Front. Med. 2025, 19(3): 474–492
https://doi.org/10.1007/s11684-025-1129-3

REVIEW

Progress of research on the gut microbiome and its metabolite short-chain fatty acids in postmenopausal osteoporosis: a literature review

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Abstract **Postmenopausal osteoporosis (PMOP) is a systemic metabolic bone disease caused by the decrease in estrogen levels after menopause. It leads to bone loss, microstructural damage, and an increased risk of fractures. Studies have found that the gut microbiota and its metabolites can regulate bone metabolism through the gut–bone axis and the gut–brain axis. As research progresses, PMOP has been found to be associated with gut microbiota dysbiosis and Th17/Treg imbalance. The gut microbiota is closely related to the development and differentiation of Treg and Th17 cells. Among them, the metabolites of the gut microbiota such as short-chain fatty acids (SCFAs) can regulate the differentiation of effector T cells by acting on molecular receptors on immune cells, thereby regulating the bone immune process. The multifaceted relationship among the gut microbiota, SCFAs, Th17/Treg cell-mediated bone immunity, and bone metabolism is eliciting attention from researchers. Through a review of existing literature, we have comprehensively summarized the effects of the gut microbiota and SCFAs on PMOP, especially from the perspective of Th17/Treg balance. Regulating this balance may provide new opportunities for PMOP treatment.**

Keywords postmenopausal osteoporosis; gut microbiota; short-chain fatty acids; Th17 cells; Treg cells

Introduction

Postmenopausal osteoporosis (PMOP) is a systemic metabolic bone disease characterized by decreased bone mass and microstructural damage, leading to an increased risk of fractures [1]. PMOP is essentially a continuous remodeling process of bone formation and bone resorption. Imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption can result in reduced bone mass or osteoporosis [2,3]. The most serious complication of osteoporosis is fragility fractures. They occur primarily in the vertebral bodies, hip joints, and distal radius, significantly increasing the disability and mortality rates of elderly individuals and thus imposing a heavy economic burden on families and society [4,5].

Currently, the treatment of osteoporosis involves a comprehensive approach combining medication and lifestyle interventions. Anti-osteoporosis medications include bisphosphonates, denosumab, teriparatide, and estrogen-replacement therapy, among others. Teriparatide works by activating or increasing osteoblast activity and promoting bone synthesis metabolism. Bisphosphonates and denosumab primarily reduce bone resorption by inhibiting osteoclast activity [6,7]. In clinical practice, attention needs to be paid to the potential complications and corresponding side effects of some medications, such as jaw necrosis and atypical femur fractures [8]. These complications of medication treatment limit the clinical application of anti-osteoporosis drugs. Therefore, researchers are exploring other potential treatment options.

Scholars from various countries have found that changes in the gut microbiota (GM) are closely related to the decrease in bone mass and the prevalence of osteoporosis in elderly individuals [9]. The GM is closely related to bone metabolism [10–12]. The GM influences bone quantity, quality, and bone strength. As a key microbial ecosystem in the human body, the GM regulates bone mass by modulating gut permeability,

Received July 14, 2024; accepted December 16, 2024
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affecting nutrient absorption and metabolism. It also regulates endocrine hormones and immune status through the "gut–bone" axis to modulate the progression of PMOP. Adjusting dietary structure, probiotic supplementation, or fecal microbiota transplantation (FMT) may effectively prevent bone loss [13]. The GM further influences bone metabolism by regulating host metabolism, immune function, and hormone secretion [14]. The GM plays an important role in regulating bone metabolism and the pathogenesis of osteoporosis by improving gut barrier function and the effects of gut metabolites [15,16]. Short-chain fatty acids (SCFAs) are the main metabolic products of the GM, and researchers have paid attention to their extensive anti-inflammatory effects. SCFAs participate in regulating bone metabolism by activating corresponding receptors, regulating the inflammation process, or other mechanisms.

In this review, we discuss the role and mechanisms of the GM in PMOP from multiple perspectives. We focus on the GM and gut metabolite SCFAs, involving the intestinal mucosal barrier, endocrine function, immune system, and gut–brain axis.

PMOP is associated with GM imbalance

GM dysbiosis in PMOP population

PMOP is a type of high-bone-turnover osteoporosis caused by a sudden decrease in estrogen levels in postmenopausal women. Previous studies have shown that the GM plays a role in improving intestinal permeability, reducing inflammation, and participating in the immune regulation of the skeletal system. The GM regulates bone metabolism through multiple pathways [17,18]. Maintaining balance in the GM is beneficial for preserving intestinal epithelial-barrier function. The intestinal epithelial barrier isolates harmful antigens and pathogens and performs immune protection functions. Intestinal epithelial cells are sealed by tight-junction proteins (TJs), such as ZO-1, claudin-1, and occludin. Imbalance in the GM can alter the expression and distribution of TJ, changing the permeability of the intestinal barrier, leading to inflammation. The crosstalk between the host and GM also extends to the regulation of bone homeostasis and bone microstructure [19,20]. Generally microbiota-mediated breakdown metabolism participates in regulating physiologic bone turnover [21,22].

The GM comprises four major coexisting phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Compared with healthy controls, the GM richness and diversity are reduced in individuals with PMOP. Changes are observed in their gut symbiotic bacteria and fecal metabolites [23–25]. In the PMOP population, the proportion of Firmicutes significantly increases, whereas the proportion of Bacteroidetes significantly decreases. At the genus level, Bacteroides, Fusicatenibacter, Ruminococcus, and Anaerostipes are enriched in the healthy control group, whereas Agathobacter and Lactobacillus are enriched in the group with decreased bone mass [26]. Wang *et al.* found that the proportion of *Prevotella histicola* is significantly lower in PMOP subjects [27]. Other cross-sectional studies on the differences in GM of postmenopausal women have found that *Bacillus luciferensis*, *Bifidobacterium breve*, Prevotella_7, Blautia, Fusicatenibacter, Romboutsia, unclassified Mollicutes, and Lachnospiraceae UCG are enriched in the control group. Conversely, bacteria belonging to the Proteobacteria phylum, such as Klebsiella, Escherichia/Shigella, and Enterobacter, are enriched in the group with decreased bone mass. Additionally, *Bifidobacterium animalis*, *Lactobacillus plantarum*, Parabacteroides, Lactobacillus, Peptoniphilus, and Propionimicrobium are enriched in the PMOP group [16,28,29].

Bacteria such as Ruminococcus and Butyricicoccus in family Ruminococcaceae are involved in the production of butyrate, which promotes the proliferation of regulatory T cells (Treg's), exerts anti-inflammatory effects, and inhibits the progression of osteoporosis [30,31]. Another study has found an increase in the abundance of Clostridium in PMOP subjects. Clostridium can promote AKT2 signaling to enhance the production of M1 macrophages, induce the inflammatory process, and promote the progression of osteoporosis [23,32,33].

The ecological imbalance in the GM is associated with bone density and bone metabolism markers, making dysbiosis a potential biomarker for bone metabolism activity and a therapeutic target for promoting bone homeostasis [32]. The GM and its metabolites can maintain intestinal-barrier function, participate in nutrient absorption, and regulate bone metabolism processes through the gut–bone axis and the brain–gut–bone axis (involving 5-hydroxytryptamine (5-HT)) [34,35]. They also regulate bone metabolism through T cell-related immune-mediated mechanisms [36,37].

Disruption of Th17/Treg cell balance in ovariectomy-induced osteoporosis animal model

The gastrointestinal tract is rich in various microbial communities, which communicate with immune cells, triggering metabolites or immune responses that affect the bone immune system. The interaction between the immune system and the skeletal system is very close. Abnormal or prolonged immune responses often affect bone metabolism. In bone immunology research, the balance of Treg's and inflammatory T cells (Th17) and related inflammatory factors is closely related to bone metabolism disorders. Estrogen deficiency leading to

impaired intestinal-barrier function and subsequent increase in circulating lipopolysaccharides (LPS) and $CD4^+$ T cells is considered as an important mechanism in the development of osteoporosis in postmenopausal women [38,39]. The bone immune processes mediated by Th17 and Treg's can influence bone metabolism by regulating the release of cytokines. Research on the crosstalk and interaction mechanisms between the skeletal and immune systems in bone immunology holds promise as the basis for developing new treatment strategies [40].

The ecological balance of the gut microbiome is beneficial for the balance of Th17/Treg's in the intestine and bone marrow (BM) [41]. In a mouse PMOP model, estrogen deficiency increases the permeability of the intestinal wall, expands Th17 cells, and upregulates the expression of the osteoclast factors interleukin (IL)-17, Tumour necrosis factor-α (TNF-α), and NF-κB receptor activator ligand (RANKL) in the intestinal and BM microenvironment. No increase in osteoclast factors or bone loss is observed in germ-free mice subjected to estrogen deprivation. Therefore, the process of estrogen deficiency-induced osteoporosis is mediated by the gut microbiome [42]. Meanwhile, the bone loss induced by estrogen deprivation depends on T cell-derived TNF-α. No bone loss is detected in T cell-deficient or T cell-depleted mice [43,44]. Under physiologic conditions, estrogen can affect the differentiation of T cells by acting on the estrogen receptor on the surface of T cells. After ovariectomy (OVX) for PMOP modeling, the decrease in estrogen increases the differentiation of initial $CD4^+$ T cells into Th17 cells, thereby changing the Th17/Treg ratio. The Th17/Treg balance in intestinal epithelial cells is disrupted, with an increase in Th17 cells and a decrease in Treg's. The pro-inflammatory factors secreted by Th17 cells induce the formation of osteoclast, leading to bone loss and imbalance in bone remodeling [45–47].

In PMOP, under the influence of impaired intestinal-barrier function and estrogen deficiency, the Th17/Treg cell balance in the intestine is disrupted. Th17 cells and TNF + T cells in the intestine, through S1P receptor 1-mediated (S1PR1-mediated) interaction, migrate to the BM under the action of the chemokines CXCR3 and CCL20 [48]. This leads to the disruption of the Th17/Treg balance in the BM. Blocking the migration of Th17 cells and TNF + T cells out of the intestine or into the BM can prevent OVX-induced bone loss [49].

Fecal microbiota transplantation improves bone loss

The gut microbiome has an immunomodulatory role and interacts with the gastrointestinal, immune, endocrine, and nervous systems, participating in many pathophysiological processes related to inflammatory responses [50]. FMT refers to the transfer of GM from a healthy donor into a recipient with an imbalanced GM to restructure the GM in the recipient and further prevent or treat diseases related to gut microbiome dysbiosis. FMT can improve the Th17/Treg cell balance in intestinal tissues by correcting GM imbalance [51]. FMT can improve intestinal permeability, restructure the Th17/Treg cell balance in the intestinal mucosal barrier, and inhibit the migration of Th17 cells into the BM. In turn, the release of pro-inflammatory cell factors mediated by Th17 cells in the BM is suppressed, which inhibits the excessive generation of osteoclasts. Accordingly, the balance between bone formation and resorption is restored, thereby protecting bone mass and preventing osteoporosis [23,51].

Clinical research on the role of GM in PMOP

Research on probiotic preparations has found that probiotics can improve bone loss by upregulating the expression of TJs in the intestine. The outcomes are increased strength of the intestinal epithelial layer, reduced antigen presentation, and the activation of intestinal immune cells [52].

Clinical studies on the GM in PMOP women are limited, with only a few focusing on probiotic formulations. Zhao *et al.* found that the combined supplementation of *Bifidobacterium lactis* Probio-M8 with calcium and vitamin D improves bone metabolism and affects the GM that produces SCFAs. However, no changes in bone mineral density (BMD) are observed [53]. Jafarnejad *et al.* conducted a multi-species probiotic supplementation study on PMOP women and found a reduction in bone turnover rate but no impact on BMD [54]. Another study on the effectiveness of a Lactobacillus probiotic supplement on BMD and bone metabolism in postmenopausal women is ongoing, with no results reported yet [55]. Among randomized clinical trials investigating the BMD of the lumbar spine, total hip, and/or femoral neck, only one 12-month probiotic intervention trial reports improvements in lumbar spine and femoral bone density [56].

Research on the role of probiotics in OVX-induced PMOP

Probiotic formulations are extensively studied in OVX-induced PMOP models. A summary of recent published data shows that probiotics prevent bone resorption by restoring GM diversity, improving the intestinal epithelial barrier, and regulating T cell-mediated inflammatory processes through their metabolites [37,42]. Probiotic supplements contain about 20 types of beneficial bacteria. They are typically classified into five categories, including Lactobacillus, Bifidobacterium, yeasts, and others [37], with Lactobacillus and Bifidobacterium being

the most commonly used probiotics [57].

The *Lactobacillus* genus regulates the balance of Th17/Treg's, suppresses inflammation in the gut and BM, inhibits the expression of inflammatory factors during osteoclast activation, and increases the expression of osteoprotegerin (OPG), thereby alleviating bone loss in OVX-induced PMOP mice [38,58–61]. In a PMOP rat model, the *Lactobacillus* genus has been shown to improve serum calcium, vitamin D, and alkaline phosphatase (ALP) levels, inhibit the expression of inflammatory cytokines, and alleviate bone loss [62-65]. Among them, *Lactobacillus rhamnosus* GG (LGG) can improve the Th17/Treg balance in the intestine and bone to ameliorate estrogen deficiency-induced osteoporosis by regulating the gut microbiome and intestinal barrier [66]. Lactic acid, as the primary metabolic product of LGG, acidifies the intestinal environment and thus helps inhibit the growth of pathogens and maintain GM balance [67]. Wu *et al.* found that lactic acid can increase histone lactylation in BM-derived mesenchymal stem cells (BMSC), inducing BMSC differentiation into osteoblasts and improving bone density in OVX mice [68]. Lactic acid also promotes the transforming growth factor (TGF)-β-induced differentiation of native $CD4^+$ T cells into Treg's, improving the microenvironment [69,70]. Rao *et al.* reported that lactic acid-induced Treg generation is pH dependent [71,72]. Lactic acid maintains Treg function and immune balance through its effect on mitochondrial N-glycosylation via *MGAT1* [73]. It also promotes the proliferation, differentiation, and oxidative phosphorylation of human Treg's [72,74]. Treg's utilize lactic acid from the microenvironment as an energy source to maintain their activity. The transplantation of LGG does not promote an increase in bone formation in mice depleted of Treg's by anti-CD25 antibody treatment, confirming that the key role of LGG in bone metabolism is mediated by Treg's [75]. The mechanism is that Treg's are involved in the iPTH-induced bone-formation metabolism [75,76].

Bifidobacterium genus studies in the PMOP model have found that it improves bone loss by increasing serum vitamin D levels, enhancing the immune regulation of Breg cells, inhibiting M1 macrophages, and modulating the gut immune process [77-79]. Among them, *Bifidobacterium longum* can increase the differentiation of Bregs and the expression of IL-10, as well as inhibit the differentiation of Th17 cells and the expression of IL-17. The bone-protective effect of *Bifidobacterium longum* on the Breg-Treg-Th17 cell axis may be a new therapeutic approach for PMOP [77]. Additionally, several studies on probiotic mixtures have been shown to increase serum calcium and vitamin D levels, regulate the Treg's, modulate the expression of inflammatory factors, improve gut and BM inflammation, reduce intestinal permeability, and inhibit bone loss [42,80,81].

The GM influences bone metabolism through multiple mechanisms, including the regulation of gut microecology, inflammation levels, hormone metabolism, and calcium absorption. Increasing the intake of beneficial bacteria such as Lactobacillus and Bifidobacterium helps slow the progression of osteoporosis [42,58,82]. Current research suggests that the effects of probiotics on bone health seem to depend on the specific strains. Further studies are needed to validate the impact of different strains on bone health and their mechanisms of action.

Role of intestinal metabolites

The delicate balance between the pro-inflammatory and anti-inflammatory mechanisms in the gut is crucial to intestinal microbiota. Simultaneously, it acts on the BM microenvironment through the gut–bone axis. Considering the significant metabolic functions of microbiota, research on the metabolic products of the GM has revealed various intestinal metabolites, including SCFAs, vitamins, 5-HT, bile acids, polyamines, and indole derivatives. These metabolites enter the systemic circulation through absorption by the intestinal wall, are transported into distant organs via the systemic circulation, and participate in the regulation of bone metabolism [83]. Intestinal microbiota metabolites such as saturated fatty acids, secondary bile acids, and indole derivatives provide energy to intestinal epithelial cells, promote the absorption of calcium and phosphorus, and play a role in bone metabolism and fracture healing [23,84]. Furthermore, metabolites derived from the intestinal microbiota significantly influence the host immune system. These metabolites regulate bone immune processes by modulating T-effector cell differentiation through molecular receptors on immune cells [85].

In the study of metabolites, SCFAs have been the focus of in-depth research due to their broad anti-inflammatory effects. SCFAs participate in maintaining the normal function of intestinal mucosal cells and provide energy for intestinal cells. They help maintain or reshape the balance of the intestinal flora and participate in the regulation of the activity of the intestinal immune system, playing an important role in immune-related intestinal diseases [86,87]. SCFAs have further been proven to improve joint inflammation in rheumatoid arthritis and collagen-induced arthritis in mice, and this inflammatory relief is related to the expansion of splenic Treg's and the increase of serum IL-10 [88,89]. However, research on SCFAs in PMOP is limited. Some clinical studies have confirmed that SCFAs are closely related to osteoporosis and bone metabolism. Cho *et al.* found that isovaleric acid improves OVX-induced osteoporosis by inhibiting osteoclast differentiation [90].

SCFAs provide energy for intestinal mucosal epithelial

cells, assist in the transport and absorption of calcium, and participate in the regulation of the body's inflammatory response, thereby maintaining the function of the intestinal mucosal barrier [91]. SCFAs are closely related as well to the differentiation and maturation of CD4T cells associated with bone metabolism. By participating in the Th17/Treg balance and regulating the release of inflammatory cytokines, they play important roles in bone metabolism [92].

Generation and structural introduction of short-chain fatty acids

SCFAs are metabolic products produced by the GM through the fermentation of dietary fibers, primarily including formate, acetate, propionate, and butyrate [93]. Little is known about the role of formate in the gut. Metagenomic studies on changes in gut microbiome diversity have identified GM that produce acetate, propionate, and butyrate. Among them, acetate is the most abundant SCFA in the gut and serves as an intermediate product in the fermentation process of most gut anaerobic bacteria, primarily produced by microbiota such as Bacteroides and Lactobacillus species [94,95]. The production of propionate, although distributed across multiple phyla, is dominated by relatively few bacterial genera. It is primarily produced by microbiota in the phylum Bacteroidetes and some species in the phylum Firmicutes, such as *Prevotella copri* and *Roseburia inulinivorans* [96,97]. Butyrate is primarily produced by microbiota in the phylum Firmicutes such as *Eubacterium rectale*, *Eubacterium hallii*, and *Coprococcus eutactus* through either the butyrate kinase route or the acetate-CoA transferase route [93,97–99]. The breakdown of amino acids and lactate also contributes to SCFA production, primarily C2 and C3 [100,101]. Essential amino acids like leucine, after microbial fermentation, can generate branched-chain fatty acids (BCFAs) such as isobutyrate with four carbons or 2-methylbutyrate and isovalerate with five carbons, as depicted in Fig. 1 [102]. Research on the production and effects of other types of SCFAs is relatively scarce, with insufficient literature detailing their metabolic processes. The cooperation and interactions between different microbiota influence the total amount and relative proportions of SCFAs. The intake and types of fiber directly affect the activity of the GM and the production of SCFAs.

SCFAs refer to volatile fatty acids containing six or fewer carbons, which can form salt structures with metals or organic groups (C1, formate; C2, acetate; C3, propionate; C4, butyrate; C5, valerate; C6, caproate) or branched-chain structures (C4, isobutyrate; C5, isovalerate and 2-methylbutyrate). Among them, acetate (C2), propionate (C3), and butyrate (C4) account for 90%–95% of the total intestinal SCFA content [103–105]. C2, C3, and C4 are the most common representative SCFAs, and they play a role in bone metabolism. Their molecular structures are shown in Fig. 1 [106,107].

SCFAs serve as an important link between the GM and host homeostasis, playing a crucial role in energy metabolism, inflammatory regulation, and glucose and lipid metabolism [108]. Colon cells primarily absorb SCFAs through H-dependent or sodium-dependent monocarboxylic acid transport proteins, serving as an important energy source for the microbiota itself and intestinal cells [109]. SCFAs then circulate through the bloodstream to various parts of the body, acting as substrates to promote sugar or lipid synthesis [110]. They can inhibit the growth of intestinal pathogens by lowering the pH. C2 provides a carbon source for lipid synthesis,

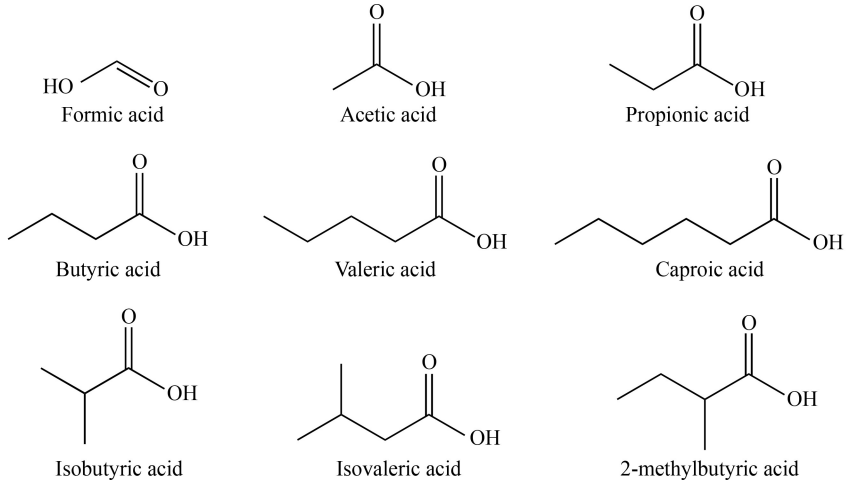

Fig. 1 SCFAs and their branched-chain structures molecular formulas. In general, the molecular formula for straight-chain SCFAs is $CH3(CH2)_{n-1}COOH$, whereas the branched-chain SCFAs contain one or more methyl (CH3) branches.

C3 serves as an energy source for colonocytes and stem cells, and C4 is the primary energy source for colonic epithelial cells [111,112]. SCFAs can enter the systemic circulation through the gut–blood barrier and exert their biological effects by acting on SCFA receptors, modulating the host's metabolism and inflammatory responses [113]. SCFAs primarily act on G-protein-coupled receptors (GPCRs). The GPR43 receptor is widely expressed on intestinal epithelial cells, enteroendocrine cells, adipocytes, and various immune cells. The GPR41 receptor is primarily expressed on neurons with a vasoconstrictor phenotype, reducing airway inflammation and inducing vasodilation. The GPR109a receptor is expressed in the intestine, some immune cells, and adipocytes. C2, C3, and C4 can act on the G-protein-coupled receptors GPR43 (also known as free fatty acid receptor 2, *FFAR2*) and GPR41 (also known as free fatty acid receptor 3, *FFAR3*); conversely, C4 can act on the G-protein-coupled receptor GPR109a (also known as *HCA2*) [114,115]. After binding to the cell surface GPR receptors, SCFAs activate downstream signaling pathways such as ERK/MAPK, Akt/PI3K, JNK, and p38, which are involved in the regulation of cell differentiation and proliferation [116].

Role and mechanism of SCFAs

SCFAs promote intestinal calcium absorption

SCFAs can promote intestinal calcium absorption to regulate bone metabolism. Calcium absorption occurs through an active transcellular pathway (ion pump) or a passive paracellular diffusion (ion channel) depending on the level of 1,25-$(OH)_2$D (1,25-dihydroxy vitamin D) [117]. Previous studies have found that C2 can prevent osteoporosis by increasing intestinal calcium absorption and the concentration of calcium in the skeleton [118]. Another study has also found that C2 and C3 can enhance the absorption of calcium in the human distal colon [119]. Calcium intake is closely related to the accumulation of bone minerals, and adequate calcium intake can prevent osteoporosis and related fracture risks [120]. The mechanisms by which SCFAs increase intestinal calcium absorption include increasing the depth of the intestinal crypt and the absorption area, reducing the pH of the intestine, increasing the solubility of Ca^{2+}, and promoting the H^+/Ca^{2+} exchange to promote calcium absorption [91,121]. SCFAs can also promote calcium absorption by increasing the function of intestinal epithelial cells Calbindin D9k, transient receptor potential vanilloid receptor 6, and Vitamin D receptor [37,122].

SCFAs regulate the inflammatory process

SCFAs, acting as signaling molecules, can bind and activate GPCRs or serve as histone deacetylase (HDAC) inhibitors. They can modulate the LPS-induced inflammatory process through GPCRs and HDAC inhibition. The anti-inflammatory effects of SCFAs can improve intestinal inflammation, joint inflammation, and bone metabolism. The immune system cells can sense SCFAs and adjust the balance between inflammatory and anti-inflammatory cells [31,123]. SCFAs can inhibit the NF-κB signaling pathway by inhibiting HDAC, thereby suppressing the secretion of the inflammatory cytokine TNF-α and exerting strong anti-inflammatory effects. C3 and C4 can upregulate the expression of intestinal TJs, block the invasion of harmful substances through the intestinal mucosa, alleviate mucosal inflammatory reactions, and effectively control intestinal inflammation [93,124]. C3 and C4 can inhibit the secretion of TNF-α and the activity of NF-κB. They can inhibit HDAC activity, activate the p38 MAPK pathway, promote the secretion of IL10 to maintain immune homeostasis, and improve intestinal inflammation and joint inflammation in mice [125]. The anti-inflammatory effects of SCFAs can improve estrogen deficiency-induced bone loss in rodents and ameliorate joint inflammation mediated by osteoclasts [42,126,127].

SCFAs selectively activate GPCR receptors, where C3 can activate GPR41 and partially GPR43, whereas C4 primarily binds and activates GPR109A and partially GPR41 [128]. Wu *et al.* found that C3 and C4 inhibit CoCrMo alloy particle-induced NLRP3 inflammasome activation, inhibiting ASC oligomerization, speck formation, and assembly in BM-derived macrophages to alleviate inflammatory bone resorption. They also found that the action of C3 does not depend on GPCR receptors or HDAC inhibitors, whereas the inhibitory effect of C4 on NLRP3 inflammasome is GPR109A receptor dependent [129]. SCFAs can directly act on osteoblasts, osteoclasts, chondrocytes, and fibroblasts involved in the bone healing or indirectly act on key cells involved in anti-inflammatory and immune regulatory responses, promoting fracture healing [130,131].

SCFAs regulate bone metabolism through the endocrine system

SCFAs promote the production of insulin-like growth factor (IGF)-1 in the liver and adipocytes, increasing serum IGF-1 levels. IGF-1, as an autocrine or paracrine growth factor, promotes the proliferation and differentiation of osteoblasts by regulating the GH/IGF axis, thereby stimulating endochondral ossification to promote longitudinal bone growth [132,133]. Information on the effects of IGF-1 on osteoclasts is limited, with studies indicating that IGF-1 binds to IGF-I receptors on the surface of osteoblasts and osteoclast precursor cells, interacting with one another. It regulates the RANKL/

RANK and M-CSF/C-FMS signaling pathways, stimulating osteoclast formation through its effects on osteoblasts/BM stromal cells [134].

SCFAs promote adipocytes to secrete leptin, a secreted protein that can reduce the number of osteoclasts in OVX-induced osteoporosis rats [135,136]. They promote the differentiation of BM mesenchymal stem cells into osteoblasts, enhance the proliferation, differentiation, and mineralization of osteoblasts, and improve the microstructure of trabecular bone, promoting bone growth [137,138]. SCFAs also bind to the surface receptors GPR43 (*FFAR2*) and GPR41 (*FFAR3*) on L cells, promoting the secretion of glucagon-like peptide-1 (GLP-1) and the neuropeptide Y family (PYY) [139]. GLP-1 enhances the viability of osteoblasts and inhibits osteoclastogenesis by suppressing the NF-κB and MAPK signaling pathways, ultimately inhibiting the expression of nuclear factor of activated T cells (NFATc1) [139 – 141]. PYY binds to the Y1R and Y2R receptors and is considered a negative regulator of bone metabolism, although its mechanism remains unclear [142,143].

Furthermore, Reigstad *et al.* found that the SCFAs produced in the intestinal lumen, such as acetate and butyrate, can increase the expression of the rate-limiting enzyme tryptophan hydroxylase mRNA and the synthesis of 5-HT in enterochromaffin cells [144]. Another study has found that SCFAs are negatively correlated with 5-HT receptors, suggesting that GM may downregulate the expression of 5-HT receptors through SCFAs [145]. The serum neurotransmitter 5-HT, a circulating serotonin with hormone-like effects, can stimulate or inhibit bone formation, exhibiting a bidirectional regulatory function. The neurotransmitter signaling system (such as 5-HT) plays an important regulatory role in bone development and maintenance [36]. 5-HT includes central and peripheral types. Peripheral 5-HT produced in the gut inhibits bone formation. Conversely, when synthesized in the brain as a neurotransmitter, it promotes bone development [146].

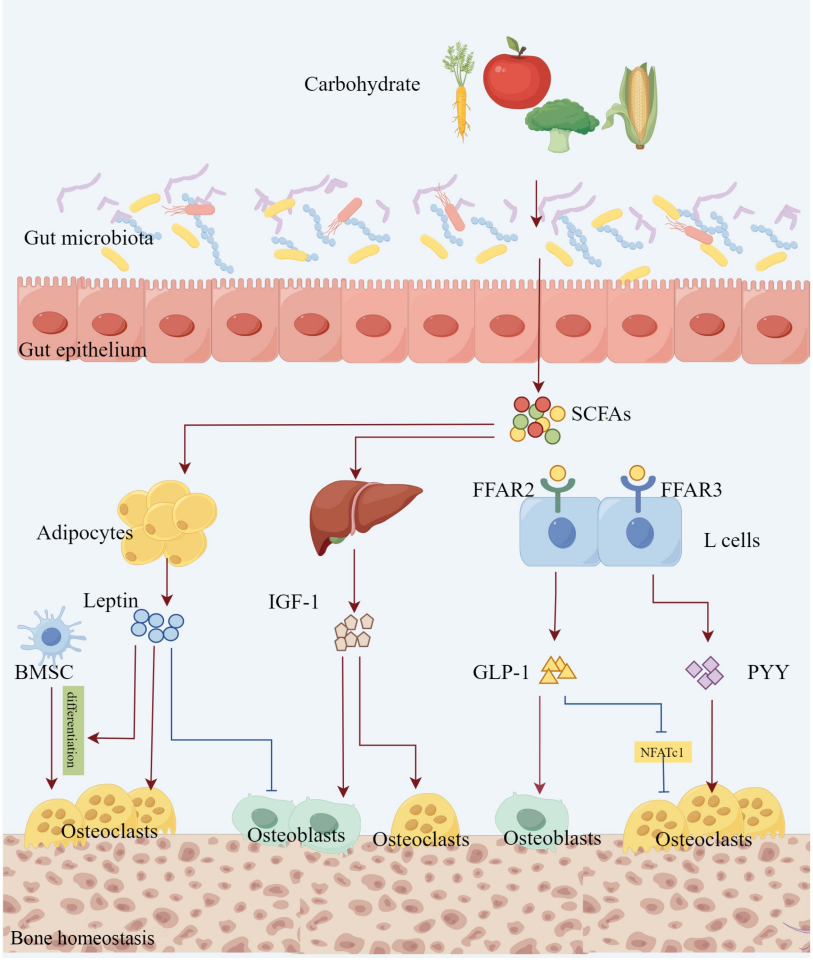

Fig. 2 SCFAs promote the production of IGF-1 in the liver and adipocytes, enhancing the proliferation and differentiation of osteoblasts, and stimulating endochondral ossification to promote longitudinal bone growth. SCFAs, by acting on the GPR43 (FFAR2) and GPR41 (FFAR3) receptors on the surface of L cells, promote the production of GLP-1 and PYY, influencing osteoblast and osteoclast activity.

SCFAs regulate the Th17/Treg cell-mediated immune process to modulate bone metabolism

SCFAs are key regulatory factors in the inflammatory reactions in the intestinal and BM microenvironment, which are crucial to the processes of bone resorption and bone formation [147]. Supplementation with SCFAs or a high-fiber diet can significantly increase bone mass and prevent bone loss in OVX mice [107]. These SCFAs exert their effects on the inflammatory process primarily through the balance between Th17 and Treg's.

T cells can differentiate into CD4 T cells and CD8 T cells. Naive CD4 T cells can be induced to differentiate into Th17 cells or Treg's. Initial $CD4^+$ T cells are induced by TGF-β to differentiate into Treg's. Through the combined action of IL-6, IL-21, and TGF-β, they are induced to differentiate into Th17 cells. IL-6 suppresses the expression of *Foxp3* by activating signal transducer and activator of transcription 3 (STAT3), thereby inhibiting the differentiation of Treg's [148,149]. The Th17/Treg balance plays an important role in maintaining bone homeostasis [47,150]. SCFAs have immunomodulatory effects, promoting the differentiation of naive CD4 T cells into Treg's and inhibiting the differentiation of Th17 cells, thereby suppressing inflammation and treating immune-related diseases [112,151]. Th17 cells expressing the transcription factor retinoic-acid receptor-related orphan γt are considered pro-inflammatory cells [149]. The inflammatory cytokines (such as IL-6, IL-17, and TNF-α) produced by Th17 cells can increase the expression of RANKL on osteoblasts and fibroblasts [152,153]. The receptor activator of RANKL is an important factor linking the skeleton and the immune system. The stimulation of RANKL activates the downstream signaling pathways of RANK, inducing the maturation of osteoclasts to promote bone resorption [154]. TH17 TNF-α + T cells secrete chemokines, thereby increasing the recruitment of monocytes into the BM and promoting the differentiation of osteoclast precursor cells into osteoclasts. The outcome is more significant bone resorption [155].

SCFAs influence DNA methylation to induce Treg cell differentiation through the GPR43/GPR109A receptors. Additionally, SCFAs are natural inhibitors of HDACs. Butyrate induces the differentiation of naive T lymphocytes into Treg's by inhibiting HDACs and increasing the acetylation of histone H3 at the *FOXP3* promoter [92,112,123]. Butyrate acts on the GPR43 receptor on dendritic cells and promotes Treg cell differentiation through GPR43 signaling on $CD4^+$ T cells [156]. Treg's express the transcription factor *Foxp3* and can be divided into thymus-derived natural Treg's (nTreg's) and induced Treg's (iTreg's), which are generated from naive $CD4^+$ T cells in the periphery [157]. nTreg's primarily induce osteoclast apoptosis through a contact-dependent mechanism involving cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and CD80/86-dependent pathways. CTLA-4 increases the expression of IκB kinase and NF-κB-inducing kinase in osteoclast precursors, leading to the activation of the NF-κB pathway and indoleamine 2,3-dioxygenase (IDO). Inducing IDO in osteoclast progenitor cells enhances the apoptosis of osteoclast precursor cells [158–159]. The co-stimulation of TGF-β and IL-2 drives the development of iTreg's in peripheral lymphoid tissues [160].

Hao *et al.* found that butyrate promotes the conversion of acetyl-CoA synthetase short-chain family member 2 into butyryl-CoA (BCoA), inhibiting the binding of malonyl-CoA (MCoA) to upregulate *CPT1A* activity, thereby promoting fatty acid oxidation and the differentiation of iTreg's through the butyrate-BCoA-CPT1A axis [161]. iTreg's secrete immunosuppressive cytokines such as granulocyte-macrophage colony-stimulating factor (CSF), IL-4, IL-5, IL-10, and TGF-β. The main cytokines secreted are IL-4, IL-10, and TGF-β, which are involved in inhibiting osteoclast generation [159]. Luo CY *et al.* found that $CD4^+CD25^+Foxp3^+$ Treg's can suppress osteoclast differentiation and bone resorption by secreting IL-10 and TGF-β1. 17β-estradiol (E2) can enhance the inhibitory effects of these cells on osteoclast differentiation and bone resorption by stimulating Treg's to secrete IL-10 and TGF-β1. IL-10 and TGF-β1 may be involved in the regulation of bone metabolism by E2 and are potential therapeutic targets for PMOP treatment [162]. They can also suppress osteoclast differentiation *in vitro* [155,163,164]. IL-10 inhibits osteoclast differentiation and maturation by upregulating OPG secretion. TGF-β activates intracellular effectors (such as MAPK and SMAD-related proteins) to induce the differentiation of mesenchymal stem cells into osteoblasts, promoting osteogenesis [47,165,166].

SCFAs participate in bone synthesis metabolism induced by intermittent PTH through Treg's.

The process of PTH stimulating bone formation and increasing bone mass is microbiota dependent [167]. SCFAs in intestinal metabolites, particularly butyrate, are involved in the Treg/Wnt10b/Wnt signaling pathway mediated by iPTH, stimulating bone formation and inducing bone synthesis metabolism [48,156]. iPTH requires butyrate to increase the number of BM Treg's. PTH also synergizes with butyrate by acting on PTH1R on $CD4^+$ T cells, promoting the differentiation of $CD4^+$ T cells into Treg's [156]. The outcome is a two- to three-fold increase in the number of Treg's in the BM. Blocking the increase of Treg's in mice can prevent iPTH-induced bone formation and increase the bone trabeculae. The increase in the number of Treg's mediated by butyrate is a key mechanism for iPTH to

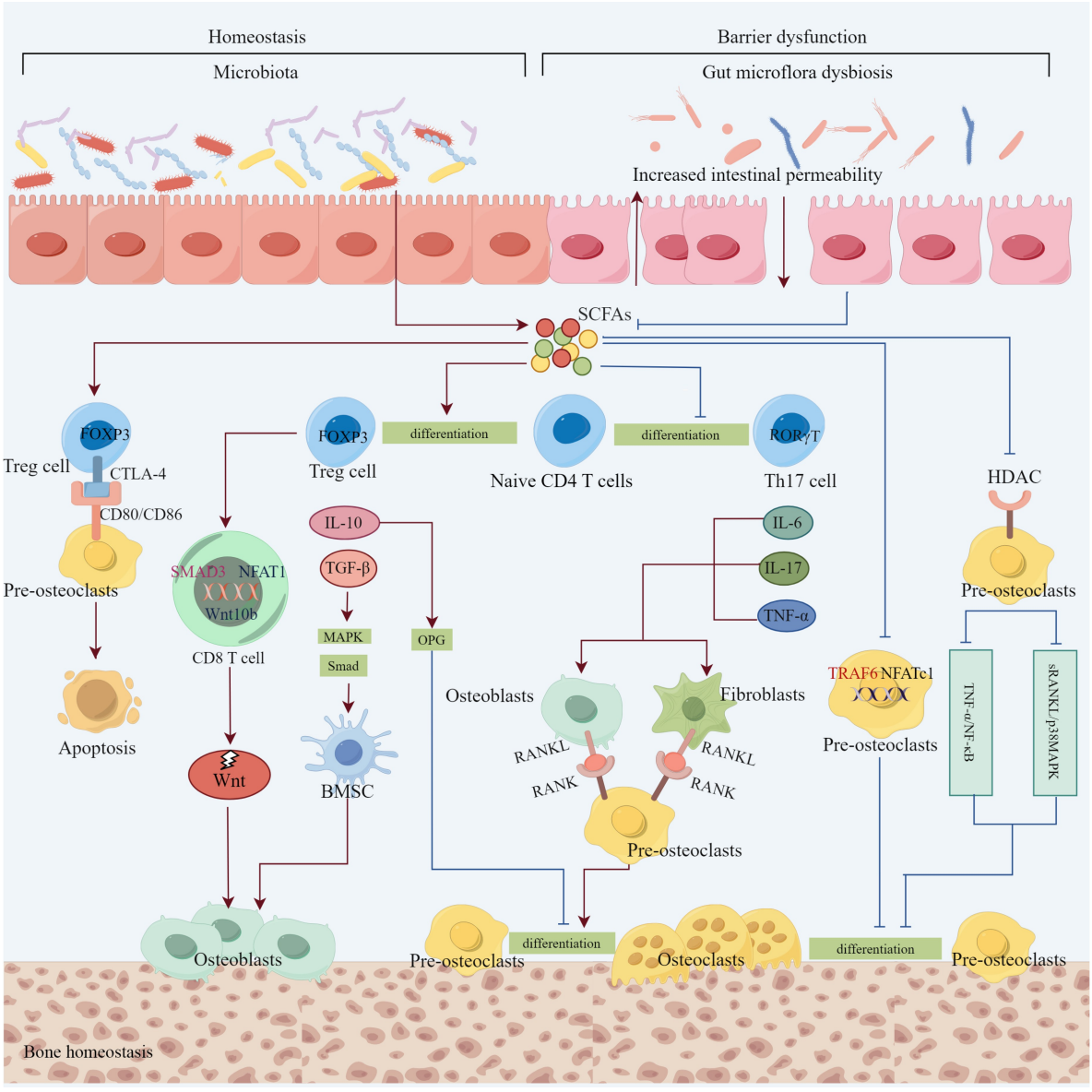

Fig. 3 After PMOP modeling, estrogen deficiency leads to GM imbalance, impaired intestinal-barrier function, increased intestinal permeability, and reduced synthesis of short-chain fatty acids (SCFAs). SCFAs can promote the differentiation of $CD4^+$ T cells into regulatory T (Treg) cells and inhibit the differentiation of Th17 cells. Treg's induce osteoclast apoptosis through a contact-dependent mechanism involving cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). They promote osteoblast differentiation and inhibit osteoclast differentiation by producing IL-10 and TGF-β. Th17 cells produce cytokines such as IL-6, IL-17, and TNF-α, which promote osteoclast differentiation. Butyrate is involved in the iPTH-mediated stimulation of the Treg/Wnt10b/Wnt signaling pathway, promoting bone formation and inducing anabolic bone metabolism. By inhibiting HDAC, it suppresses TNF-α-induced NF-κB nuclear translocation and sRANKL-induced p38 MAPK signaling activation, thereby inhibiting osteoclastogenesis. Treg: regulatory T cell, SCFAs: short-chain fatty acids.

exert its bone-synthesis metabolic activity [168]. Butyrate primarily induces Treg differentiation through the intrinsic epigenetic regulation of the Foxp3 gene in T cells [92]. It also regulates Treg cell differentiation and function by acting on the cell surface FFAR2 receptor [123]. Treg's promote the assembly of the NFAT1-SMAD3 transcription complex in $CD8^+$ T cells. NFAT1-SMAD3 drives the expression of the Wnt pathway ligand *Wnt10b*, thereby regulating bone synthesis metabolism and activating Wnt-dependent bone formation [75,169].

Role of SCFAs in osteoblasts

SCFAs can directly act on osteogenic differentiation. In rat osteoblast-like ROS17/2.8 cells, butyrate as an effective inhibitor of HDAC acts on the fibroblast growth factor 2 response element of the bone sialoprotein (BSP) gene promoter, increasing the transcription of the BSP gene. BSP plays a role in the initial mineralization of bone, and it may promote osteoblast differentiation and bone-matrix mineralization [170]. Studies on osteoblasts

from normal populations have found that butyrate increases the formation and calcium content of mineralized nodules in a dose-dependent manner. However, it has no significant effect on osteoblast proliferation and ALP activity. It can increase the gene and protein expression of BSP, osteopontin (OPN), and OPG, with no significant effect on the expression levels of type I collagen and M-CSF. Butyrate-stimulated osteoblast-derived OPG can inhibit osteoclast differentiation [171]. Studies on MC3T3-E1 cells have shown that C2 and C3 can upregulate ALP activity in MC3T3-E1 cells, where C2 can upregulate ALP mRNA expression, promoting the differentiation of primary osteoblasts and maintaining the balance of bone turnover. C2, C3, and C4 can increase the expression of OPN in MC3T3-E1 cells, promoting osteogenic differentiation [172]. C4 has no significant effect on the proliferation of ROS17/2.8 cells. However, it can promote the expression of extracellular matrix proteins (such as type I collagen and OPN) in ROS17/2.8 cells, which is associated with the induction of prostaglandin receptor expression by butyrate [173]. In studies on ROS17/2.8 cells, high concentrations of sodium butyrate inhibit their osteoblast differentiation and bone mineralization [174]. The regulation of butyrate on the osteoblast differentiation process is hypothesized to be concentration dependent, with low concentrations exerting a promoting effect and high concentrations exerting an inhibitory effect.

Role of SCFAs in osteoclasts

SCFAs inhibit osteoclast differentiation *in vitro*. Isovaleric acid (IVA), also known as 3-methylbutyric acid, is a 5-carbon BCFA. IVA can inhibit the differentiation of BM-derived macrophages into osteoclasts by inhibiting RANKL[90]. C3 and C4 induce metabolic reprogramming in osteoclasts, shifting their metabolism from oxidative phosphorylation to glycolysis at early stages of osteoclast differentiation, causing cellular stress and preventing osteoclast differentiation. They significantly inhibit the expression of two essential osteoclast signaling genes, *TRAF6* and *NFATc1*, at the early time points after RANKL stimulation [107]. *In vitro* studies have found that C3 and C4 inhibit osteoclast differentiation in a dose- and time-dependent manner, reducing osteoclast formation and bone resorption [175]. C3 and C4 can inhibit IL-1β-promoted osteoclast differentiation, reducing the expression of osteoclast differentiation-related proteins such as TRAF2, TRAF6, NFATc-1, and c-Fos. Further verification using HDAC inhibitors (TSA, Panobinostat) and GPR41, GPR43, and GPR109A agonists (AR420626, 4-CMTB, niacin) found that the inhibitory effect of C3 and C4 on osteoclast differentiation depends on HDAC inhibition rather than GPCR activation [129]. Other studies have also confirmed that C4 inhibits osteoclast-specific signaling pathways by inhibiting the activity of HDACs, thereby directly inhibiting bone resorption [176,177].

However, Montalvany-Antonucci CC's study on the impact of the SCFA/*FFAR2* axis on alveolar bone has found that the pretreatment of osteoclasts with histone deacetylase inhibitors does not alter the inhibitory effect of SCFAs on osteoclasts. BM cells from *FFAR2*-deficient mice (*FFAR2*$^{-/-}$) show a differentiation process toward osteoclasts. The effects of SCFAs on osteoclasts depend on *FFAR2* activation and are independent of HDAC inhibition [178].

Butyrate intervention in OVX mice significantly reduces the $CD5^{-}CD19^{+}B220^{+}$ cells in the BM, inhibiting the expression of RANKL in B lymphocytes and RANK on the surface of osteoclasts. C4 intervention in RAW264.7 cells significantly inhibits the expression of the osteoclastogenesis-related genes *CTSK*, *Acp5*, and *c-Fos*, as well as the expression of *F-actin*, *MMP9*, and *NFATc1* [179]. Wallimann *et al.* found that butyrate promotes the fracture-healing process, significantly reducing the formation and resorption activity of osteoclasts in a dose-dependent manner. Calcium deposition in mesenchymal stromal cell cultures also increases [130]. In the RAW264.7 cell line, butyrate inhibits osteoclast differentiation and bone resorption by inhibiting TNF-α-induced RANKL nuclear translocation and the sRANKL-induced p38 mitogen-activated protein kinase signaling pathway [19,180].

The inhibitory effect of SCFAs on osteoclastogenesis is the result of a multi-pathway combined action, including GPR signaling, HDAC inhibition, immune-related signaling, and alterations in cellular metabolism.

Interactions between SCFAs and pharmacological or nonpharmacological treatment

SCFAs regulate gut health, improve immune system function, and modulate inflammatory responses, thereby influencing the bone-remodeling process. SCFAs regulate calcium absorption in the gut, inhibit osteoclast differentiation, and promote osteoblast activity to improve bone health [181]. Research on the interaction between SCFAs and anti-osteoporosis drugs (such as estrogens, selective estrogen receptor modulators, bisphosphonates, denosumab, teriparatide) is limited. Some studies have confirmed that butyrate plays an important role in the bone anabolic metabolism process induced by iPTH [48,156]. SCFAs may promote the absorption of calcium in the gut, improve intestinal permeability, and regulate inflammatory processes. Thus, they enhance the absorption of anti-osteoporosis drugs and play a synergistic role in bone formation and osteoclast inhibition. Additionally, several studies on the interaction between SCFAs, dietary intake, and probiotics

Table 1 Effects and mechanisms of SCFAs in bone metabolism

SCFAs	Bone cell types	Receptors	Effects on bone cells		Effects on bone		Reference
			Poliferation	Differentiation	Bone formation parameters	Bone resorption parameters	
C2	MC3T3-E1	/	/	Promotes osteogenic differentiation	Promotes osteopontin expression and alkaline phosphatase activity	/	[172]
C3	MC3T3-E1	/	/	Promotes osteogenic differentiation	Promotes osteopontin expression and alkaline phosphatase activity	/	[172]
C3	Osteoclast precursors	/	/	Inhibits osteoclast differentiation	/	Shifts their metabolism from oxidative phosphorylation to glycolysis, inhibiting *TRAF6* and *NFATc1* signaling	[107]
C3	Osteoclast stimulated by IL-1β	HDAC	/	Inhibits osteoclast differentiation	/	Inhibits the expression of *TRAF2*, *TRAF6*, *NFATc-1*, and *c-Fos*	[129]
C4	MC3T3-E1	/	/	Promotes osteogenic differentiation	Promotes the expression of type I collagen and osteopontin	/	[172]
C4	ROS17/2.8	/	/	Promotes osteoblast differentiation and bone matrix mineralization	Promotes BSP gene transcription and extracellular matrix proteins expression	/	[170,173]
C4	Human osteoblasts	/	/	Increases the formation of osteoblast, inhibits osteoclast differentiation	Increases the gene and protein expression of OPN, OPG, and BSP	Stimulates the expression of OPG	[171]
C4	Osteoclast precursors	/	/	Inhibits osteoclast differentiation	/	Inhibiting *TRAF6* and *NFATc1* signaling	[107]
C4	Osteoclast stimulated by IL-1β	HDAC	/	Inhibits osteoclast differentiation	/	Inhibits the expression of *TRAF2*, *TRAF6*, *NFATc-1*, and *c-Fos*	[129]
C4	Bone marrow-derived monocyte/macrophages (BMMs)	HDAC	/	Inhibits osteoclast differentiation	/	Inhibits the expression of carbonic *anhydrase II*, *TRAP*, *integrin β3*, *DC-stamp*, *MMP-9*, and calcitonin receptor	[177]
C4	Bone marrow cells from *FFAR2*$^{-/-}$ mice	FFAR2	/	Inhibits osteoclast differentiation	/	Activates *FFAR2*	[178]
C4	RAW264.7	/	/	Inhibits osteoclast differentiation	/	Inhibits the expression of *CTSK*, *Acp5*, and *c-Fos*, *F-actin*, *MMP9*, and *NFATc1*	[179]
IVA	Mouse bone marrow-derived macrophages	Gi-coupled GPCRs	/	Inhibits osteoclast differentiation	/	Stimulates AMPK phosphorylation, inhibits *RANKL*, *RANK*, *TRAF6*, *NFATc1*, and *c-fos*, and downregulates the expression of *OC-STAMP*, *DC-STAMP*, and *Atp6v0d2*	[90]

have found that increasing the intake of dietary fiber, probiotics (such as lactobacilli and bifidobacteria), and prebiotics can increase SCFA production, collectively improving bone metabolism [182–184].

Research on the interaction between SCFAs and osteoporosis treatment is ongoing. Future studies may further reveal how SCFAs can be combined with pharmacological and nonpharmacological interventions, providing new perspectives on comprehensive osteoporosis treatment and approaches to it.

Current research challenges and future directions

An increasing number of basic studies have found that SCFAs play an important role in the pathophysiology of

osteoporosis by optimizing mitochondrial function [185–187]. Butyrate upregulates the melatonin pathway in intestinal epithelial cells, and melatonin is an inhibitor of osteoporosis [188,189]. Another major trigger of osteoporosis is the activation of glucocorticoid receptors (GRs). Butyrate and melatonin can inhibit the nuclear translocation of GR-alpha, thereby suppressing the progression of osteoporosis [190,191]. Changes in melatonin produced by the pineal gland at night, SCFAs generated by the gut microbiome, and adrenal cortisol levels may significantly impact osteoporosis development [192]. This reveals the role of aging in osteoporosis. Future research will further explore the role of the GM-SCFA axis in age-related diseases.

Emerging evidence supports the role of SCFAs as key mediators of cellular function in gut and bone metabolism, highlighting their importance in the diet–gut microbiome–bone metabolism axis. However, can SCFAs be considered the critical molecular link between the gut microbiome and bone health?

The production and effects of SCFAs are influenced by various factors, and the specific molecular mechanisms through which they affect bone metabolism have not been fully elucidated. The optimal concentration range and dose–response relationship in the body remain unclear. Current research primarily focuses on animal and cell experiments, with a lack of large-scale human clinical trial data to support the findings. The long-term impact of SCFA supplementation on bone health in the PMOP population is not yet clear. Its safety and effectiveness require long-term follow-up studies. Evidence that can be used to formulate appropriate, evidence-based clinical or public health interventions using SCFA preparations and to clearly define outcomes are currently insufficient.

Large-scale, long-term follow-up human clinical trials are needed to assess the safety and effectiveness of SCFAs in the treatment of PMOP. With the development of multiomic integration analysis techniques, the effects of SCFAs can be comprehensively evaluated by combining GM genomics, metabolomics, and transcriptomics. Based on the characteristics of the GM and metabolite profiles, individualized SCFA intervention plans should be developed. Further research should explore the synergistic effects of SCFAs with existing osteoporosis treatments to develop new combined therapeutic strategies.

Research on SCFAs holds broad application potential. Properly regulating their ratio to improve gut–bone health is a relatively simple and cost-effective intervention.

Conclusions

The influence of GM and its metabolites SCFAs on bone metabolism is evident. The regulatory effect of probiotics and probiotic preparations on bone metabolism is primarily through their metabolites. As the main metabolite of GM, SCFAs play wide-ranging anti-inflammatory roles in immune-system diseases such as intestinal inflammation and arthritis. SCFAs reportedly play an important role in the bone metabolism through the gut–bone axis and gut–brain-bone axis. SCFAs, especially propionate and butyrate, can regulate bone metabolism by modulating intestinal calcium absorption, regulating endocrine processes, and directly affecting the cells involved in bone metabolism (such as osteoblasts and osteoclasts). They can also indirectly affect bone metabolism through the Th17/Treg immune-regulation response and the production of anti-inflammatory cytokines. Supplementation of SCFAs can reshape the balance of the gut microbiome and improve the balance of Th17/Treg's in the intestine, spleen, and BM.

Considering the close relationship and plasticity between the gut microbiome, gut metabolites, Th17/Treg's, and bone metabolism, we believe that in-depth research on the mechanism of action of SCFAs on osteoporosis and the factors regulating the Th17/Treg cell balance help further identify new drug targets for osteoporosis, which are crucial to maintaining human health. Regulating this balance also profoundly affects the treatment of chronic inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis.

However, most current studies have been conducted in animal models. More high-quality clinical studies are needed in the future to further explore the efficacy and safety of GM and its metabolites SCFAs in osteoporosis treatment.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 82273294) and the 1.3.5 project for discipline of excellence, West China Hospital, Sichuan University (No. 2020HXFH008).

Compliance with ethics guidelines

Conflicts of interest Yao Chen, Ying Xie, and Xijie Yu declare that they have no conflict of interest.

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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