs are correlated with a lower incidence of graft vs. host disease and improved bone marrow immune regulation (55,56). The functional consequences of this Treg expansion, specifically its coupling to osteoblast activation and suppression of osteoclastogenesis, are integrated below.

Osteoblast gene upregulation [Runx2, osterix (Sp7), alkaline phosphatase (ALP) and osteocalcin (Bglap)]. HDAC inhibition and SCFA signaling are responsible for upregulating the expression of key osteogenic transcription factors and markers, such as Runx2, Osterix, ALP and osteocalcin. These genes are necessary for the development, maturation and mineralization of cells in bone formation (osteoblasts). For example, some materials such as Chitosan nanofiber scaffolds and natural substances such as *Petasites japonicus* extract have been demonstrated to enhance bone repair and osteoblast development through enhancing the expression of key markers including Runx2, Osterix, ALP and osteocalcin (57-59). Additionally, epigenetic mechanisms, such as enhanced acetylation of histone proteins at the promoters of these genes further stimulate their activation and transcription (60). How this osteoblast gene activation is coordinated with Treg-mediated suppression of osteoclastogenesis within a unified bone-remodeling framework is discussed below.

Coupling of bone formation and resorption: Immuno-epigenetic integration. Bone remodeling is carefully regulated by the balance between the actions of osteoblasts and osteoclasts. Previous research has suggested that SCFAs from the gut are important regulators of this balance, regulating it via immune and epigenetic mechanisms (55-58). As aforementioned, SCFAs in particular butyrate promote the expansion and stabilization of $Foxp3^+$ Tregs in the bone marrow through both GPCR-mediated and HDAC inhibitory mechanisms (54-56). These Tregs then secrete anti-inflammatory mediators such as IL-10 and TGF-β, which slow the production of osteoclasts and attenuate signals that accelerate bone destruction, ultimately resulting in an immune environment that is protective of bone and reduces resorption (55-58).

Osteo-blastogenic gene activation: Epigenetic and transcriptional control. As aforementioned, SCFA-mediated HDAC inhibition upregulates key osteogenic transcription factors including Runx2 and Osterix, and matrix proteins such as ALP and osteocalcin (57-60). Mechanistically, butyrate's potent inhibition of class I and IIa HDACs results in histone H3K9 and H4K12 hyperacetylation (52,60), which increases the accessibility of gene promoters to osteogenic transcription factors, supporting osteoblast development and matrix formation. These integrated immune and epigenetic processes ensure that bone formation and resorption are tightly coupled. Tregs inhibit the activity of osteoclasts, and SCFAs stimulate osteoblast activity, which in concert ensures strong bone integrity and normal turnover. Disruption of this balance through dysbiosis or reduced SCFA availability can result in unregulated bone remodeling, and any such disruption can lead to OP and fragile bones.

Direct suppression of osteoclastogenesis by metabolic reprogramming TRAF6/NFATc1 pathway. In addition to indirect immune modulating roles, SCFAs directly impair development and function of osteoclasts. Specifically, SCFAs, especially butyrate, directly suppress osteoclastogenesis induced by receptor activator of nuclear factor kB ligand (RANKL). This is achieved by dampening the signaling pathway of TRAF6/NF-kB/MAPK, which is required for the activation of NFATc1, a key transcription factor in the formation of osteoclasts. It has been found that when the expression of NFATc1 is decreased, the expression of certain osteoclast markers including tartrate resistant acid phosphatase (TRAP), dendritic cell specific transmembrane (DC-Stamp) and c-Fos are also decreased (52,60).

Metabolic reprogramming of osteoclast precursors beyond the transcriptional repression. SCFAs promote a metabolic transition in osteoclast precursors from aerobic glycolysis to oxidative phosphorylation. This change in metabolism reduces the available energy for osteoclast development and bone-resorbing function, decreasing further the availability of building new osteoclasts to inhibit bone density reduction.

Synthesis and therapeutic implications. Collectively, these results show that SCFAs maintain bone density through multiple interconnected mechanisms: (i) SCFAs increase Treg cells to suppress osteoclasts; (ii) SCFAs epigenetically promote osteoblast development; and (iii) SCFAs directly inhibit signals and metabolic processes that lead to osteoclast formation. These insights underscore the therapeutic potential of SCFA-based interventions or microbiota-based interventions for OP and other bone loss-related conditions (55-60). The dual immuno-metabolic and epigenetic mechanisms by which SCFAs modulate these bone cell populations specifically through GPR43/GPR109A signaling and HDAC inhibition are detailed in Fig. 2.

4. Polyamines in bone homeostasis: From biosynthesis to osteogenesis

Polyamines are vital organic cations involved in numerous cellular functions, such as cell growth, differentiation and metabolism. Their diverse functions also contribute to maintaining physiological balance, and recent studies have highlighted their significance in bone homeostasis. Notably, endogenous polyamines such as putrescine, spermidine and spermine have been demonstrated to inhibit osteoclast migration and fusion, acting through signaling pathways such as Ca^{2+}-PYK2-Src-NFATc1 to suppress osteoclast differentiation (61).

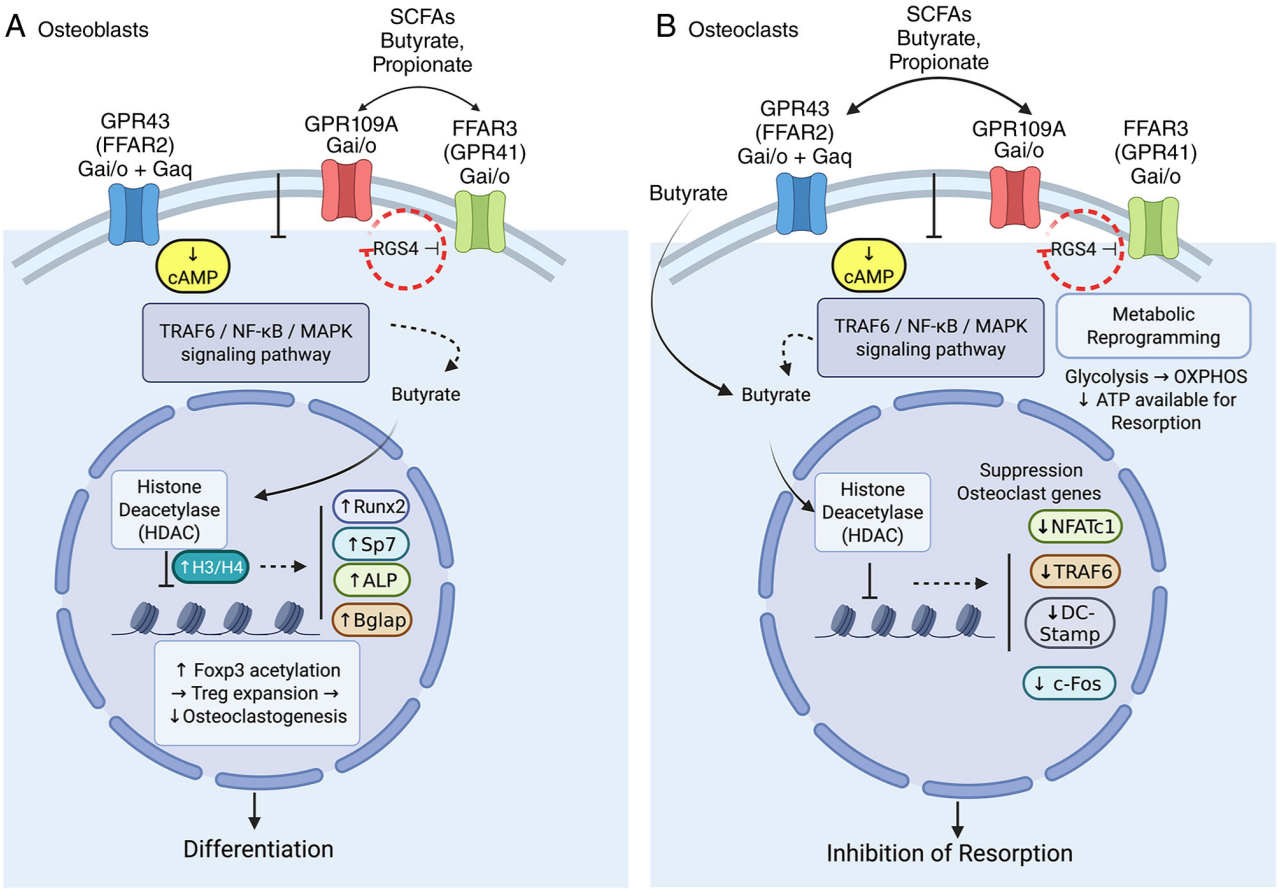

Figure 2. Dual mechanisms of SCFA-mediated bone cell regulation. (A) In osteoblasts, SCFAs activate GPR43/FFAR2 (Gαi/o + Gαq), GPR109A (Gαi/o), and FFAR3/GPR41 (Gαi/o; negatively regulated by RGS4), suppressing cAMP and attenuating TRAF6/NF-κB/MAPK signaling. Independently, butyrate inhibits HDACs, increasing H3/H4 acetylation at promoters of Runx2, Osterix (Sp7), ALP and osteocalcin (Bglap), promoting differentiation. HDAC inhibition also stabilizes Foxp3$^+$ Tregs, indirectly suppressing osteoclastogenesis. (B) In osteoclast precursors, the same receptor cascade attenuates TRAF6/NF-κB/MAPK signaling. Butyrate-mediated HDAC inhibition downregulates NFATc1, TRAF6, DC-Stamp and c-Fos. SCFAs additionally reprogram metabolism from glycolysis to OXPHOS, reducing ATP availability for resorption. HDAC, histone deacetylase; ALP, alkaline phosphatase; OXPHOS, oxidative phosphorylation.

Polyamines also promote osteoblast differentiation; exogenous administration of putrescine, spermidine and spermine has been shown to upregulate ALP activity and the expression of osteogenic genes (for example, Runx2 and osteocalcin) in mesenchymal stem cells (62). Furthermore, polyamines enhance bone formation *in vivo*, as evidenced by increased mineralization and bone volume, alongside suppression of osteoclast activity in animal models (63).

The physiological significance of polyamine homeostasis is further highlighted by the observation that mutations in polyamine-synthesizing enzymes such as spermine synthase in Snyder-Robinson syndrome result in severe bone defects, reduced osteoblast numbers and diminished bone volume (64). Similarly, genetic inactivation of spermine synthase in murine models leads to osteopenia, attributable to impaired osteoblast function (65). Polyamines such as putrescine, spermidine and spermine are derived from multiple sources: Endogenous host synthesis, dietary intake and intestinal microbial production. Both diet and the composition of the gut microbiota exert a significant influence on luminal polyamine concentrations.

Microbiological de novo synthesis from arginine. The gut microbiota plays an important role in the *de novo* synthesis of polyamines. Diverse bacterial taxa have the enzymatic ability to synthesize polyamines from precursors such as arginine. While the canonical ornithine decarboxylase 1 (ODC1)-dependent pathway (arginine - ornithine - putrescine) is a major route for polyamine biosynthesis, in the event of ODC1 deficiencies alternative pathways [for example the arginine decarboxylase (ADC) agmatinase (AGMAT) dependent route] may play a supplementary role (66). The biochemical mechanisms by which arginine biosynthesis and catabolism are completed are well characterized in bacteria such as *Escherichia coli* (*E. coli*), in the following manner: This provides an example of the proficiency of microorganisms in the metabolism of polyamine precursors (67). Numerous gut bacteria function as serious polyamine producers. For example, *Staphylococcus epidermidis* FB146 isolated from fermented foods (for example, miso) is an avid producer of putrefying substances and thus highlights the potential for these organisms to be dietarily important sources of polyamines for human consumption (68). Additional gut microbes such as *Akkermansia muciniphila*, *Bacteroides spp.* and *Clostridium spp.* (for example *Clostridium lavalense*) represent recognized components of the gut microbiota that contribute to the homeostasis of the immune system and

overall gut health (69-71). These groups of bacteria take part in various metabolic processes in the gut, such as the biosynthesis of beneficial substances such as polyamines.

Bioavailability studies depletion of antibiotic and restoration from fecal microbiota transplantation (FMT). Research studying antibiotic-induced gut microbiota depletion and its subsequent restoration through FMT is offering more information into the relationship between the gut microbiota and the host's polyamine status and well-being. Extensive antibiotics administration causes severe changes in immune cells and the intestinal epithelium, including the decrease of antimicrobial peptides and intestinal atrophy (72,73). Such perturbations are often accompanied by large changes in the abundance of bacterial inhabitants of the gut. FMT has been shown to be able to effectively reinstall the intestinal microbiota and thus also its variety of host functions. For example, FMT can help to replenish immune cell populations in numerous different areas of the small and large intestines after the antibiotic-induced depletion (72,74). In the context of *Clostridioides difficile* infection, FMT has been shown to be efficacious in normalizing a healthy gut microbiota while antibiotic therapy does not yield the same result (75). The reconstitution of microbial communities by FMT suggests a feasible approach with which to reinstate microbial polyamine production and its availability to the host, thereby underscoring the importance of a crucial relationship between a healthy gut microbiota and the polyamine status of the host.

Distinction from dietary source and host synthesis. Polyamines are not only produced by microbes in the gut but also come from dietary inputs and endogenous biosynthesis of host cells. Both dietary polyamines as well as arginine are recognized as nutritional determinants that influence physiological functions such as fertility (76). Fermented foods are especially rich in polyamines, presumably from the fermentative bacteria involved in its manufacture (68). Lactic acid bacteria (LABs) which is often found in foods such as curd, pickles, milk and wheat dough is considered to be beneficial microorganisms that help to supplement polyamine content of those foods (77). Host cells express the enzymatic capacity required to synthesize polyamines, which are mainly formed in an ODC1-dependent pathway, which changes arginine into ornithine and then into putrescine (66). The total pool of polyamines available to the organism is controlled by a mixture of endogenous production, consumption of the diet and microbial synthesis. This collective reservoir modulates a variety of physiological processes including processes that are important for skeletal health.

SSAT1 as the key catabolic enzyme. Intracellular polyamine concentrations are governed by a dynamic equilibrium between biosynthesis, transport and catabolism. An enzyme that plays a central role in polyamine catabolism is spermidine/spermine N^1-acetyltransferase (SSAT1, or SAT1). SSAT1 acts as an acetylase of molecular weight polyamines (spermidine and spermidine) to earmark them for either efflux or further degradation by polyamine oxidase. This acetylation process is central in polyamine export and cellular polyamine homeostasis. SSAT1 is distinct from other N-acetyltransferases such as SSAT2, which acetylates thialysine and is not part of the polyamine metabolism. Previous biochemical investigations confirmed that SSAT1 performs these reactions and controls the level of polyamines in the cell (78,79). On acetylation, polyamines are released from the cell, limiting the intracellular concentrations. Although the aforementioned studies do not specifically refer to the use of acetylated polyamines in feces or urine as biomarkers, the fact that the metabolites are being exported suggests that forms of these metabolites that are excreted could potentially reflect general polyamine metabolism and SSAT1 activity within an organism. Indeed, the increased activity of SSAT1 under physiological conditions results in an increase in the release of acetylated polyamine derivatives, making them potential biomarkers (80,81). Further studies should be conducted to determine their specific utility as biomarkers for bone-related conditions.

Dysregulation of SSAT1 activity has been implicated in various pathological conditions, particularly in cancer. For instance, elevated SAT1 expression has been linked to cell radio-resistance in glioblastoma multiforme, suggesting that SAT1 could be a therapeutic target to sensitize cancer cells to radiation therapies (82,83). Furthermore, overexpression of SSAT1 modulates tumorigenesis, with SSAT1 knockdown shown to reduce tumor growth *in vivo* (23,83).

While the provided studies do not directly address the dysregulation of SSAT1 in osteoclastogenesis, the established role of SSAT1 in regulating polyamine levels and its potential as a therapeutic target in other diseases suggests that its modulation could be relevant in bone metabolism. Given the critical roles of polyamines in cellular processes, investigating the specific impact of SSAT1 dysregulation on osteoclast differentiation and function, and its potential as a therapeutic target for bone diseases, warrants further exploration. Emerging data in immune and metabolic contexts (for example, in T-cell subsets) support a broader role for SAT1 beyond cancer (84).

Eukaryotic initiation factor 5A (eIF5A) hypusination: A novel mechanism for osteogenic translation. The eIF5A undergoes a unique post-translational modification called hypusination, which is absolutely dependent on the polyamine spermidine. In a two-step enzymatic reaction, deoxy-hypusine synthase (DHPS) transfers the aminobutyl moiety from spermidine to a conserved lysine residue of eIF5A, generating deoxy-hypusine; deoxy-hypusine hydroxylase (DOHH) then completes the modification to form hypusine (85).

The hypusinated form of eIF5A (hyp-eIF5A) is the only known protein to contain hypusine, and this modification is essential for eIF5A's function as a translation elongation factor. Specifically, hyp-eIF5A facilitates ribosomal transit through mRNA sequences encoding polyproline motifs by resolving ribosomal stalling at the peptidyl-transferase center, enabling efficient synthesis of proteins containing consecutive proline residues. Beyond polyproline resolution, eIF5A has also been implicated in translation termination efficiency and cellular differentiation (86,87). DHPS and DOHH are evolutionarily conserved from yeast to humans, underscoring the fundamental importance of this modification (88).

In the context of bone biology, eIF5A hypusination represents a mechanistically plausible link between gut microbial spermidine availability and osteogenic protein synthesis,

given that collagen and other structural bone matrix proteins, as well as transcription factors such as Runx2, contain poly-proline stretches whose efficient translation may depend on hyp-eIF5A activity (86-89). However, direct experimental evidence for this pathway in osteoblasts or osteoclasts remains to be established; this represents a high-priority area for future mechanistic investigation, as discussed below.

Direct effects of polyamines on bone cells. Polyamines exert direct effects on both major bone-remodeling cell lineages, osteoblasts and osteoclasts collectively recalibrating the balance of bone remodeling in favor of bone formation and stability. Effects on osteoblasts polyamines (putrescine, spermidine and spermine) have been demonstrated to promote differentiation of bone marrow derived stromal cells (BMSCs) into osteoblasts. In human BMSCs, exogenous polyamines increase ALP activity and enhance mRNA expression of key osteogenic transcription factors, including Runx2 and osteocalcin (Bglap) (62). At the same time, they suppress genes associated with adipogenesis, such as PPARγ, thereby redirecting cellular fate toward the osteoblastic lineage rather than adipocytes (62). Although direct evidence for activation of the PI3K-Akt pathway by polyamines in osteoblasts is not well documented in the literature, this signaling axis is well known to support osteoblast survival, proliferation and differentiation (90). Effects on osteoclasts in experiments with osteoclast precursors, natural polyamines (spermidine and spermine) strongly inhibit RANKL-stimulated osteoclastogenesis, reducing expression of NFATc1 and other osteoclast marker genes, and decreasing TRAP activity (89). In RAW 264.7 macrophage-derived osteoclasts, exogenous putrescine and spermine similarly reduce RANKL-induced expression of NFATc1 and RANK, and suppress TRAP activity, indicating impaired maturation toward resorbing osteoclasts (63). Mechanistically, these anti-osteoclast effects involve suppression of the Ca^{2+}-PYK2-Src-NFATc1 signaling axis, which is normally activated by RANKL during osteoclast differentiation, thereby limiting osteoclast migration, fusion and differentiation (61). Moreover, *in vitro* and *in vivo* work demonstrates that exogenous polyamines not only enhance bone formation via osteoblast activation but also suppress osteoclastogenesis, as evidenced in ovariectomized rat models (63). Collectively, by enhancing osteoblast differentiation and inhibiting osteoclastogenesis, polyamines contribute to a net bone-anabolic effect, favoring bone formation over resorption. The specific signaling pathways driving these polyamine-mediated effects, including the upregulation of osteogenic markers (Runx2 and ALP) and the suppression of the Ca^{2+}-PYK2-Src-NFATc1 axis in osteoclasts, are depicted in Fig. 3.

5. Microbial metabolomics for OP: Discovery and integration

Recent advances in microbiome research have elucidated the critical role of the gut microbiota in modulating bone metabolism, thereby establishing the gut-bone axis as a central paradigm in OP pathophysiology. The study of microbial metabolomics, that is, the global analysis of small molecules that are either produced or modified by gut microbes, has become an important methodological tool to unravel the mechanistic basis of this link. By determining the metabolic products of the gut microbes, researchers can understand how gut microbial activities affect bone remodeling and fracture susceptibility and, more importantly, determine novel biomarkers or targets for therapy. Notably, there is accumulating evidence to denote that metabolites accessed from the gut including SCFAs, bile acids and several bioactive compounds play a critical direct and indirect role in modulating osteoblast and osteoclast function (91,92). A comprehensive comparison of the biosynthetic origins, signaling targets, and cellular effects of these key metabolite classes is provided in Table I.

Microbiological metabolomics analytical platforms. The microbial metabolome is extremely chemically heterogeneous, requiring careful consideration before choosing the most suitable analytical tools to provide exhaustive and accurate identification. The following advanced technologies are currently being used in the study of microbial metabolomics, and each has its own strengths and limitations.

Liquid chromatography tandem mass spectrometry (LC-MS/MS). LC-MS/MS is one of the most basic technologies in microbial metabolomics that is appreciated for its remarkable precision, selectivity and versatility. Reversed phase LC-MS/MS is well suited to the separation and quantification of hydrophobic and moderately polar entities such as lipids, bile acids and xenobiotics. By contrast, hydrophilic interaction chromatography coupled with MS/MS is effective for the detection of highly polar molecules; for example, amino acids, nucleotides and SCFA derivatives. High-resolution mass spectrometry in combination with MS/MS provides the option for even stronger detection of multiple metabolic markers per sample to distinguish between structurally similar compounds often observed in biological matrices. These methodological advances have played a key role in the recent study into the metabolic complexities of gut microbiota and its implications on bone health (18).

Gas chromatography-MS (GC-MS). GC-MS spectrometry is highly respected for the fact that the analysis of volatile and semi-volatile substances produces excellent repeatability and precision. Chemical derivatization allows for the separation and detection of metabolites SCFAs, for example acetate, propionate, butyrate, organic acids and some amino acids. Comprehensive electron ionization spectral libraries that provide reliable identification and quantification of metabolites, are available. This approach is often used in gut-bone axis research, especially for SCFAs and other related compound-targeted analysis (93).

Nuclear magnetic resonance spectroscopy (NMR). Although NMR spectroscopy is less sensitive than MS-based approaches, it allows to get unmatched structural detail and allows precision of quantification with no need of external references. Its low sample preparation, as well as non-destructive nature render NMR a fantastic compliment to MS platforms, particularly when investigating the structures of new microbial metabolites. Integrated NMR- MS studies have been shown to provide more robust identification and quantification of metabolites, which will further enable understanding of gut-bone interactions (92). The coming together of a variety of metabolomic platforms with more

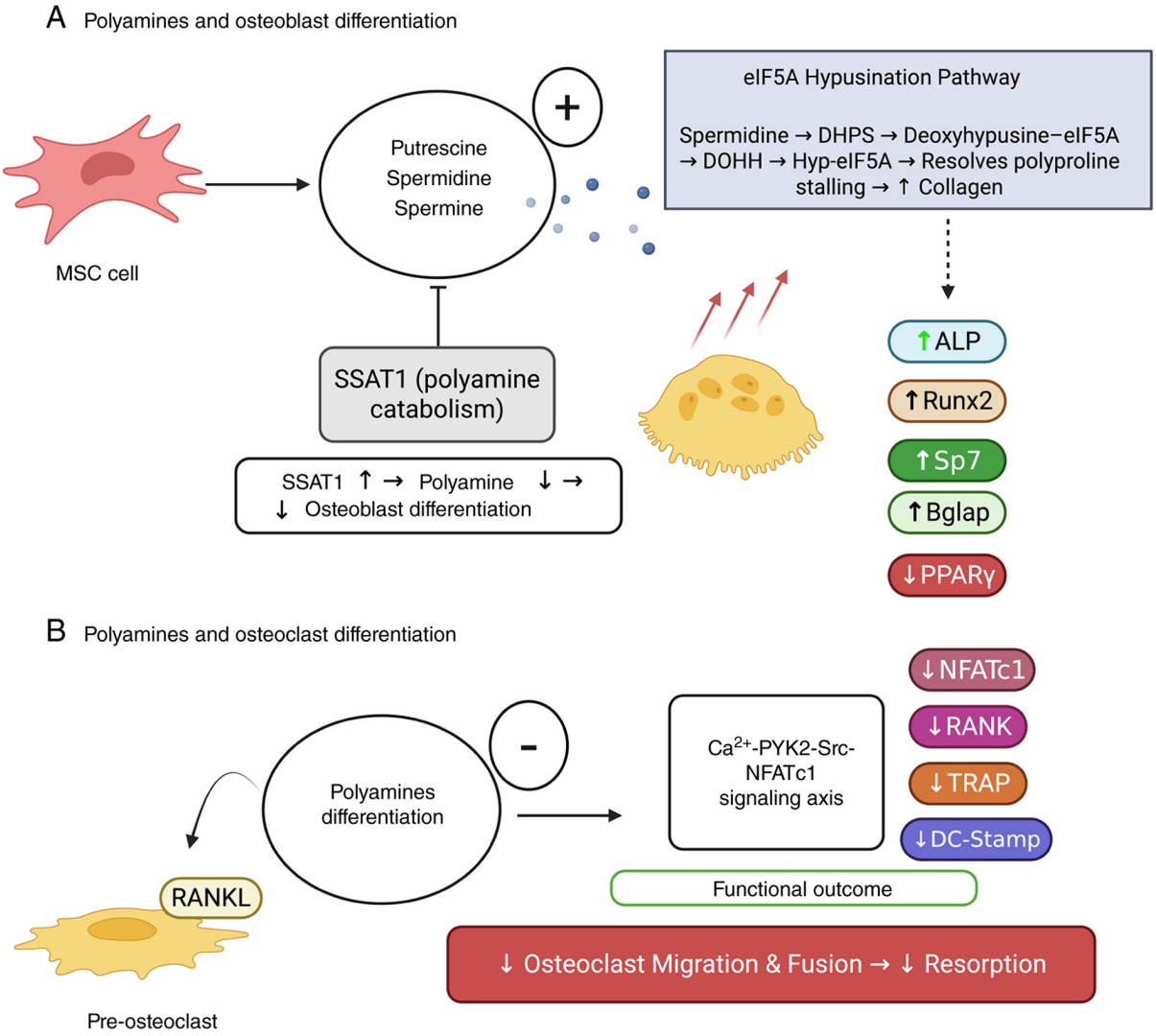

Figure 3. Polyamine-mediated regulation of osteoblast and osteoclast differentiation. (A) Gut-derived polyamines (putrescine, spermidine and spermine) promote MSC osteoblast differentiation, upregulating ALP, Runx2, Osterix (Sp7) and osteocalcin (Bglap), while suppressing the adipogenic regulator PPARγ. Spermidine drives the eIF5A hypusination pathway (DHPS → deoxyhypusine-eIF5A; DOHH → Hyp-eIF5A), resolving polyproline ribosomal stalling and enhancing collagen and osteogenic protein synthesis. SSAT1-mediated catabolism governs intracellular polyamine availability; SSAT1 overactivation depletes the polyamine pool and impairs differentiation. (B) In pre-osteoclasts, polyamines suppress RANKL-induced differentiation by inhibiting the Ca^{2+}-PYK2-Src-NFATc1 signaling axis, reducing RANK, NFATc1, TRAP and DC-Stamp expression and impairing osteoclast maturation, migration and fusion. ALP, alkaline phosphatase; DHPS, deoxy-hypusine synthase; DOHH, deoxy-hypusine hydroxylase; SSAT1, spermidine/spermine N^1-acetyltransferase 1; TRAP, tartrate-resistant acid phosphatase; MSC, mesenchymal stem cell.

advanced bioinformatics and systems biology is expected to further facilitate the identification of gut microbiota-derived biomarkers and therapeutics for OP. Comprehensive metabolite profiling in combination with metagenomic, transcriptomic and clinical data allows the construction of predictive models to understand the complex relationship between host and microbiome in skeletal health. In order to validate potential biomarkers and investigate causal associations, future studies should focus on standardization of analytical methods, extension of exhaustive metabolite databases, and conduct longitudinal and interventional studies.

Integrative challenges and multi-omics strategies. A major difficulty in microbial metabolomics is accurately identifying the features that are found. The vast quantity of signals, numerous of which represent unknown compounds, necessitates thorough verification using fragmentation spectra, matching retention times, and, if feasible, stable-isotope standards. Integrating metabolomic data with microbiome sequencing approaches such as 16S rRNA amplicon profiling and metagenomics has proven instrumental in establishing functional links between specific metabolites and bone outcomes, as demonstrated by an increasing body of MR and observational evidence. Recent MR studies have offered genetic proof of causal relationships between gut microbiota, plasma metabolites and the risk of OP (10). In the aforementioned review, specific bacterial taxa (such as Desulfobacterota) exhibited associations with OP susceptibility, mediated in part by plasma metabolites such as 3α-androstanediol monosulfate, accounting for up to ~9% of the effect (10). Another MR investigation focused on post-menopausal OP and osteoclast biology, identifying the Burkholderiales order as inversely linked with OP risk and positively correlated with osteoclast number; plus, two

Table I. Overview of key microbial metabolites, their biosynthetic origins, and their molecular targets in bone remodeling.

Metabolite class	Key examples	Primary orecursor	Key bacterial producers	Receptor/ signaling target	Effect on osteoblast	Effect on osteoclast
SCFAs	Acetate, Propionate, Butyrate	Dietary Fiber (Complex Carbohydrates)	*Faecalibacterium prausnitzii*, *Roseburia hominis*	GPR43 (FFAR2), GPR109A (HCAR2), HDAC Inhibition	Upregulates *Runx2*, *Osterix*, *Alp*, *Bglap* via histone acetylation	Inhibits NFATc1 via TRAF6/NF-κB pathway; induces metabolic shift from glycolysis to OXPHOS
Polyamines	Spermidine, Spermine, Putrescine	Dietary and Endogenous Arginine	*Staphylococcus epidermidis*, *Bacteroides* spp.	eIF5A hypusination, Ca^{2+}-PYK2-Src signaling	Enhances translation of polyproline-motif proteins (for example, Collagen, Runx2)	Inhibits migration and fusion by suppressing Ca^{2+}-PYK2-Src-NFATc1 signaling
Secondary bile acids	Deoxycholic acid, lithocholic acid	Primary bile acids (host-derived)	*Clostridium* spp. (via 7α-dehydroxylation)	FXR, TGR5	Indirectly modulates bone via immune cell regulation	Modulates resorption activity

OXPHOS, oxidative phosphorylation; HDAC, histone deacetylase.

osteoclast-related genes, FMNL2 and SRBD1, were implicated as potential mediators (91). Observational studies also provide strong support: In a cohort of 108 post-menopausal women, gut microbiota composition and fecal metabolome were significantly altered in those with OP, with metabolites such as L-pipecolic acid correlating closely with BMD (94). More recently, a study in Frontiers in Microbiology used large-scale GWAS and MR to explore 196 microbial taxa and 1,400 plasma metabolites, identifying ~96 metabolites potentially causally associated with OP, and delineating six gut-microbe-metabolite-bone causal pathways (95). Collectively, these studies illustrate how the combined deployment of metabolomics, metagenomics and genetic causal inference provides a methodological foundation for both biomarker discovery and mechanistic validation in gut-bone axis research.

Toward biomarkers and therapeutic targets. Combining metabolomic and genomic data improves our understanding of biological mechanisms and speeds up the identification of biomarkers. For instance, certain plasma metabolites found through MR could act as indicators of OP risk or how it progresses. Meanwhile, microbial groups involved in these pathways might be targeted for gut microbiota-focused treatments, such as interventions (for example, probiotics, prebiotics, or even specially engineered bacteria). Additionally, recent research has started investigating new connections within the gut-bone relationship. A 2025 study highlighted how *Terrisporobacter othiniensis* protects against OP by influencing ferroptosis-related proteins (specifically the MDM4/p53 pathway), strongly suggesting a link between the gut, microbes, ferroptosis and bone (96). These insights into mechanisms could lead to the development of entirely new treatments for OP.

Feature annotation and metabolite identification challenges. Despite significant progress in analytical methods, precisely identifying and labeling metabolites continues to be one of the toughest hurdles in microbial metabolomics. The immense chemical variety of microbial metabolites, numerous of which are new and uncharacterized, makes it very difficult to confidently assign compounds (97,98). The usual method for identifying metabolites starts by comparing experimental MS/MS spectra with existing public databases. Widely utilized resources include the Human Metabolome Database (HMDB), METLIN, MassBank and molecular networking platforms such as GNPS (Global Natural Products Social Molecular Networking) (97,99). These platforms facilitate the comparison of experimental spectra to known reference spectra, support structural annotation, and enable the exploration of related but unknown compounds through network-based similarity analyses (97). To promote consistency and transparency in metabolite reporting, the Metabolomics Standards Initiative has established a tiered framework for identification confidence. Level 1 (confirmed identification) requires concordance with an authentic standard in terms of retention time, mass accuracy and fragmentation pattern. Level 2 (putatively annotated compounds) is based on MS/MS spectral matching to library entries or contextual evidence. Level 3 (putatively characterized compound class) indicates assignment to a known chemical class (for example, bile acids and fatty acids) without full structural elucidation. Level 4 ('unknown compounds') encompasses features for which no plausible structural assignment can be made often referred to as the 'dark matter' of the metabolome (99,100). A significant proportion of features identified from gut microbiome metabolomics studies are still at Level 4. This phenomenon can be explained by two main reasons, which are the occurrence of novel molecules of microbial origin and the lack of spectra attached to extant

reference databases (98,101). Although these unidentified metabolites could have biologically important roles, for example in bone metabolism, the accurate identification of such metabolites requires sophisticated and heterogeneous analytical methods. Such techniques include high resolution NMR spectroscopy, *ex novo* synthetic generation of candidate molecules, and computer-based techniques such as *in silico* fragmentation simulations and statistical modeling (102). Recent methodological reviews emphasize that this so-called metabolic 'dark matter' continues to be a formidable obstacle within the field (98). In the specific context of the gut-bone axis, this dark matter problem carries particular consequence. Given that established microbial metabolites such as SCFAs and polyamines exert potent, receptor-mediated and epigenetically driven effects on osteoblast and osteoclast function, it is plausible that the pool of Level 4 features detected in gut metabolomics studies harbors additional, yet uncharacterized bone-regulatory molecules. This inference is supported by evidence already discussed in the present review: Observational cohort studies correlating fecal metabolomes with BMD have identified novel metabolite associations such as that of L-pipecolic acid for which mechanistic bone pathways remain undefined (94). Similarly, MR analyses have implicated gut microbial taxa in OP risk through metabolic mediators that are not yet fully characterized (10,95), suggesting that causally relevant bone metabolites may reside within the current dark matter pool. Targeted strategies to address this gap include: (i) Stable-isotope labelling approaches (for example, ^{13}C-labelled dietary fiber) to trace microbial fermentation products into systemic circulation and bone tissue; (ii) co-culture systems pairing gut microbial communities with osteoblast or osteoclast cell lines to enable functional screening of conditioned media fractions; (iii) molecular networking via GNPS to identify structural analogues of known bone-active metabolites within unannotated spectral clusters (97,99); and (iv) longitudinal metabolomic profiling in fracture cohorts with concurrent bone histomorphometry, enabling correlation of unidentified features with bone turnover endpoints. Advancing these approaches will be essential to bridge the gap between metabolomics discovery and mechanistic bone biology.

Multi-successor series integration: Integration of metabolites and microbial taxa and function. Integrating microbial metabolomics and microbiome sequencing techniques (that is, 16S rRNA gene amplicon sequencing and shotgun metagenomics) significantly increases the usefulness of these studies with regard to OP research. The use of the multivariate, multi-omics framework provides a more granular and mechanistic understanding of how the compositional architecture and functional repertoire of microbial consortia modulate metabolite synthesis, thereby affecting skeletal homeostasis. Taxonomic correlation using 16S rRNA sequence data or metagenomic datasets, research publications have been able to link relative abundance of certain clades of organisms (for example, SCFA producing organisms) such as *F. prausnitzii*, or *Roseburia* with quantified levels of SCFAs (acetate, butyrate and propionate) in either fecal or plasma samples. SCFAs have been shown to modulate bone remodeling through changes in immune signaling pathways as well as direct effects on osteogenic and osteoclastic cell populations (20).

Functional linkage. Shotgun metagenomics provides fine gene level information, which reveals the metabolic ability of the microbiome - for example genes for butyrate synthesis. When combined with metabolomics, this synergy allows us to reconstruct metabolic pathways using of resources such as KEGG (https://www.kegg.jp/), or CAZy (https://www.cazy.org/) in order to link genetic potential with actual metabolite production.

Co-occurrence networks. Advanced network-centric methods to construct co-occurrence models incorporating microbial taxa, metabolites and clinical phenotypes (such as BMD) Recent multi-omics studies of fracture patients have indeed shown strong links between specific types of bacteria, metabolites associated with inflammation (for example, N-acetylneuraminate) and low bone mass, using network analysis to show putative pathogenic trajectories (103).

Mediation and causality. MR studies further strengthen mechanistic insights by exploring possible mechanisms in which microbial taxa may mediate their effects on bone health by way of specific metabolite intermediates. For example, a contemporary MR analysis revealed numerous gut bacterial lineages, including those in the phylum *Desulfobacterota*, that have an impact on OP susceptibility partially mediated by plasma concentrations of 3a-radioligand androgens, namely 3a-dihydro-5a-androstanediol monosulphate, and explained ~9% of the effect (10). The ongoing challenge of identifying the majority of features of the metabolome, or the so-called 'dark matter' as these are colloquially called, continues to hinder the discovery of new biomarkers and targets for drug therapy. Consequently, a comprehensive characterization of these cryptic metabolites by integrated multi-omics, sophisticated analytical chemistry and computational strategies is an imperative. As is evident from recent multi-omics studies focused on OP, vector use of such synergistic methodologies can yield microbial metabolite signatures with favorable correlation with BMD and risk of fracture, opening up biomarker mining and microbiome-centric therapeutic interventions.

6. Translational strategies and therapeutic interventions

The aforementioned mechanistic insights toward a hierarchy of microbiota-targeted interventions span from broad dietary modifications to precision pharmacological strategies. For clarity, these are grouped into four tiers of increasing mechanistic specificity: Dietary fibers and prebiotics, probiotics and synbiotics, postbiotics, and next-generation pharmacological approaches.

Dietary fibers and prebiotics. Inulin-type fructans (ITF) and microbiota-targeted modulation ITF including inulin and oligofructose fulfil the operational definition of prebiotics by being selectively utilized by the gut microbiota to confer a health benefit (104). Human intervention studies demonstrate that daily intakes in the 10-20 g range reproducibly enrich saccharolytic taxa such as *Bifidobacterium* and *F. prausnitzii*, with downstream effects on systemic metabolism that are directly relevant to OP risk. In obese women, 16 g/day of an inulin/oligofructose 50:50 mixture for three months induced selective microbiota shifts characterized by increased *Bifidobacterium* and other beneficial taxa, alongside modest

improvements in metabolic endotoxemia and metabolomic signatures related to lipid and glucose handling (105). A large systematic review of human ITF trials confirms that such doses consistently increase *Bifidobacterium*, *Lactobacillus* and *F. prausnitzii* across diverse populations, while also influencing intestinal functions (barrier and immune tone) and extra-intestinal outcomes including glucose homeostasis, lipid profile, mineral absorption and bone health endpoints (104). Ecosystem-wide analyses in healthy adults have further shown that inulin supplementation reshapes the colonic microbiota beyond the classical 'bifidogenic' effect, with coordinated changes in several genera and parallel shifts in fecal metabolites, including SCFAs (106). Such SCFAs, particularly butyrate and propionate, engage the receptor-mediated and epigenetic mechanisms aforementioned, representing key mechanistic links between ITF, gut microbiota and bone remodeling. Evidence specific to post-menopausal women remains limited but is biologically compelling. ITF improve cardiometabolic risk factors that are over-represented in post-menopausal OP, including insulin resistance, dyslipidemia and low-grade inflammation, as shown in randomized trials in overweight/obese women with polycystic ovary syndrome (107) and in type 2 diabetes mellitus (T2DM) populations (108,109). Given the recognized association between T2DM, BMD and fracture risk (110), sustained ITF use in post-menopausal women may offer a dual benefit: Correction of dysmetabolism and indirect mitigation of diabetic bone fragility. From a translational standpoint, a pragmatic target dose for long-term use in post-menopausal women is 10-15 g/day, titrated upward based on gastrointestinal tolerance. At this range, human studies consistently demonstrate robust microbiota responses (104-106) with acceptable tolerability, while meta-analytic data support improvements in glycemic control and lipid profile that are relevant to bone health and fracture risk reduction (108,109).

RS and other fermentable fibers. RS represents another class of microbiota-accessible carbohydrate with demonstrable effects on bone in preclinical models. In meat ducks, graded inclusion of RS derived from raw potato starch (RPS) produced linear and quadratic increases in tibial strength and ash content, accompanied by elevated cecal propionate and butyrate levels (111). Micro-CT and microbiota profiling indicated that RPS-induced gains in bone mass correlated with higher *Firmicutes* abundance and enhanced SCFA production, as well as improved phosphorus absorption and reduced digestive tract pH (111).

In a model of *E. coli*-induced bone loss in ducks, a diet containing 12% RPS improved bone quality, suppressed bone resorption and attenuated inflammatory responses in both ileum and bone marrow (112). These benefits coincided with increased *Firmicutes* abundance, higher SCFA (especially propionate) production, and downregulation of Malt1/NF-κB inflammasome activation, together with expansion of Treg cells (112). Collectively, these data provide mechanistic proof-of-concept that RS-driven microbiota remodeling and SCFA generation can ameliorate inflammation-related bone loss, highly relevant to post-menopausal and inflammatory OP.

Although direct human evidence on RS and skeletal endpoints is sparse, these preclinical findings support cautious translation of RS-enriched foods (for example, high-amylose grains, cooled potatoes and legumes) or purified RS as adjuncts to standard OP management. Dosing regimens should likely mirror those used in metabolic trials (10-30 g/day fermentable fiber), with careful attention to gastrointestinal tolerance, fiber type and interactions with other components of the habitual diet (104,113).

High-fiber dietary patterns and the Mediterranean diet. Beyond isolated fibers, dietary patterns characterized by high intake of plant foods and diverse fibers appear favorable for bone health. A comprehensive review of dietary patterns and bone outcomes underscores that diets rich in fruits, vegetables, whole grains and legumes patterns overlapping substantially with the Mediterranean diet are associated with higher BMD and lower fracture risk, whereas Western-type patterns high in sugar, fat and refined grains are detrimental (113).

Specifically in menopausal women, intervention studies with Mediterranean-style diets demonstrate improvements in body weight, blood pressure, lipid profiles and omega-6: omega-3 ratios (114), and broader reviews implicate this pattern in the targeted prevention of multiple chronic diseases, including OP (115). A recent review focusing on nutrition and post-menopausal OP concludes that, in addition to calcium and vitamin D, dietary patterns featuring abundant plant foods, moderate high-quality protein, and low ultra-processed food intake are likely to support bone health and should be prioritized in non-pharmacological management (116).

The inflammatory tone of the diet is an additional, mechanistically pertinent dimension. A systematic review and meta-analysis using the Dietary Inflammatory Index showed that more pro-inflammatory diets are significantly associated with lower lumbar spine and total hip BMD, and with higher risks of OP and fractures (117). These findings offer a coherent framework whereby high-fiber, plant-rich, low-inflammatory dietary patterns (for example, Mediterranean diet) exert skeletal benefits in part via microbiota-mediated and immunomodulatory mechanisms.

Nevertheless, the relationship between very high fiber intake and bone is not unequivocally beneficial in all contexts. In older individuals with T2DM, a 12-week high-fiber diet intervention (38 g/day) improved metabolic outcomes but reduced biochemical markers of bone formation, without detectable alterations in bone microarchitecture or Wnt signaling, suggesting potential suppression of bone turnover in an already high-risk group (118). Given the elevated baseline fracture risk and complex BMD trajectory in T2DM (110), these findings call for careful titration of fiber in diabetic OP, with monitoring of both metabolic and skeletal endpoints.

Prebiotics: Dosage and clinical considerations. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and inulin are well established prebiotic fibers. These carbohydrates resist digestion in the small intestine and are accordingly fermented by colonic microbiota, favoring (i) the increase of populations of useful bacteria such as B*ifidobacterium*; (ii) the generation of SCFAs; (iii) the decrease of gastrointestinal pH; and (iv) the use of minerals such as calcium and magnesium, which might be responsible for the increase of the whole mineral intake (104,113,116). A thorough review of human studies uncovered the fact that ITF such as short-chain FOS as well as longer-chain

inulin, in general, reliably show prebiotic activity. These compounds appear to have a positive effect on glycemic control, lipid metabolism, mineral absorption, inflammatory status and body composition (104). Further meta-analyses have suggested the modest ability of ITF supplementation to enhance lipid profile and glucose metabolism overall, with more profound effects seen in individuals with T2DM, in which improvements in HDL-cholesterol and glycemic control are significant (108,109). Clinical trials with women with metabolic disturbance support these results. For example, overweight and obese women, diagnosed with polycystic ovary syndrome, who consumed ITF (10 g/d) for 12 weeks showed ameliorations in insulin resistance, lipid parameters, anthropometric measures and androgen levels as well as clinical symptomatology (107). These variables are implicated in the risk of OP, through effects on adiposity, inflammation and sex hormones. In obese women, a 3-month regimen of ITF supplementation caused changes in the composition of gut microbiota and systemic metabolic profiles with specific bacterial taxa that were linked to markers of metabolic endotoxemia (105). This body of evidence highlights a crucial conclusion; prebiotics should not be considered only as 'gut health' supplements but as metabolic modulators that can exert their biological effects on multiple fracture risk determinants including insulin resistance, dyslipidemia and inflammation, in post-menopausal women and patients with T2DM (108-110,116). Nevertheless, large interindividual differences in microbiota sensitivity require a more customized strategy for dosing and patient consideration. A crossover study showed that the increase in *Bifidobacterium* that accompanied an ITF prebiotic was significantly higher in people who consistently ate more dietary fiber than those who consumed less, suggesting that an individual's baseline diet and resulting gut microbiota configuration influences prebiotic activity (119). Parallel investigations in obese women reveal intricate associations between species of bacteria, metabolic biomarkers and indices of metabolic endotoxemia after ITF treatment (105).

These findings support the emerging notion of 'responders' vs. 'non-responders' to prebiotic interventions. For OP-focused translation, this suggests that: (i) Prebiotic dosing (typically 5-20 g/day for FOS/inulin) may need to be individualized based on habitual fiber intake, baseline microbiota composition and co-morbidities (104,119); (ii) Stratification by baseline dysbiosis (for example, low *Bifidobacterium*/SCFA producers, high pro-inflammatory taxa) and metabolic status (obesity, T2DM and PCOS) should be incorporated into trial design (105,107-109); (iii) Bone-relevant endpoints (BMD, bone turnover markers, microarchitecture and fracture risk) must be systematically integrated into future prebiotic RCTs, particularly in post-menopausal and diabetic cohorts in whom metabolic and skeletal risks co-cluster (110,116,118).

Probiotics and synbiotics. In contrast to the relatively robust human evidence for prebiotics, data on probiotic effects on human BMD and fracture risk remain limited and were not directly captured in the research papers reviewed here. Nonetheless, the mechanistic logic for targeting specific probiotic strains in OP is supported indirectly by the prebiotic and dietary fiber literature.

Preclinical work with RS shows that modifying the gut microbial community toward SCFA-producing Firmicutes improves bone mass and protects against pathogen-induced bone loss, in part by dampening pro-inflammatory signaling (Malt1/NF-κB inflammasome) and promoting regulatory T-cell expansion (111,112). These results align with the general idea that certain probiotic strains, especially *Lactobacillus* species and *Bifidobacterium* species, could improve bone health by modulating the immune system, strengthening gut barriers, and increasing the production of beneficial postbiotic compounds (including SCFAs, indoles and polyamines). Considering this, the following strategies can be envisioned at the strain level (though comprehensive bone-related data for these specific strains are largely beyond the scope of this current research): (i) *Lactobacillus reuteri* (DSM 17938), widely studied in gastrointestinal and immune indications: This strain exerts anti-inflammatory effects and can enhance mucosal barrier function and regulatory T-cell responses, mechanisms that mirror those implicated in RS-mediated protection against bone loss (111,112); (ii) *Bifidobacterium longum* (for example, BB536): *Bifidobacterium* species are prominent responders to ITF (104-106) and are known producers of acetate and certain polyamines, molecules implicated in mucosal integrity, osteoblast activity and regulation of oxidative stress; (iii) *Lacticaseibacillus paracasei*: Members of this taxon display immunomodulatory properties and may bias host responses toward anti-inflammatory phenotypes, conceptually aligning with the suppression of NF-κB-driven bone resorption observed in RPS-fed, *E. coli*-challenged ducks (112); (iv) *Akkermansia muciniphila* although not directly modulated by ITF in all studies, barrier-strengthening and metabolic benefits attributed to this mucin-degrading bacterium are congruent with the broader evidence that improved gut integrity and reduced metabolic endotoxemia accompany ITF and high-fiber interventions (104-106,113); (v) translationally, synbiotic formulations that combine structurally compatible prebiotics (for example, ITF or FOS) with carefully selected probiotic strains represent a rational next step. Such combinations may enhance colonization or persistence of beneficial strains, amplify SCFA and other postbiotic production, and exert more consistent immuno-skeletal effects than either component alone. The robust prebiotic literature reviewed (104,105, 108,109,111,112,119) provides an essential scaffold for designing and rationally titrating these synbiotic interventions in osteopenic and osteoporotic populations.

Postbiotics: Metabolites and fermented foods. Postbiotics defined as functional bioactive compounds produced by microorganisms and released into the host environment, include SCFAs, polyamines, exopolysaccharides, cell wall fragments and extracellular vesicles. From the perspective of OP, three classes of postbiotics stand out:

SCFAs (acetate, propionate and butyrate). The RPS studies in ducks elegantly demonstrate that increases in cecal propionate and butyrate are tightly associated with improved tibial strength, ash content and bone microarchitecture, as well as with reduced intestinal and bone marrow inflammation (111,112). These data strongly support SCFAs as effector molecules linking fermentable fiber, the microbiota and bone mass.

Polyamines and related metabolites. Although not directly quantified in the cited animal studies, polyamines are recognized microbial products that can influence osteoblast differentiation, collagen maturation and redox balance. The enrichment of *Bifidobacterium* and other saccharolytic taxa by ITF (104-106) suggests a plausible, if as yet under-explored, route by which prebiotic-induced changes in microbiota-derived polyamines could contribute to bone anabolism.

Extracellular vesicles and exosomal cargo. Milk-derived exosomes provide a striking example. Oral administration of bovine colostrum-derived exosomes in an OP-induced mouse model improved bone health *in vivo* and promoted osteoblast proliferation and differentiation *in vitro*, suggesting that dietary exosomal cargo can modulate bone remodeling (120). These vesicles, while not microbiota-derived, exemplify the concept that orally delivered nanoscale particles, and their molecular cargos (microRNAs, proteins and lipids) can act as 'postbiotic-like' agents in bone tissue.

Fermented foods such as kefir, yogurt, miso and sauerkraut deliver complex mixtures of live microbes and their metabolites, potentially acting as natural synbiotic matrices. While the specific studies summarized in the present review do not provide direct RCT evidence for fermented foods and BMD, broader nutritional reviews emphasize that dietary patterns rich in minimally processed plant foods and fermented dairy are associated with improved bone outcomes in post-menopausal women (113,116). Kefir and cultured dairy may be particularly promising due to their combined provision of calcium, vitamin K2, bioactive peptides and live LABs, though rigorously controlled OP-focused trials remain scarce. From a translational perspective, cell-free supernatants from probiotic cultures and standardized postbiotic preparations (purified SCFAs, exopolysaccharides and exosomes) offer a route to harness microbial bioactivity while avoiding viability, safety and colonization issues associated with live organisms. The SCFA-mediated skeletal benefits observed in RPS-fed ducks (111,112) and the bone-protective effects of bovine colostrum exosomes in mice (65), together suggest that targeted delivery of defined postbiotic cocktails could complement existing OP pharmacotherapies, particularly in patients unable or unwilling to consume high-fiber or fermented foods.

Next-generation pharmacological strategies. The convergent evidence that SCFAs and other microbial metabolites modulate bone mass via immunological and osteoclast/osteoblast pathways (111-113,116) provides a rationale for more pharmacologically precise approaches that move beyond dietary modulation alone. Emerging strategies can be conceptually grouped as follows:

Direct administration of SCFAs (for example, sodium butyrate) or prodrugs such as tributyrin has the potential to recapitulate numerous of the bone-protective effects observed with fermentable fibers, but with more predictable pharmacokinetics. The duck studies highlight butyrate and propionate as plausible mediators of improved bone mass and reduced inflammatory bone loss (111,112). However, unencapsulated SCFA salts are rapidly absorbed in the upper gut, limiting distal colonic exposure and potentially increasing systemic side effects.

Advances in pH-dependent coatings and encapsulation technologies allow for colon-targeted SCFA delivery. By formulating tributyrin or butyrate-releasing polymers that disintegrate at colonic pH, it may be possible to locally increase SCFA concentrations in proximity to the gut-associated lymphoid tissue and portal circulation, thereby more precisely engaging the osteo-immune axes implicated in bone remodeling. Such approaches are particularly attractive for patients in whom high-dose fermentable fiber is poorly tolerated or contraindicated [for example, severe irritable bowel syndromes (IBS) and advanced diabetic gastroparesis].

SCFAs signal through GPCRs such as FFAR2/GPR43 (acetate/propionate) and GPR109A (butyrate/niacin), which are expressed on immune cells, intestinal epithelial cells and potentially bone cells. The RS-induced suppression of Malt1/NF-κB inflammasome activation and promotion of Treg expansion in ducks (112) align well with known SCFA-GPCR signaling networks that favor anti-inflammatory, tolerogenic immune states.

These insights support the preclinical development of selective FFAR2/GPR43 and GPR109A agonists as candidate therapeutics for inflammatory and post-menopausal OP. Such agents might: (i) Reproduce the Treg-expanding, NF-κB-suppressing effects of SCFAs observed in RPS-fed animals (111,112); (ii) circumvent gastrointestinal side effects of high-dose fibers, and be readily combined with anti-resorptives or anabolics in high-risk patients (for example, those with T2DM-related bone fragility (110); (iii) rigorous preclinical work is needed to define optimal receptor selectivity, dosing, bone tissue pharmacodynamics and long-term safety, with particular attention to potential off-target effects in carcinogenesis or immune suppression.

HDAC inhibition and bone-specific epigenetic modulation. Butyrate is also a potent inhibitor of HDACs, linking microbial fermentation to host epigenetic regulation. The SCFA-mediated improvements in bone mass and suppression of inflammatory pathways in RPS-fed ducks (111,112) are consistent with a model in which HDAC inhibition skews gene expression toward anti-inflammatory, pro-osteogenic programs in immune and stromal cells.

This raises two translational possibilities:

Repurposing existing HDAC inhibitors (HDACi). Several HDACi are already in clinical use for cancer and other indications. However, their systemic toxicity and pleiotropic actions necessitate caution. Any attempt to leverage HDACi for OP would require bone-targeted delivery or very low dosing to avoid hematologic, neurologic or oncogenic adverse effects.

Designing bone-specific HDAC modulators. By integrating knowledge from dietary and prebiotic studies, showing that localized gut-derived SCFA exposure can beneficially influence bone via immune and endocrine axes (111-113,116). It may be possible to develop novel HDAC modulators with preferential accumulation in bone marrow or osteoblast/osteoclast lineages. Such agents could emulate the epigenetic profile induced by physiologic SCFA exposure but with greater pharmacologic precision. In both scenarios, mechanistic insights derived from prebiotic, RS and diet-pattern studies provide crucial guardrails: Therapies should aim to mimic the spatially restricted, temporally pulsatile SCFA exposure generated by

Table II. Key preclinical and clinical studies demonstrating the efficacy of microbiota-targeted interventions.

Authors, year	Intervention type	Study model	Dosage/regimen	Key observed effects on bone/microbiota	(Refs.)
Zhang *et al*, 2022	Resistant starch (RPS)	Animal (meat ducks)	Graded inclusion of RPS	Increased tibial strength and ash content; elevated cecal propionate and butyrate; increased *Firmicutes* abundance.	(111)
Zhang *et al*, 2022	Resistant starch (RPS)	Animal (*E. coli*-challenged Ducks)	Diet containing 12% RPS	Suppressed bone resorption and attenuated inflammation; downregulated Malt1/NF-κB activation; expanded Treg cells.	(112)
Dewulf *et al*, 2012	Inulin-type fructans	Human (obese women)	16 g/day of inulin/ oligofructose for 3 months	Selective microbiota shifts (increased *Bifidobacterium*); modest improvements in metabolic endotoxemia.	(105)
Lee, 2024	Spermine synthase KO	Animal (mouse model)	Genetic inactivation	Caused osteopenia attributable to impaired osteoblast function, highlighting the role of endogenous polyamines.	(63)
Yun *et al*, 2020	Postbiotics (exosomes)	Animal (mouse model)	Oral administration of bovine colostrum-derived exosomes	Improved bone health *in vivo* and promoted osteoblast proliferation and differentiation *in vitro*.	(120)

RPS, raw potato starch.

a healthy microbiota, rather than induce chronic, high-level systemic HDAC inhibition.

Safety considerations and limitations. While the aforementioned interventions demonstrate compelling mechanistic and preclinical rationale, responsible translational implementation requires explicit consideration of their safety profiles and clinical limitations.

Dietary fibers and prebiotic supplements are generally well tolerated; however, dose-dependent gastrointestinal side effects including bloating, flatulence, abdominal cramping and altered bowel habits are consistently reported, particularly at intakes above 15 g/day. Patients with IBS, IBD, or advanced diabetic gastroparesis may require careful dose titration or may not be suitable candidates for high-dose fiber interventions (104,113). As aforementioned, very high fiber intakes in older individuals with T2DM may paradoxically suppress bone formation markers (118), underscoring the need for bone-specific endpoint monitoring alongside metabolic outcomes in this population.

Probiotic interventions carry a low but clinically relevant risk of bacteremia or fungemia in severely immunocompromised individuals, including those receiving chemotherapy, post-transplant immunosuppression, or with advanced HIV infection. Strain-specific safety documentation is therefore essential prior to widespread clinical adoption. Postbiotic preparations including purified SCFAs, extracellular vesicles and exosomes face additional challenges of production standardization, dose quantification and shelf stability that must be addressed before they can be responsibly administered in clinical settings (120).

For pharmacological approaches (GPCR agonists and HDAC inhibitors), the risk of off-target effects is the primary concern. HDAC inhibitors in particular carry well-characterized risks in oncological contexts including hematologic toxicity and immunosuppression that would require bone-targeted delivery strategies or ultra-low dosing to be acceptable in an OP indication (111-113,116). Selective GPCR agonists targeting FFAR2/GPR43 and GPR109A require rigorous preclinical characterization of receptor selectivity, bone tissue pharmacodynamics, and long-term oncogenic and immune safety before clinical development is warranted.

Finally, across all intervention tiers, large interindividual variability in gut microbiota composition means that therapeutic responses will be heterogeneous. Biomarker-guided patient stratification incorporating baseline dysbiosis indices, SCFA producer abundance profiles and metabolic comorbidity status is essential to ensure that future clinical trials generate interpretable and generalizable findings (105,107-109,119).

Collectively, the evidence from prebiotic fibers, RS, dietary patterns and emerging postbiotic concepts suggests a rich translational landscape in which microbiota-targeted strategies can complement established pharmacological therapies for OP. Key preclinical and clinical findings supporting the efficacy of these specific interventions are summarized in Table II. ITF and RS stand out as immediately actionable

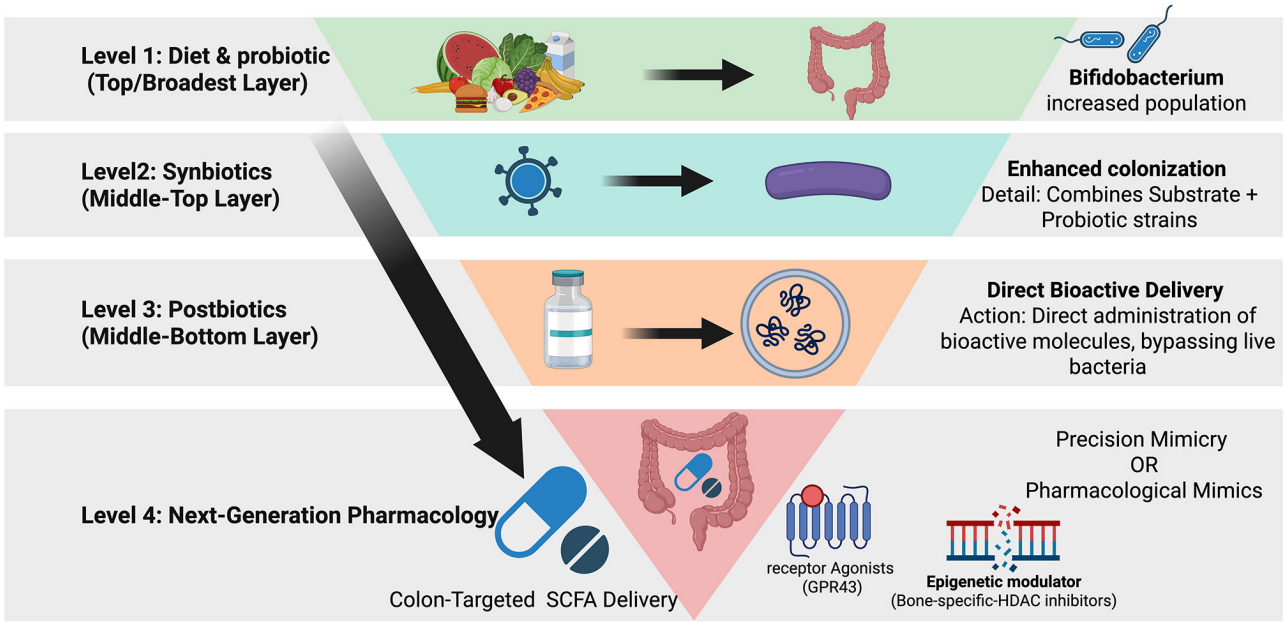

Figure 4. Hierarchical framework of microbiota-targeted therapeutic strategies for osteoporosis. Therapeutic interventions are organized by increasing mechanistic specificity. Level 1 (Diet/Prebiotics): High-fiber diets and prebiotics (inulin, resistant starch) enrich SCFA-producing taxa (for example, *Bifidobacterium*), increasing luminal SCFA production. Level 2 (Synbiotics): Prebiotic-probiotic combinations enhance targeted microbial colonization and SCFA yield. Level 3 (Postbiotics): Direct administration of purified bioactive compounds (SCFAs, polyamines and extracellular vesicles) delivers bone-protective signals independently of live bacteria. Level 4 (next-generation pharmacology): Colon-targeted SCFA delivery systems, selective GPR43/GPR109A receptor agonists and bone-specific HDAC inhibitors provide precise pharmacological mimicry of microbiome-derived bone-protective signaling. SCFAs, short-chain fatty acids; EVs, extracellular vesicles; HDAC, histone deacetylase.

interventions with plausible skeletal benefits mediated by SCFAs, improved metabolic control and reduced inflammation (104,105,107-109,111-113,116). Future work should prioritize stratified, mechanistically informed clinical trials in post-menopausal and T2DM populations, integrating microbiome, metabolomic, immunologic and bone structural endpoints to rationally advance from dietary modulation toward next-generation microbial-derived therapeutics. A hierarchical framework for these translational interventions, classifying strategies from broad ecological modulation (diet/prebiotics) to precision pharmacology (postbiotics/mimetics), is presented in Fig. 4.

7. Discussion

OP remains a major public-health challenge marked by high fracture incidence, chronic morbidity and substantial healthcare costs; its management is further complicated by various guideline recommendations, inconsistent treatment adherence, and heterogeneous patient responses. The emerging view of the gut microbiota as an influential metabolic organ offers a compelling, mechanistic complement to classical endocrine and mechanical models of bone biology. Accumulating preclinical and human evidence suggests that microbial metabolites, particularly SCFAs and polyamines, may act as effectors that translate dietary and microbial ecology into bone-remodeling signals. SCFAs generated from fiber fermentation modulate osteoclastogenesis, bolster osteoblast function, and temper systemic inflammation (9).

Polyamines derived from amino-acid metabolism support osteogenic differentiation, collagen maturation and translational control (for example, eIF5A hypusination), while antagonizing RANKL-driven osteoclast activation (121). These metabolite-centric mechanisms dovetail with observations from germ-free, antibiotic and fecal-microbiota transplantation models, and are supported by MR analyses linking specific taxa and plasma metabolites to BMD and OP risk (122).

Translational prospects are promising but nascent. Dietary modulation (prebiotics, fermented foods), rational probiotic strains, standardized postbiotics (purified SCFAs, polyamines, extracellular vesicles), and cell-free supernatants each represent feasible routes to harness microbiota-derived bone-protective activities while minimizing safety and colonization concerns inherent to live-microbe therapies (123). Early animal and limited human studies (for example, fiber-driven SCFA increases associated with improved bone strength; cohort correlations between fecal metabolites and BMD) provide preliminary support but stop short of definitive clinical evidence (124,125).

Several methodological and knowledge gaps temper enthusiasm and must be prioritized. Microbial metabolomics is indispensable for mapping effector molecules, yet the field faces substantial annotation challenges: A large proportion of detected features remain 'dark matter'. Robust identification requires combinatory analytics (LC-MS/MS, GC-MS and NMR), high-quality fragmentation spectra, retention-time standards, and, where possible, stable-isotope labeling. Integration of metabolomics with metagenomics, host genomics and functional readouts (immune, endocrine and bone histomorphometry) is essential to resolve microbe-metabolite-host causal chains and discover actionable biomarkers an approach exemplified by recent MR studies that identified microbial taxa and mediating

plasma metabolites (for example, 3α-androstane derivatives) accounting for measurable OP risk (103).

Clinical translation further requires rigorous causal study designs, adequately powered RCTs of defined dietary, probiotic or postbiotic interventions with bone-specific endpoints (BMD, bone-turnover markers, fracture incidence) and mechanistic sub study arms (metabolomics, microbiome sequencing and immune profiling). Special populations (post-menopausal women, chronic kidney disease and inflammatory comorbidities) demand tailored strategies given their distinct microbiota signatures and metabolic milieus (123). Safety surveillance particularly for long-term modulation of microbial metabolism and systemic polyamine exposure will be critical.

Overall, the gut bone axis reframes OP as a multi-compartmental disorder in which diet, microbial ecology and small-molecule mediators converge on skeletal homeostasis. Translating these insights into precision prevention and therapy requires coordinated advances in metabolomic annotation, integrative multi-omics, causal human trials, and the development of standardized, scalable microbiota-derived interventions. If these priorities are addressed, microbiota and metabolite-based strategies could meaningfully complement existing pharmacologic approaches and broaden the armamentarium against OP.

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Authors' contributions

FC and JS drafted the manuscript, edited and revised the manuscript and participated in the literature search and analysis of the data to be included in the review. XW designed the review and revised the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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