

REVIEW

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The interplay of estrogen, gut microbiome, and bone immunity in osteoporosis

Maohua Chen^{1†}, Jiaxu Wang^{2†}, Yuanhang Yang^{3†}, Yingge He^{4*} and Liqi Li^{5*}

Abstract

Osteoporosis is a global health concern arising from estrogen deficiency, intestinal dysbiosis, and dysregulation of the bone immune system. The absence of estrogen after menopause created environmental changes within the gut, where the microbial growth of the body was disturbed by the endocrine hormone, and inflammation increases due to the gut-bone axis, which leads to heightened bone loss. The gut microbiota, composed of bacteria and their metabolites such as short-chain fatty acids, play a crucial role in bone immune regulation by modulating immune cell function, influencing bone cell activity, and mediating epigenetic modifications. Malfunction of this system, characterized by the activation of immune cells and the imbalance of cytokines, also leads to the development of osteoporosis. Treatment strategies involved the regulation of the dysbiosis of the gut microbiota, reposition estrogen to the body, and immune modulation. The future direction should be the clarification of these interactions between estrogen and intestinal microflora, identification of new therapeutic targets, and personalization of osteoporosis management.

Keywords Estrogen, Gut microbiota, Osteoporosis, Osteoimmunity, Therapeutic strategies

Graphical abstract

The gut microbiota has a significant hand in the development of osteoporosis. They affect bone density and bone health in different ways by immune modulation, which is the production of metabolites and hormonal regulation. Dietary changes, consumption of probiotics, and prebiotics, among others, maintain healthy gut microbiota, which will become part of the fight against osteoporosis.

Introduction

Bone health has been increasingly gaining attention with age, mainly due to its negative effects on the resistance of bones to fractures and in general [1, 2]. This mechanism was important for women in the period after menopause who experienced a significant decrease in estrogen levels [3–5]. Estrogen is a critical hormone that offers protection to the bones by influencing the activities of those cells and evaluating the necessary chemicals and growth factors for the bone strength and calcium reserve [6, 7]. During menopause, the tremendous reduction in estrogen level disturbs the balance of bone cell activity and function, leading to bone resorption and increased

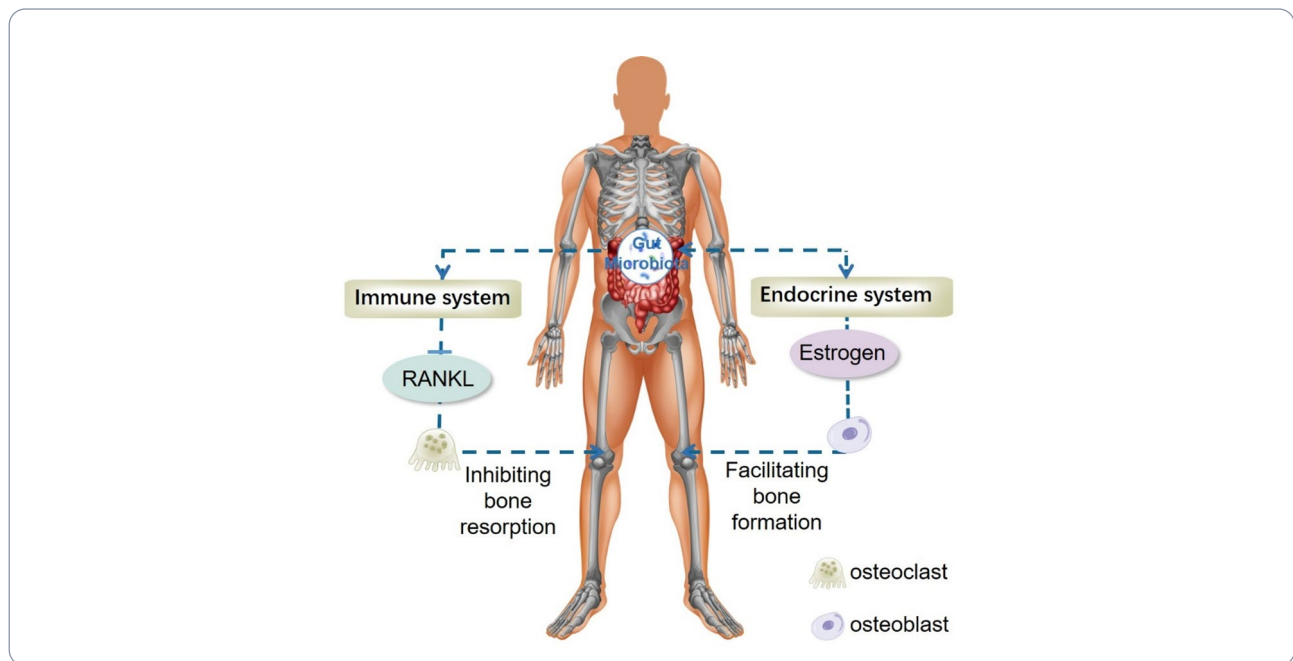
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formation of bones [8]. To simplify, the multi-factorial elements of estrogen deficiency are interrelated, and they can be characterized as a phenomenon across different bodily networks. In detail, low estrogen levels stimulate expression of inflammatory cytokines comprising IL-6 and TNF alpha, which act on osteoclast progenitors, promote osteoclastogenesis, and enhance osteoclast activity, causing accelerated bone turnover and eventual bone loss [9–11]. Moreover, this lack of hormone also compromises the role of osteoblasts leading to low production of bone and brittle bone structure [12]. Studies have shown that estrogen deficiency affects gut microbiota, as observed in postmenopausal women or ovariectomized (OVX) rat models [13, 14]. Notable changes in the diversity and functionality of gut microbes have been documented in these cases. Through multiple mechanisms, it potentially affects the intestinal barrier mechanisms to indirectly impact microbial populations and by regulating local immune responses through estrogen receptors present in intestinal epithelial cells and immune cells [15–18]. This hormone plays a role in shaping the gut's ecological environment. For instance, studies in mice have shown that when estrogen levels decline, beneficial strains, such as *Lactobacillus* and *Bifidobacteria* decrease, while potentially harmful bacteria proliferate [19]. This shift in the gut ecosystem can exacerbate inflammation, impair nutrient absorption, and disrupt the natural bone metabolism balance, potentially triggering the progression of osteoporosis [17, 20, 21].

Over the last few years, there has been a growing interest in investigating the gut microbiome and how it affects various aspects of human health and well-being [22, 23].

This heterogeneous collection of organisms, which is hosted in the digestive tract, collaborated with the host to achieve gut microbiome homeostasis, regulate a number of physiological functions, and influence nutrient metabolism [24, 25]. Not only do these microorganisms have the ability to produce compounds like short-chain fatty acids (SCFAs) [26], but they also secrete other metabolites. From the findings of the previous studies, these metabolites have been shown to improve bone strength through facilitating calcium absorption, stimulating osteoblast proliferation, and inhibiting the formation of osteoclasts [27, 28]. Recent studies have also highlighted the estrogen and gut bacteria connection, which contributes to maintenance of health by prevention of osteoporosis [14, 29, 30]. Such a complicated relationship was both for the composition of the gut microbiota and importantly also for the period of the body's metabolism and signaling. Homeostatic balance may be promoted or impeded by interactions of gut bacteria and the host, which are of importance for regeneration processes [31, 32]. When the immune system is impaired, bone loss may be evident or the formation of new bone tissue may be inhibited. Gut microbiota exert a profound influence on systemic responses by producing specific metabolites and regulating immune cell activation and specialization. This factsheet also concentrates on the role of the gut microbiome in the process of osteoporosis associated with estrogen deficiency [33]. Alterations in the interactions between gut microbiota and the immune system may lead to increased osteoclast activity and reduced osteoblast function [34, 35]. The current study focuses on the link between the level of estrogen, gut bacteria,

and osteoporosis in postmenopausal women, with the emphasis being on how the gut abundances altered by the low estrogen level might possibly affect bone density. It analyzes the consequence of changing the gut ecosystem in terms of a possible innovative intervention for prevention of bone loss. By investigating the interplay among these factors, the study aims to provide insights into the underlying mechanisms of osteoporosis and thereby inform the development of effective interventions.

Estrogen-gut microbiota interaction

Estrogen's effect on gut microbiota

Estrogen is a class of key hormones that regulate multiple systems including the reproductive, skeletal, and immune systems. Recent studies have shown that it also indirectly participated in systemic physiological and pathological processes by influencing the structure and function of the gut microbiota (Fig. 1). Specifically, estrogen can regulate

multiple systems by altering the abundance of specific microbiota and their metabolic activity. For example, in the reproductive system, estrogen can promote the proliferation of beneficial bacteria such as *Bifidobacterium*, inhibit the relative proportion of bacteria like *Lactobacillus*, and activate the hypothalamic-pituitary-gonadal axis through microbial metabolites such as SCFAs (e.g., butyrate, propionate), thereby participating in the regulation of sexual maturation [36]. In the skeletal system, estrogen deficiency leads to a reduction in butyrate-producing bacteria (e.g., *Lachnospiraceae*), which in turn affects bone mineral density (BMD). Estrogen supplementation can partially restore the microbiota structure and alleviate osteoporosis through anti-inflammatory mechanisms [37]. In terms of immune regulation, estrogen can upregulate intestinal alkaline phosphatase, inhibit lipopolysaccharide-producing bacteria (e.g., *Proteobacteria*), and simultaneously promote the proliferation of the

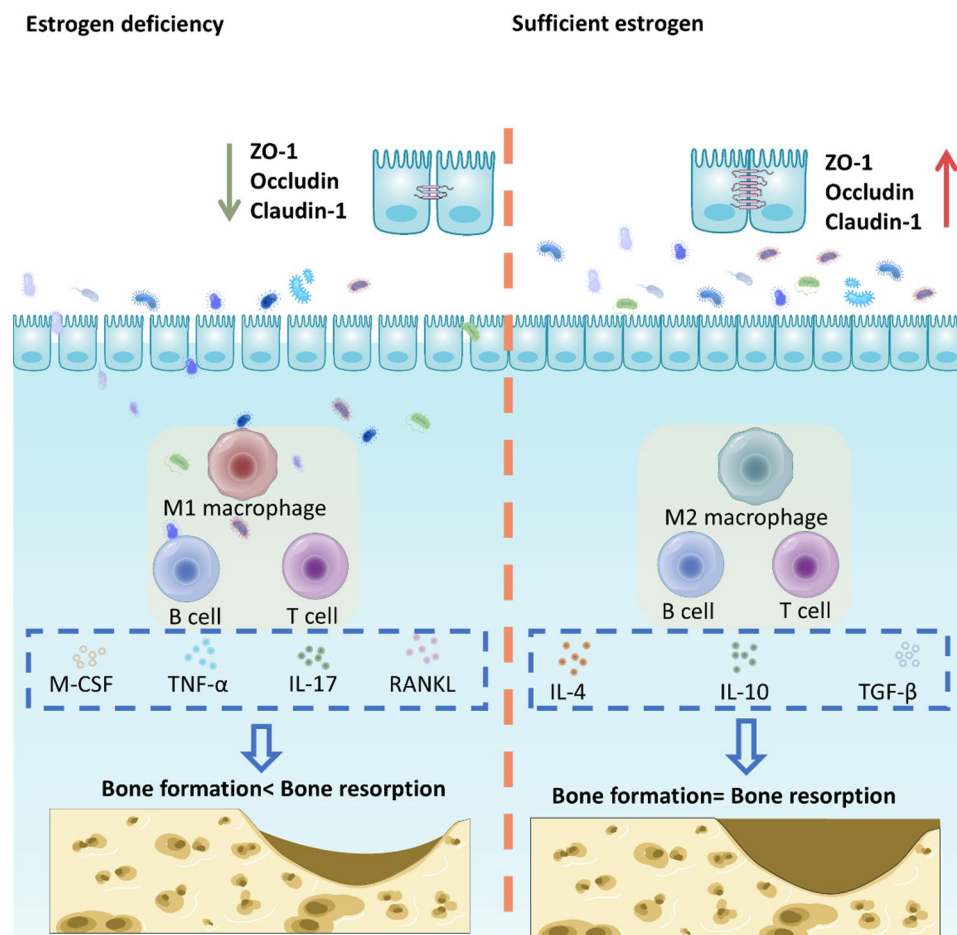


Fig. 1 Estrogen, an integral pairing hormone, regulates not only the reproductive system but also serves, as it were, the immune system. Inadequate estrogen may come to disturb barrier function of the intestine, resulting in the invasion of lipopolysaccharides from gut microbiota into the blood circulation. It is these actions that lead to the modulation of the function of both the innate immune cells (such as macrophages, dendritic cells, and NK cells) and the adaptive immune cells (e.g., T and B lymphocytes), with the concomitant effect of perturbing the inflammatory response, the immune tolerance, and the immune defense. This leads, in turn, to a whole body inflammatory response, which is initiated by the stimulation of the osteoclasts, and this accelerates the process of bone loss

mucus-degrading bacterium *Akkermansia muciniphila* and barrier-protective *Bifidobacterium*, thereby reducing systemic low-grade inflammation [38]. Estrogen receptors are widely present in intestinal epithelial cells and immune cells, providing a structural basis for the bidirectional regulation between estrogen and the microbiota.

Estrogen directly influences the structure and function of the gut microbiota through multiple mechanisms. It can alter the gut environment (such as pH, mucus layer, and bile acid metabolism) or act directly on specific bacteria, thereby changing their abundance and metabolic activity. For instance, estrogen supplementation (e.g., in an ovariectomized rat model fed a high-fat diet) increased the numbers of *Lactobacillus* and *Bifidobacterium*, upregulated tight junction proteins, and enhanced intestinal barrier function. Estrogen can upregulate intestinal alkaline phosphatase, inhibit lipopolysaccharide-producing bacteria (e.g., the phylum *Proteobacteria*), and simultaneously promote the proliferation of the mucus-degrading bacterium *Akkermansia muciniphila* and barrier-protective *Bifidobacterium* [38]. In addition, estrogen can affect the gut microbiota by regulating signaling pathways. Treatment with estrogen (e.g., β -estradiol) alters the diversity of the microbiota and regulates the function of the gut microbiota by activating signaling pathways such as PI3K/Akt/mTOR in estrogen receptor β and G protein-coupled receptor 30 (GPR30). Estrogen-related receptor α (ESRRA) also plays a role in maintaining intestinal stability and autophagic processes. Estrogen can also affect the function of immune cells (e.g., increasing the secretion of the anti-inflammatory cytokine IL-10), thereby indirectly shaping the composition of the gut microbiota, inhibiting pro-inflammatory microbiota, and creating an environment more conducive to the growth of beneficial bacteria [39–45].

Estrogen affects the production of microbial metabolites, particularly SCFAs such as butyrate and propionate, by altering the structure of the microbiota. These metabolites are key mediators for estrogen to exert distal effects, including the activation of the hypothalamic-pituitary-gonadal axis, maintenance of BMD, and anti-inflammatory effects [36].

The drop in estrogen levels experienced during menopause has been proven to exert a powerful influence on the composition of the intestinal microbiota. These changes affect our bone health and the probability of developing osteoporosis. As Yu et al. observed, the intestinal tract of OVX mice was found to contain more specific bacterial strains such as segmented filamentous bacteria (SFB) [46]. This alteration caused an increase in the number of TNF- α positive T cells and Th17 cells in the gut, which, in consequence, leads to a decrease in bone density. Through these studies, it can be concluded that the imbalance in gut microbiome composition due

to low estrogen levels can greatly impair the immune system's ability to maintain bone homeostasis.

In addition, Cline Smith et al. made a significant contribution by showing that OVX-induced estrogen deficiency caused the shift of gut bacteria toward the low relative abundances of some taxa and the high relative abundances of others [47]. Consequently, it was found that such changes resulted in the production of pro-inflammatory cytokines including interleukin-7 (IL-7) and interleukin-15 (IL-15) by the immune cells in the bone marrow. It is hypothesized that the gut microbiome acts as an important instigator of memory cell activation and initiator of chronic low-grade inflammation, which builds up in the course of time, contributing to the promotion of bone loss and the development of osteoporosis. Through these studies, interlocking pieces of the complex mechanisms of estrogen depletion, gut microbial dysbiosis, and inflammatory pathways are provided along with the vital need for a balanced gut microbiome for optimal bone health, where low-grade inflammation can lead to bone pathology. Yersin et al. have made a detailed examination of the way the intestinal microbiota links to osteoporosis, particularly when there is insufficient estrogen secretion [48]. They mediated this point by mentioning that the lowered estrogen levels may destabilize the gut ecosystem, resulting in malabsorption and non-metabolism of nutrients. Such an imbalance could affect the gut-bone axis functioning, thus making the progress of osteoporosis more severe. Ge et al. conducted a research study on the influence of gut microbiota on osteoporosis, which had been triggered by lead poisoning, in an animal model with the involvement of estrogen deficiency worsened [49]. Findings showed that the variation of gut microbiome composition is in turn correlated to low levels of SCFA, butyrate in particular, which has been known to be vital for maintaining bone integrity. Hence, inadequate butyrate synthesis can possibly serve to drive the development of osteoporosis and beyond. This observation is a reminder of why it is essential to maintain proper gut health and to produce butyrate in sufficiently high concentration to avoid bone resorption due to low estrogen levels.

In addition, Chen et al. observed the diminished presence of some species such as *Lactobacillus* and *Bifidobacterium* in a menopause model mouse [42]. This perturbation of the microbial community was associated with an upsurge of pro-inflammatory cytokines, through which bone density was lost rapidly. It is suggested by scientists that the low estrogen level changes the conditions in the intestines, which leads to the appearance of wrong bacteria and the disturbance of the relative ratios of the existing microbial community. Researchers such as Guan and colleagues demonstrated that the absence of estrogens causes not only modifications of gut composition

but also a boost in intestinal permeability [30]. The lipopolysaccharide (LPS) concentration in the blood increased, leading to the activation of TLR4/NFκB signaling pathway and the subsequent increase in osteoclastogenesis, which, eventually, caused the huge loss of bone mass. Hu et al. investigated the action of gut microbes through the enzyme β-glucosidase about the concentration of estrogen [50]. Their findings imply that a decline of estrogen levels leads to the disruption of the enzyme activity, which is fundamental for the conversion of the estrogen metabolites. Deficiency in β-glucosidase might lead to the disturbance of estrogen levels and may even have a role in osteoporosis development as reported in the studies of Kim and colleagues [43]. It was shown that estrogen deficiency-induced dysbiosis can affect the cellular engulfment pathway. A dysfunctional autophagy can be associated with the compromised gut integrity and enhanced inflammation, the things which may facilitate the development of osteoporosis indirectly through gut-bone axis. In short, there are a sequence of alterations in the intestinal microbiome coming with the decreasing level of estrogen, including the changes in composition, function, and their interaction with the host. Those factors contain, in total, one of the key factors increasing the risk for some of the diseases like osteoporosis associated with the estrogen level reduction. One of the keys to finding therapies for these diseases is understanding how the gut microbiome adjusts to lower estrogen levels. This perturbed intestinal environment is not merely a local

event; rather, it serves as a link that remotely disrupts the homeostasis of the bone microenvironment by releasing microbial metabolites and immune signals (Table 1).

Estrogen and bone health

Role of estrogen in maintaining bone density

Estrogen exerts its effects in maintaining bone strength through multiple mechanisms: it promotes the proliferation and differentiation of osteoblasts (cells that form bone) while inhibiting the activity of bone-resorbing cells such as osteoclasts (cells that absorb bone); regulates the immune response within bone tissue to reduce the release of inflammatory mediators; and influences osteocytes, which are responsible for sensing physical strain and regulating bone remodeling activities (Fig. 2). These processes collectively contribute to the maintenance of BMD and support overall bone health [51–54].

However, a growing body of evidence indicates that these protective effects of estrogen are largely achieved by maintaining the homeostasis of the gut microbiota. Bone loss triggered by estrogen deficiency is closely associated with gut dysbiosis and the subsequent systemic inflammation it induces [55, 56]. For instance, probiotics can produce SCFAs, which may exert beneficial effects on BMD. Estrogen deficiency leads to gut microbiota dysbiosis and consequent dysregulation of the gut-bone axis system. Xu et al. [44] pointed out that a diverse gastrointestinal microbiota can produce SCFAs to promote bone health. A study by M. Zhang et al. found that estrogen deficiency causes gut microbiota imbalance (characterized by a significant increase in Bacteroidetes, a marked decrease in Firmicutes, and an overall reduction in α-diversity), and this dysbiosis is closely linked to subsequent bone loss; after restoring microbiota balance through the supplementation of alginate oligosaccharides, bone loss was alleviated, suggesting that bone loss induced by estrogen deficiency is closely associated with gut dysbiosis [57]. Similarly, a study by Guan et al. demonstrated that bone loss in mice with estrogen deficiency (induced by ovariectomy) is closely related to a significant increase in the Firmicutes/Bacteroidetes ratio in the gut microbiota. Further verification through experiments involving gut microbiota depletion with antibiotics or fecal microbiota transplantation (FMT) confirmed that this dysbiosis is a key link in estrogen deficiency-induced bone loss [30]. Therefore, the regulation of the balance between bone formation and resorption by estrogen is dependent on the support of a healthy and diverse gut microbiota. The latter plays an indispensable role in maintaining bone health by producing beneficial metabolites such as SCFAs. In-depth exploration of the estrogen-microbiota-bone axis relationship will provide a novel theoretical basis and intervention strategies for the

Table 1 Effects of Estrogen deficiency on intestinal microbiota

Impact Level	Specific Changes	Downstream Effects	References
Microbial Composition	Decrease in beneficial bacteria: <i>Bifidobacterium</i> <i>Lactobacillus</i> Butyrate-producing bacteria (e.g., <i>Lachnospiraceae</i>)	Disrupts microbial balance, reduces the synthesis of short-chain fatty acids (e.g., butyrate), and impairs anti-inflammatory and barrier protection functions.	[37, 38, 42, 46]
	Increase in harmful bacteria: LPS-producing bacteria (e.g., phylum <i>Proteobacteria</i>) Segmented filamentous bacteria		
Metabolic Function	Decrease in SCFAs: Reduced synthesis of butyrate and propionate	Decreased bone mineral density and imbalance of bone homeostasis	[36, 49]
	Dysregulation of β-glucosidase activity: Affects the conversion of estrogen metabolites	Abnormal conversion of estrogen metabolites, exacerbating estrogen level imbalance	

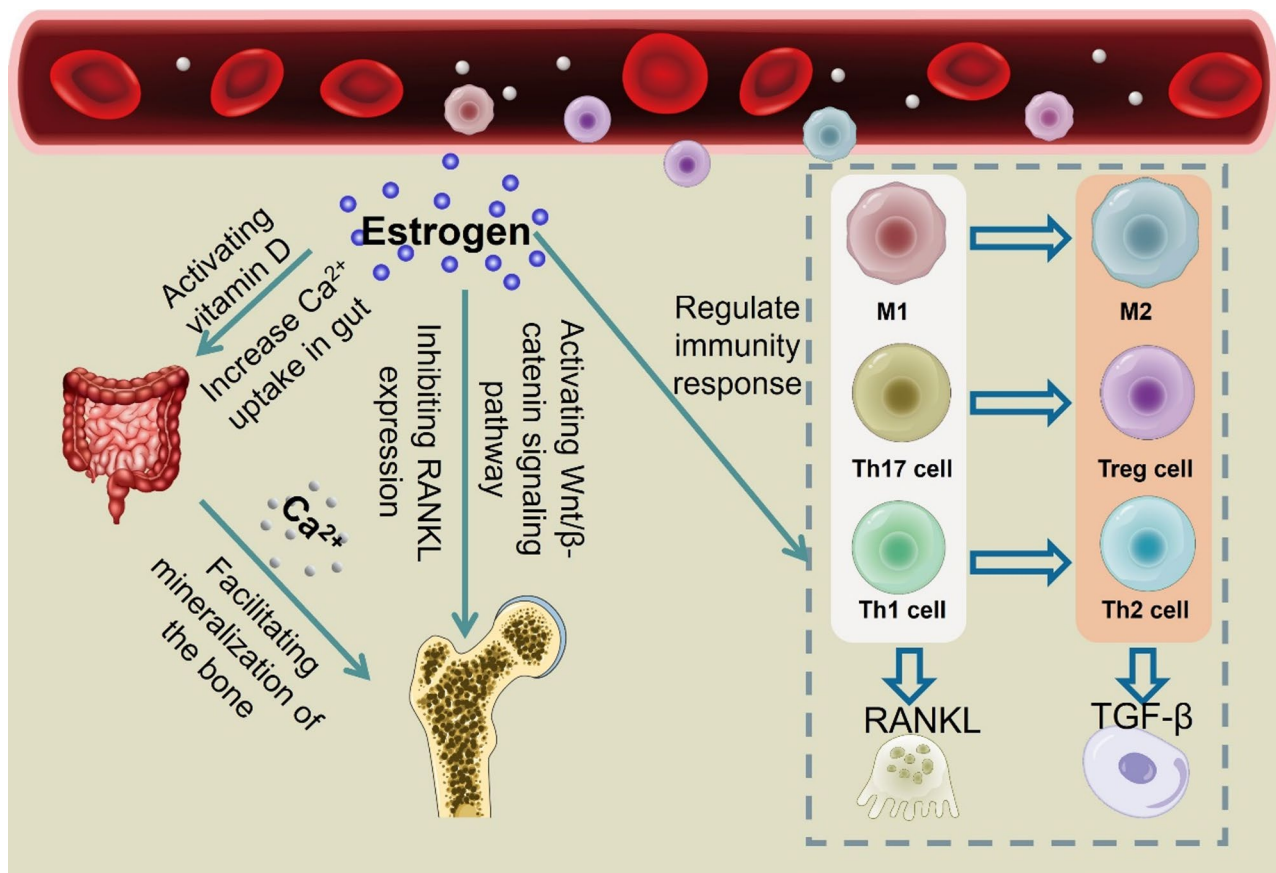


Fig. 2 This section is focused on both the role of estrogen in the preservation of gut microbiota and the amelioration of osteoporosis. It influences bone health through the modulation of the composition and activity of gut bacteria, which, in turn, regulates the metabolism of bones. Estrogen helps to convert the microbial vitamin D into its active form and, consequently, enhances the absorption of calcium ions through the gut. On top of that, estrogen stops the process of bone resorption by decreasing the expression of RANKL (the receptor activator of nuclear factor κB ligand), or regulating the processes of osteoclastogenesis and osteoclast activity. Besides this, estrogen stimulates bone formation by promoting osteoblast differentiation, which occurs primarily through the activation of the Wnt/ β -catenin signaling pathway. Estrogen could also exert regulatory effect on both innate and adaptive immune system, which leads to the inflammatory process, and thus, the osteoblasts and osteoclasts activities

prevention and treatment of osteoporosis associated with estrogen deficiency.

Gut microbiota in health and disease

Current studies suggest that the role of these microorganisms in bone health is by regulating immune interactions, which lead to abnormal bone remodeling. Gut bacteria create compounds that affect the roles of osteoblasts and osteoclasts, hence affecting bone strength. Alterations of the gut bacteria have been connected with the onset and advance of osteoporosis. Recent studies, such as that by Mann et al. are focused on the key function of gut bacteria and their metabolites like SCFAs [58]. SCFAs like acetate, propionate, and butyrate, produced by gut microbes, are imperative in mucosal barrier and immune system regulation. Butyrate boosts the integrity of the intestines by enhancing the protective lining, modulating inflammation, and keeping immune cells tolerant via directing the differentiation of regulatory T

cells (Treg) cells and inhibiting the pro-inflammatory T cells. Also, to add, butyrate enhances the formation of new osteoblasts and remodels osteoclasts [33, 59]. Yersin et al. investigated the link between the microbiota of the small intestine and osteoporosis and consequently postulated that variations in its composition or activities might be detrimental to the absorption of vital nutrients, for example, calcium, which might accelerate the development of osteoporosis [48]. Such study is imperative in showing how the microbiota live in the small intestine influence the health of bone. During healthy conditions, gut microbes are crucial for digestion and manufacturing SCFAs from indigestible carbohydrates, which offer manifold beneficial effects to the host. Kim et al. also demonstrated the critical role of gut microbial homeostasis in the estrogen-controlled receptor α and its associated effects on the overall intestine health and stability [43]. The SCFAs not only carry out the dual role of regulating energy levels and possessing anti-inflammatory

properties that help in maintaining gut and other organ health but also promote bone health such as propionic acid calcium metabolism. In addition, gut bacteria are highly interconnected with the immune system and are integral to immune provision. Training the immune system to discriminate between harmful foreign proteins and innocuous substances benefits health by preventing unwarranted immune reactions.

However, gut microbiota imbalance, known as dysbiosis, is one of the results of the deviation of gut flora caused by reduced estrogen levels, which can, in turn, contribute to osteoporosis and other diseases, according to Guan et al. [30] It includes a raising gut permeability, which helps the entry of LPS from the gut to the blood circulation where it triggers the TLR/NF- κ B signaling pathways. This activation stimulates osteoclastogenesis, eventually resulting in bone loss. Gut microbiota dysbiosis causes IBD, but the latter is not directly related to the proposed estrogen-gut microbiota-osteoporosis connection inherent in the article. In IBD, it is evident that having a healthy gut bacteria is critical. IBD patients show notable alterations in gut microbiota composition: increased presence of deleterious bacteria and decrease of valuable ones. This microbial imbalance promotes chronic gut inflammation. It may have far-reaching impacts that are indirectly linked with bone health, such as the gut-bone axis.

The gut microbiota contributes to human health by manipulating important physiological processes,

including mucosal immunity, nutrient transportation, and bone strength. Comprehending these correlations is necessary to cultivate creative therapeutic means for combating disorders entangled with gut microbiota dysbiosis. Interventions targeting the gut microbiota, like dietary modifications, probiotics, and prebiotics, present exciting options for addressing conditions induced by disturbances in the gut microbiome. The future of research should be dedicated to analyzing the nuanced relationships linking gut microbiota and host physiology and their contribution to health and disease. This effort will fill the gap in knowledge and promote the development of more efficient interventions.

The impact of gut microbiota on osteoimmune

Microbiota-derived metabolites and osteoimmune regulation

Microbiota-derived metabolites are determining factors in the modulation of bone health. Such metabolites can bind directly to bone cells, like osteoblasts and osteoclasts, and thus determine their physiological behaviors (Fig. 3). As a further note, these metabolites can control the immune response taking place within the bone microenvironment. This process in turn has an impact on the balance in the levels of pro-inflammatory and anti-inflammatory cytokines. Added to this, metabolites yielded from microbiota can mediate the communication of the gut-bone axis, accentuating or impairing the processes of bone remodeling that result in the preservation

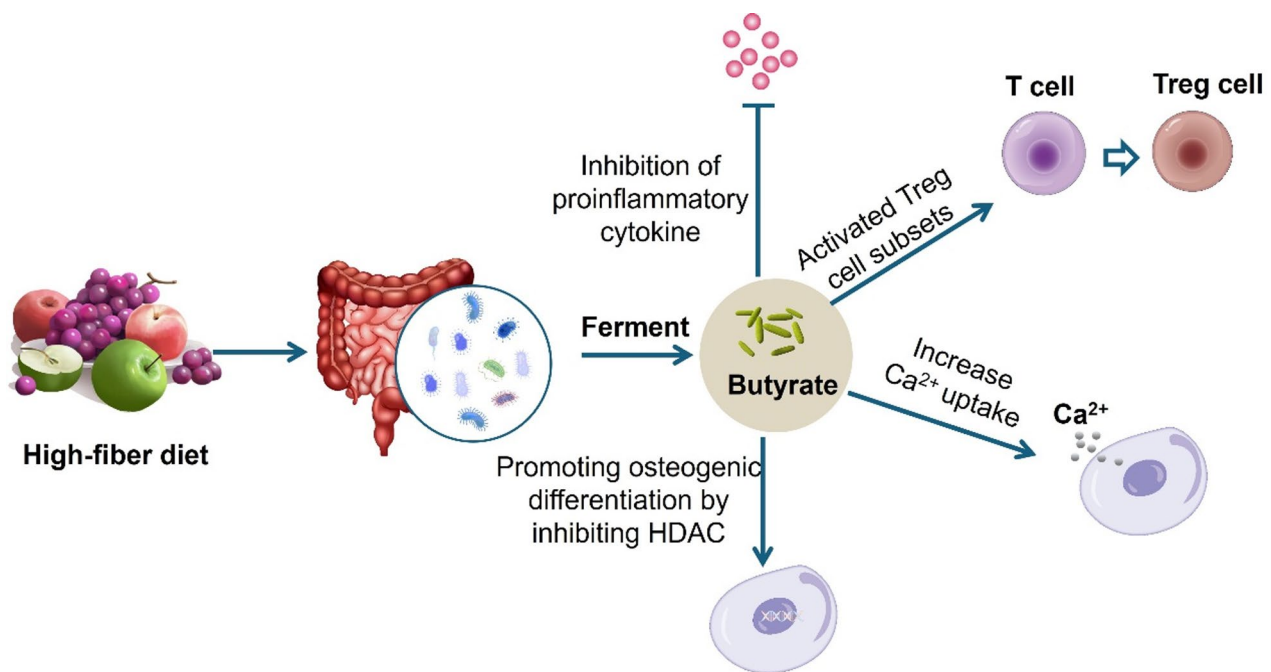


Fig. 3 Butyrate, a type of SCFA predominantly formed by gut microbes in the process of fermenting fiber from the diet, plays a pivotal role in the maintenance of gut health and the regulation of whole-body metabolism. It regulates osteoporosis through: Reduces inflammatory factors; Modulates immune responses; Promotes intestinal calcium absorption; Influences epigenetic regulation of bone metabolism genes (as an HDAC inhibitor)

or deterioration of bone homeostasis [60]. Propionate showed to dampen T cell activation in some way, which could contribute to a less inflammatory milieu in the bones that eventually prevents bone resorption as well. The secondary bile acids generated by gut microbiota interact with the nuclear hormone receptors like FXR and VDR in immune and bone cells. The activation of FXR in macrophages results in the modulation of their polarization status, which subsequently has an effect on osteoclastogenesis. Metabolites produced by the gut microbiome have an immense influence on the function of the intestinal barrier. For instance, the role of SCFAs, particularly butyric acid, extends beyond the strengthening of the epithelial barrier. They also help reduce systemic-level inflammation that may eventually affect bone health negatively [61]. The action of gut microbiomes with their deriving metabolites, includes modulation of immune cells and cytokines that are associated with the activities of both osteoclasts and osteoblasts [62].

Importantly, there are different classes of metabolites, which are able to act as pro-osteoclastogenesis inhibitors. Remodeling of bile acids, which are not normally conserved by the gut microbiota, entails immune responses and bone metabolism through changes in nuclear receptor activation and subsequently in gene expression related to bone formation and resorption. In conclusion, the metabolites derived from the gut microbiome are bound to different cells and signaling pathways through their interaction, ultimately resulting in a well-defined process of bone formation and resorption. The study on the impact of such metabolites on bone-immune interactions is requisite for the development of groundbreaking therapies for osteopenia, osteoporosis, and other bone-related disorders. There is a need to understand the details of these interactions at the molecular level and check the metabolites or metabolite derivatives' efficacy for the prevention and treatment of bone disorders in the coming years.

Gut microbiota and inflammatory responses in the bone microenvironment

There are complex interactions between the gut microbiota and bone microenvironment. This microbiota drives inflammatory pathways within bones through several mechanisms, including metabolites production that can modulate the activity of immune cells in bones and signaling along the gut-bone axes. A gut bacterium's imbalance can promote aberrant inflammatory pathways in the bone microenvironments, thereby impairing normal bone homeostasis and potentially leading to bone diseases such as osteoporosis. Estrogen deficiency alters the gut microbiota composition, leading to the activation of memory T cells, as demonstrated by Cline-Smith et al. [47]. In OVX mice, the changes in gut microbiota

have been shown to be associated with the increased levels of pro-inflammatory factors such as interleukin-17 A (IL-17 A) and tumor necrosis factor- α (TNF- α), which are released by the activated memory T cells. These cytokines are crucial for not only osteoclast formation but also aspects of the resorption of bones. Chronic low-grade inflammation, which may be triggered by an imbalanced gut microbiota or a hyperactive T cell, compromises the delicate balance between bone formation and resorption, thereby encouraging osteoporosis. With this study, we show that gut microbiota dysbiosis is significantly associated with the activation of skeletal inflammatory pathways.

Based on the study by Yu et al., the mechanisms by which gut microbiota may influence the inflammatory processes involved in bone health [46]. In particular, it examines the interaction between the parathyroid hormone (PTH) and the gut microbiota, as it uncovers the fact that some of the gut bacteria, especially the segmented filamentous bacteria (SFB), exert a considerable effect on the efficacy of PTH. As the result of this interaction, there would be an increment in the regional TNF⁺ T cells and Th17 cells that are involved in inflammation. The enlarged T cell populations subsequently enter the bone marrow, which in turn drives bone resorption. The SFB presence in the gut microbiota is revealed to be a critical factor that indicates that such a specific composition of the microbiota can modulate the immune response in bone tissue and therefore facilitate the process of bone mass loss, according to this study. This study underscores the influence of gut microbiota on the hormonal effects on the bone immune system and picks specific microorganisms that incite the bone inflammatory processes. The changes of gut microbiota composition are also able to affect the expression of the chemokines and adhesion molecules in the bone microenvironment, which are important for facilitating the recruitment of immune cells to the sites of inflammation or remodeling.

Dysbiosis of the gut microbiota may further disrupt the chemokine signaling pathway that would lead to the accumulation of immune cells in the bone, thereby intensifying the inflammatory response and hindering normal bone rebuilding [63]. For instance, increase in the concentrations of certain chemokines may lead to enhanced recruitment of additional progenitor cells that harbor the potential to differentiate into osteoclasts, therefore expediting osteoclast differentiation and enhancing bone resorption. The gut microbiota can also shape the behaviors of innate immune cells, like macrophages and dendritic cells, in the bone microenvironment. Macrophages are critically involved in orchestrating immune responses in the bone; however, by stimuli from the external environment, they might polarize towards M1 (pro-inflammatory) or M2 (anti-inflammatory) macrophages [64].

Evidence has been accumulated to support the view that the gut microbiota is a major player in macrophage polarization through the production of metabolites and cell-to-cell communication. In a healthy gut microbiome, some metabolites promote M2 polarization, which helps cut down on inflammation and speeds up tissue repair.

However, in the context of gut dysbiosis, pro-inflammatory metabolites will be likely to be produced, thus driving macrophages towards the M1 phenotype that escalates inflammation and bone resorption. Dendritic cells, which are mainly responsible for presenting antigens and orchestrating immune responses, are influenced and governed by gut microbiota. This effect gives rise to T cell activation and can drive the overall inflammatory responses in the bone microenvironment. Balancing the immune response, including those in the bone tissues, is sustained by communicating with the gut microbiota and directing the production of anti-inflammatory mediators. For instance, Kim et al. [43] suggested that with the help of the ESRRA, modulation within a well-balanced gut microbiota to maintains both gut and systemic homeostasis is critical. This balance might favor the bone microenvironment since it helps to prevent the overproduction of inflammation that will antagonize the normal remodeling process. However, with conditions such as the estrogen deficiency or changes in the composition of gut microbiota, these immune processes would be dysregulated in the bone. In the study undertaken by Chen et al. [42], the deficiency of estrogen was observed to change gut microbiota. In this study, the changes in the gut microbiota were linked to the increased levels of pro-inflammatory cytokines within the bone microenvironment. One of the possible outcomes of the heightened levels of inflammation is the stimulation of osteoclastogenesis, which is the increase of osteoclasts number and activity, subsequently leading to the reduction of bone mass. For example, pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) can exacerbate osteoclastic activities, thereby interfering with the balance between bone formation and resorption.

Guan et al. [30] demonstrated that, when the estrogen is deficient and the gut microbiota is imbalanced, there is an increase in intestinal permeability that further allows the influx of LPS to the bloodstream. As a powerful stimulator of the immune system, LPS is capable of triggering inflammation in the bone microenvironment. In the end, when bound to the TLR4 receptors on the cell's surface, this leads to the activation of the NF- κ B signaling pathway, which mainly causes the increase in the production of pro-inflammatory cytokines and the promotion of osteoclastogenesis. Therefore, there would be a very strong link between the state of the gut microbiota and the inflammatory processes within the bone

microenvironment. The maintenance of a healthy gut microbiome becomes essential for the regulation of the inflammation. In contrast, a drop in estrogen levels is able to create an excessive inflammation condition and cause bone loss. The production of pro-inflammatory cytokines from the activated immune tissue may be leveraged to degenerate the composition of gut microbiota and mediate the infection of the bones [65, 66]. As a consequence, the increased inflammation leads to osteoclastogenesis and subsequent bone loss. This is truly the reason we need a proper composition of gut microbiota for healthy bones.

Gut microbiota and bone cell function

The recent research confirms that, among many influences, gut microbiota have a very large impact on the functioning of osteoclasts and osteoblasts, in particular, during bone remodeling (Fig. 4). Yang and colleagues [67] explored the modulatory effects of epigenetic factors on bone loss caused by microbiota changes with the onset of ovariectomy. They found that gut microbiota compositional shifts after ovariectomy might modulate the expression of genes involved in osteoclast development via epigenetic mechanisms. In particular, the alteration of gut microbiota may impair the autophagy control in osteoclasts, which may in turn affect their growth and function. These results indicate that the entire microbial community, including bacteria, in the intestine plays a significant part in controlling the genetic responses regarding bone health. It offers novel perspectives on the workings of gut microbes in the context of osteogenesis and the immune system. Some gut bacteria, or their by-products, might interact with molecules like DNA methyltransferases or histone deacetylases to modulate the chromatin structure and thus the expression of genes involved in osteoclast activity.

Furthermore, human gut microbiota is associated with the modulation of osteoblast function. Certain species of gut microbes and their metabolites may have possible roles in regulating osteoblast proliferation and activity by boosting protein synthesis for formation of the bone matrix and altering the processes of mineralization [68]. Though not exhaustively covered in each study summarized, investigations carried out recently indicate that gut microbiome likely exerts a marked influence on local environment surrounding osteoblasts. Such effect is probably achieved due to discharging of substances that could directly affect osteoblast function (making them more inhibited or activated) or by affecting bone tissue responses (increasing or decreasing their effect in the process). A healthy gut microbiota can create favorable conditions for the secretion of growth factors and cytokines, which collectively help to maintain osteoblasts' viability as well as their activity. On the other hand, the

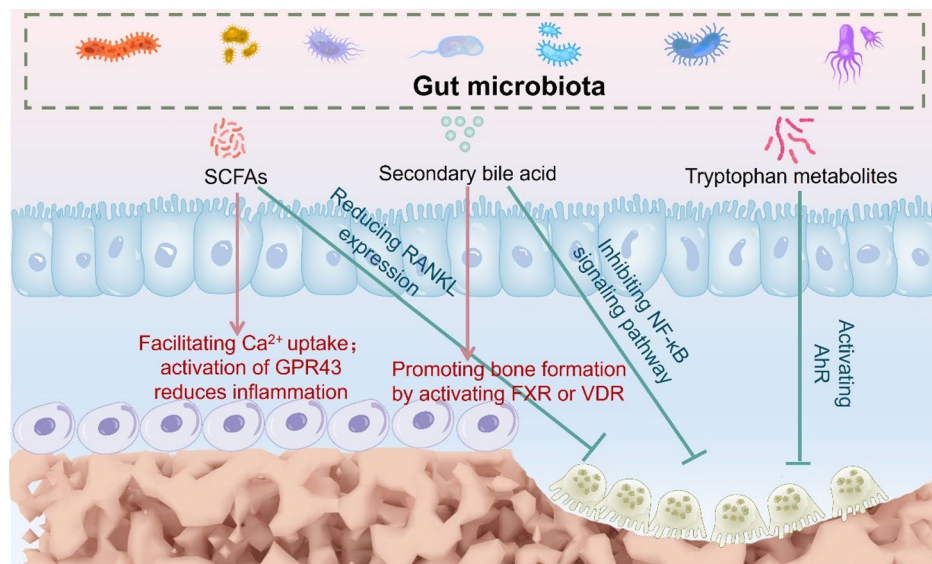


Fig. 4 Metabolites arising from gut microbiota abundantly contribute to osteoporosis pathophysiology. Through several pathways, which include immune system modulation, bone metabolism influencing, hormone level modulation, and gut barrier function enhancement, these metabolites affect bone health. Acetic acid, propionic acid, and butyric acid are examples of SCFAs that are mostly formed as a result of the fermentation of the dietary fiber by the intestinal microbes. SCFAs demonstrate their ability to induce calcium absorption, downregulate RANKL expression, augment bone density, and display anti-inflammatory properties. Bacterial enzymes in the intestines convert primary bile acids into secondary bile acids, such as deoxycholic acid and lithocholic acid. These secondary bile acids modulate bone metabolism by promoting osteoclast activity and exerting anti-inflammatory effects. Indoles and serotonin, bioactive compounds such as tryptophan metabolites that are produced by gut microbes, are the products of this process. Indoles activate AhR (aryl hydrocarbon receptor) through inhibition of osteoclastogenesis and promotion of osteoblast differentiation. It is the serotonin (5-hydroxytryptamine) that controls the immune activity within the gut and further affects the bone metabolism indirectly

shift in the gut bacteria can lead to production of substances that could potentially block or impair such functions [69]. The gut microbiota holds an important place in finagling the interplay between osteoblasts and osteoclasts and, consequently, the integrity of the bones. These two cell types interact through direct contact with each other as well as by the exchange of signaling molecules [70]. Enteric microbes can interfere with communication by regulating the expression or function of those signaling molecules. For example, certain metabolites, produced by intestines bacteria, can influence how other proteins, such as RANK Ligand and OPG, are acting in a bone cell. RANK Ligand is an important component in development of osteoclasts that are responsible for bone resorption. Specifically, OPG acts as the decoy receptor that binds with RANK Ligand and contributes to the inhibition of osteoclastogenic activity. An elevated RANKL/OPG ratio promotes excessive osteoclast activity and bone resorption [71, 72].

According to Chen et al., maintaining gut microbiota homeostasis may influence RANKL and OPG expression or indirectly affect their production through the modulation of immune responses [54]. Their study demonstrates a correlation between shifts in gut microbiota composition and changes in bone density, suggesting that a well-maintained gut microbiota can enhance osteoblast function and inhibit osteoclast formation. A balanced gut

microbiota has the potential to produce growth factors and cytokines that promote osteoblast proliferation and function. Specific gut bacteria may generate substances that stimulate osteoblasts to synthesize bone matrix proteins, thereby maintaining bone strength, as reported by Guan and colleagues [30]. Research findings indicate that decreased estrogen levels can alter the gut microbiome, leading to an increase in osteoclast numbers. This disturbance is associated with compromised gut barrier function and the translocation of LPS into the systemic circulation. Xu and colleagues suggest that LPS activates the TLR4/NF-κB pathway in osteoclast precursor cells [44], promoting their differentiation into mature osteoclasts and accelerating bone resorption. They also propose that gut microbial balance influences the gut-bone axis by modulating immune responses within the bone microenvironment.

Immune components, including T cells and macrophages, produce cytokines affecting the activities of a wide range of cells, such as osteoblasts and osteoclasts. Th17 cells secrete IL-17, which promotes osteoclastogenesis, and Tregs release IL-10 and TGF-beta, which inhibit osteoclastogenesis and promote osteoblasts. The gut microbiota composition is integral to the immunological balance of different immune cells, which ultimately affects the cellular function concerning the skeletal system [73, 74]. Using various approaches, such

as epigenetic regulation, alteration of the local microenvironment, and interaction between different bone cells, this protein is involved in the modulation of osteoclast generation and osteoblast activity. To build up a solid foundation for studying the associations of these ingredients, a comprehensive understanding is needed. Also, the identification of the novel therapeutic approaches to address the disorders of bone requires that understanding. Future studies should be aimed primarily at probing the effect of gut microbiota on bone health and the possibility of its increase to bone strength.

Epigenetic regulation by the gut microbiota in osteoimmunity

Epigenetic regulation assumes a very important function in determining the response of the skeletal immune system. The most up-to-date research has shown that the involvement of the gut microbiota is of vital significance, and multiple mechanisms impact bone health as well. The study by Yang et al. [67] has revealed that gut microbiota epigenetically controls the osteoclasts' formation. Their findings suggest that after ovariectomy, gut microbiota composition could be altered, and they may play a role in the regulation of the expression levels of genes involved in the formation of osteoclasts through epigenetic pathways. Hence, the specific mechanism that was studied was that of TET2, an enzyme responsible for demethylation of DNA [75]. Modifications in the gut microbiota composition may potentially affect the way TET2 works, giving rise to variations in the methylation status of genes linked to osteoclast function. Because of this, TET2 may alter the methylation of genes related to the process of autophagy, which is an essential step for the differentiation of osteoclasts and their activity. Perturbations in our gut microbiota may lead to altered patterns of histone modifications as well as impaired osteoclast-mediated bone resorption. Hence, such changes should be viewed in the context of epigenetic regulation and skeletal immunity. Besides the histones, there is another essential factor called the 'microbiota' which also interferes with the histone deacetylases (HDACs). These HDACs are inhibited by a metabolite called butyrate, which is produced by gastrointestinal bacteria and is known for its effect on histone. As per the findings of Mann et al., butyrate can be taken up by cells and directly interact with HDACs, which reduces their activity. This obstruction results in increased levels of histone acetylation, thereby having an effect on the expression of immune and bone cellular genes [58]. As a matter of fact, acetylation of the histone induced by butyrate has a special bearing on Tregs' development and activity. Butyrate triggers histone acetylation at the FoxP3 promoter as well as at the other specific regions within the gene sequence corresponding to FoxP3, therefore serving to nourish Tregs' differentiation.

Consequently, the production of butyrate by gut microbiota and its impact on histone acetylation may alter the balance of the osteoimmune system. Emerging research indicates that gut microbiota can potentially influence epigenetic modifications such as histone methylation and noncoding RNA expression, leading to changes in osteoimmune response patterns. For instance, certain gut bacteria may produce substances or chemical messengers that interact with enzymes responsible for histone methylation, thereby altering the methylation profiles of genes involved in bone cell function and immune regulation [76]. Additionally, non-coding RNAs, particularly microRNAs, have been shown to regulate osteoimmune functions. Gut bacteria may influence the activity of these noncoding RNAs, which can target genes that affect the functions of both osteoblasts and osteoclasts, as well as immune cell activity. In a study conducted by Chen and colleagues [42], although not specifically focused on DNA methylation, they identified a correlation between alterations in gut microbiota and changes in gene expression associated with bone health.

Immune cells remain within this microenvironment so that they can inhibit excessive immune responses and preserve immune tolerance in the bone microenvironment. Changes in the gut microbiome composition are likely to alter the patterns of DNA methylation and this indirectly swings the gene expression for both osteoblasts and osteoclasts in the bones. As a matter of course, the OPG, RANKL, or other key proteins' methylation status in the genes may be responsible for the development of new bone and reabsorption of the already existing one. For example, the rise of methylation of the OPG gene due to gut microbiome disturbance may lead to a decrease in its expression, which in turn will trigger the production of more osteoclasts and consequent loss of bones [77, 78]. Through its activities, the gut microbiome impacts bone health by adjusting immune and bone cells through epigenetic processes such as DNA methylation, histone modifications, and noncoding RNAs [79, 80]. By doing so, it plays a key role in maintaining homeostasis and stimulating immune reactions in the context of bone regeneration. Hence, a detailed study addressing gut microbiota and its role in well-being of bones is necessary to come up with therapies targeting osteoporosis and related disorders. The upcoming investigations should specifically focus on the action of bacteria in the intestines, so as to help participants optimally manage their bone immune responses.

Osteoimmune helps alleviate osteoporosis **Immune cell and cytokine network: bone immune imbalance driven by intestinal dysbiosis**

The complex network composed of immune cells and the cytokines they secrete plays a crucial role in regulating bone remodeling. Among these components, the

functional balance between T lymphocytes (such as pro-inflammatory Th17 cells and anti-inflammatory Treg) and macrophages (such as pro-inflammatory M1 type and anti-inflammatory M2 type) is essential for maintaining bone homeostasis [47, 53, 54]. However, this delicate balance is completely disrupted under conditions of estrogen deficiency. Studies have shown that intestinal dysbiosis is a key upstream event that triggers this pathological cascade [81]. Estrogen deficiency directly leads to changes in the composition of intestinal flora (dysbiosis); on one hand, it promotes the translocation of pro-inflammatory substances such as bacterial LPS into the bloodstream, and on the other hand, it reduces the production of beneficial metabolites like SCFAs, thereby inducing a state of systemic low-grade inflammation [42].

This systemic inflammation spreads to the bone microenvironment, causing severe disorders of immune function, with the core manifestations being the functional polarization of immune cells and the imbalance of the specifically secreted cytokine network. In the pro-inflammatory direction, Th17 cells and M1-type macrophages are abnormally activated and become the main sources of pro-inflammatory cytokines, secreting large amounts of key mediators such as IL-17, TNF- α , IL-6, and IL-1 [44, 47, 82]. Among these mediators, TNF- α not only strongly promotes the differentiation and activation of osteoclasts but also inhibits the activity of osteoblasts, and this dual effect exacerbates bone loss [47, 54]; IL-17 is a potent inducer of osteoclast formation [46]; elevated IL-6 levels facilitate the maturation of osteoclasts and play a fundamental role in regulating the systemic inflammatory environment [54]; IL-1 mainly stimulates the proliferation of osteoclasts and enhances their activity, which further disrupts the balance of bone remodeling in the context of infection or inflammation. Collectively, these factors form a powerful pro-bone resorption signaling environment. In the anti-inflammatory direction, the functions of Treg cells and M2-type macrophages are impaired, resulting in reduced secretion of protective factors. IL-10 specifically inhibits the formation of osteoclasts by interfering with the differentiation of osteoclast precursor cells and downregulating genes related to osteoclast function and bone resorption enzymes; at the same time, IL-10 also supports the survival and function of osteoblasts, and this dual effect is of significance for maintaining the balance of bone homeostasis. As a cytokine with dual roles, transforming growth factor- β (TGF- β) can promote the activity and differentiation of osteoblasts in the bone microenvironment while inhibiting the development of osteoclasts [83].

The weakening of such anti-inflammatory signals further exacerbates the deterioration of the bone immune microenvironment. The disruption of Th17/Treg and M1/M2 balance driven by intestinal dysbiosis, along

with the subsequent imbalance of the pro-inflammatory and anti-inflammatory cytokine network, is the core link leading to chronic inflammation and accelerated bone resorption. In view of this mechanism, therapeutic strategies have two dimensions: the first is to directly target specific cytokines, and preclinical and clinical studies have shown that antibodies against IL-17a or TNF- α as well as small-molecule inhibitors are promising for inhibiting bone loss and increasing BMD [81, 82, 84–88]; in addition, increasing the production or utilization of effective anti-inflammatory cytokines (such as IL-10) in the bone microenvironment (for example, through exogenous supplementation or promotion of endogenous secretion) is also a potential therapeutic approach. The second is more fundamental ecological regulation, that is, correcting intestinal dysbiosis through probiotics, prebiotics, and other means to reshape immune homeostasis at the source [44, 54]. Therefore, the management of intestinal flora has become an emerging key strategy for regulating bone immunity and maintaining bone health. Ultimately, clarifying this “gut-to-bone” immune mechanism will provide direct evidence for the development of targeted therapeutic strategies, thereby contributing to the comprehensive management of osteoporosis [89, 90].

Intestinal flora-mediated bone immune imbalance and bone remodeling disorders

There is an intimate “crosstalk” between the immune system and bone tissue. Among this interaction, immune cells (such as T cells and macrophages) exert critical regulation on the activity of osteoblasts and osteoclasts by secreting cytokines and chemokines, collectively maintaining the balance of bone remodeling [83, 91–93]. Estrogen is one of the core factors regulating this balance. A decrease in estrogen levels directly disrupts the osteoimmunological crosstalk and indirectly exacerbates this imbalance by inducing gut microbiota dysbiosis, ultimately leading to bone diseases such as osteoporosis [53].

Specifically, reduced estrogen levels affect the bone marrow and activate memory T cells. The activated T cells release factors that regulate the activity of osteoblasts and osteoclasts, thereby inhibiting bone formation while promoting bone resorption by osteoclasts. Additionally, osteoporosis induced by low estrogen levels may cause macrophages to promote inflammation and disrupt bone metabolism. Regulatory T cells regulate immune responses by sending signals to osteoblasts, balancing bone growth and bone resorption which are influenced by hormones such as estrogen [47, 51].

Under the state of estrogen deficiency, gut microbiota dysbiosis plays a crucial role in driving the imbalance of osteoimmunological crosstalk. Specifically, the research findings by Guan et al. [30] indicated that the composition of gut microbiota undergoes significant changes

after estrogen deficiency, suggesting that gut microbiota adds another layer of complexity to osteoimmunological interactions. This dysbiosis may increase intestinal permeability, followed by the release of LPS into the circulation. LPS then activates the immune system in the bone microenvironment, leading to intensified local inflammation and promotion of osteoclastogenesis. Furthermore, Xu et al. [44] proposed that changes in gut microbiota affect macrophage polarization in bones; an increase in M1-type macrophages may enhance osteoclast activity and result in bone loss. In contrast, M2-type macrophages possess anti-inflammatory properties and promote tissue repair by supporting osteoblast activity while inhibiting osteoclastogenesis, thereby facilitating bone formation and repair processes. Chen et al. [54] demonstrated that changes in gut microbiota caused by estrogen deficiency alter T cell function and cytokine production, affecting the osteoblast-osteoclast balance. Regulatory T cells secrete anti-inflammatory cytokines such as interleukin-10 (IL-10) and TGF- β , which inhibit osteoclastogenesis while supporting osteoblast activity, thereby maintaining bone homeostasis.

Therefore, the gut microbiota, immune system (cells and factors), and bone cells (osteoblasts and osteoclasts) together form a closely interactive network known as the “microbiota-immune-bone axis”. In this tripartite network, the homeostasis of gut microbiota is key to maintaining normal osteoimmunological crosstalk and the balance of bone remodeling. Estrogen deficiency drives the imbalance of this network through direct effects and induction of gut microbiota dysbiosis, which serves as an important mechanism underlying the occurrence and development of osteoporosis.

Therapeutic strategies for osteoporosis based on the intestinal flora-bone immunity axis

In recent years, the gut microbiota has been found to influence the host immune system, and an overactive immune system is closely associated with bone resorption. The “gut-immune response-bone” axis has been proposed as a new potential approach for the prevention and treatment of postmenopausal osteoporosis (PMOP). Zongjun Ma et al. recruited postmenopausal women, performed 16 S rRNA gene sequencing on the participants to investigate their gut microbiota, and analyzed the frequencies of Treg cells and Th17 cells in lymphocytes, as well as the levels of inflammatory factors and LPS in serum. The results showed a decrease in bacterial richness in PMOP, with significant differences in the relative abundance of the gut microbial community, including an increase in Bacteroidetes, Proteobacteria, and Campylobacterota, and a decrease in Firmicutes, Blautia, and Faecalibacterium. Additionally, the concentration of Treg cells and related interleukin-10 (IL-10) in the peripheral circulation was reduced, while

pro-inflammatory cytokines were elevated [94]. Metabolites of the gut microbiota are important regulators of immunity. Xiaoyu Cai et al. analyzed the gut microbiota of PMOP patients using 16 S rDNA sequencing, and determined the levels of metabolites and the frequencies of immune cells. They found that the gut microbiota diversity of PMOP patients was decreased, and the restoration of the gut microbiota in these patients increased the level of the gut microbiota metabolite glyoursodeoxycholic acid (GUDCA). Circulating GUDCA enhanced the differentiation of CD4⁺ T cells into Treg cells via the constitutive androstane receptor (CAR), increased the number of Treg cells, promoted osteoblastic differentiation, and alleviated osteoporosis [95].

Probiotics and prebiotics can effectively increase BMD and bone strength, reduce bone loss, and improve osteoporosis through the “gut-immune-bone” axis. Tingyang Ai et al. found that treatment with Bifidobacterium improved BMD, bone volume/total volume ratio (BV/TV), and trabecular number in ovariectomized rats, and effectively inhibited bone loss. Furthermore, Bifidobacterium treatment could suppress the expression of inflammatory cytokines in the gut, alleviate intestinal inflammation, and thereby inhibit the excessive production of osteoclasts. Its mechanisms of action include the protection of mucosal barrier factors (including occludin, zonula occludens-1, claudin-2, and mucin 2) and a reduction in pro-inflammatory M1 macrophages [96]. In addition, Jhih-Hua Jhong et al. found that after treatment with heat-killed *Lactobacillus paracasei*, the serum levels of IL-17 and LPS in ovariectomized mice decreased; the mRNA level of RANKL in tibial tissue was reduced, while the levels of TGF- β and IL-10 were increased. These changes further improved osteoporosis through the “gut-immune response-bone” axis [97]. Regarding prebiotics, Ang Li et al. found that 2-fucosyllactose, as a prebiotic, could not only restore gut microbiota diversity but also reduce M1-polarized macrophages and alleviate inflammation, thereby preventing osteoporosis through the dual approaches of regulating gut microbiota and immunity [98]. A study by Tingyang Ai et al. showed that the prebiotic konjac oligosaccharide promoted the growth of Bifidobacterium longum, restored the balance of Treg/Th17 in bone marrow, facilitated intestinal barrier repair, reduced pro-inflammatory cytokines, and decreased osteoclastogenic activity, thereby inhibiting inflammation-related bone loss [99].

Therapeutic implications

Modulating gut microbiota for osteoporosis

Alterations in gut microbiota are known as a strategic method for osteoporosis management and prevention. The reason behind this is the critical role that gut microbes play in bone health by orchestrating immune system interplay and metabolic function. Probiotics are the most efficient

method of achieving this. Provided in adequate levels, these probiotic bacteria can be referred to as living microorganisms that promote health benefits to the host. For instance, the results from a study by Chen et al. [54] show that specific probiotics regulate microbial homeostasis in the host room and improve bone strength. Special bacterial strains, including *Lactobacillus* and *Bifidobacterium*, are associated with modifying the gut microbiome structure through stimulating the synthesis of the anti-inflammatory SCFAs. Therefore, suppressing gut inflammation and, possibly, modulating bone apposition or resorption, these beneficial bacterial strains might be able to avoid the development of bone-resorbing cells and promote new bone production in the human body.

Probiotics can also influence immunity by controlling the activities of immune cells located in the gut and probably inside the bones. For instance, the population of Tregs might be augmented through probiotic supplementations. These cells have the characteristic of issuing anti-inflammatory signals, which are responsible for a healthy equilibrium between bone apposition and resorption. In an independent study, Guo et al. [100] demonstrated that *Lactobacillus rhamnosus* GG supplements for OVX rats augmented their bone strength. This work proposed that such a probiotic modulated both the Th17 and Treg response, which, in turn, supported the gut microbiota stability. Moreover, the variations in the Th17/Treg balance were associated with the reduction of systemic inflammation, thus, the modulation of gut microbiota composition could be attributed to improved nutrient absorption as well as anabolic and metabolic functions, ultimately leading to the enhancement of bone strength. Therefore, these possible results indicate that some probiotics can facilitate interactions between bones and the gut, and they can be employed as a novel treatment to prevent and cure osteoporosis.

Apart from probiotics, targeted nutritional strategies such as increased prebiotic fiber intake (e.g., inulin from chicory root, fructooligosaccharides in onions/garlic) or consumption of polyphenol-rich foods (e.g., berries, green tea) are effective approaches for modulating gut microbiota composition. Prebiotics are compounds that carry the property of feeding or improving the activity of beneficial bacteria in the digestive system. Dietary fiber is the utmost prevalent instance. As per Kim et al. [43], the prebiotic enriched diet could, in fact, promote the growth of healthy gut bacteria that in turn contribute to overall health and positively benefit to bone health by assisting in the creation of essential metabolites that play an important role in bone metabolism. Some studies corroborate that SCFAs synthesized by gut bacteria during the process of fermentation, especially when prebiotics are involved in the process, comprise a crucial effect on the behavior of osteoblasts and osteoclasts. SCFAs are not only enriched via osteoblast activity but still

diminished osteoclast activity, meanwhile, promoting the formation of bone tissue and increasing the old bone mass loss, respectively.

Synbiotics link probiotics and prebiotics, the proper use of which allows it to maintain microbial equilibrium in the gut. It is anticipated that the results of combining pro- and prebiotics will improve gut bacteria balance and promote bone strength due to the synergistic effects of synbiotics. To exemplify, a synbiotic blend, comprising of certain probiotics and additional prebiotics that are required for probiotic functioning, can assist in securing stable and continuous alternations in the gut microbiome, thereby enhancing the production of beneficial metabolites in the gut and skeletal systems and providing tangible immune regulation effects [101]. In addition to probiotics and prebiotics mentioned above, FMT is an emergent treatment that deserves attention in future studies. This technique includes the transfer or transplantation of the stool samples from healthful donors to the painful recipients in order to recuperate their bowel microbiome. Albeit this technology is more commonly associated with studying several diseases and shows high potential in this field, its power to prevent and treat osteoporosis is currently unknown [20, 102].

The perspective that probiotics mismatching in gut microbes could contribute to better osteoporosis conditions via contributing to the restoration of the gut microbes' ecosystem is gaining a stronghold. If the findings are in favor, then FMT could be a novel way for preventing the development of osteoporosis alongside connecting the wellbeing of the gut with bone strength [103, 104]. Therefore, interacting with microbes of the gut through probiotic supplementation, prebiotic-enriched diets (e.g., high-fiber or polyphenol-rich foods), synbiotics, and FMT promises a novel way for osteoporosis treatment and prevention. These methods not only alter the gut microbiome composition and its functions but also help in minimizing systemic inflammation levels and improve nutrient absorption, which, in turn, enhances bone strength. Finally, widespread extra studies are needed have a clear understanding of the underlying mechanisms as well as methodology of how to improve their practicability. Researchers should be directed towards the identification of the most effective probiotics strains, extraordinary components, most appropriate ratios, and the most convenient delivery and duration of synbiotics medication regimens to maximize their outcomes in treating osteoporosis.

Estrogen replacement and gut microbiota

Due to its wonderful effectiveness in alleviating menopausal discomfort and protecting the bones from dissolution, there is a history of the use of estrogen replacement therapy (ERT). Several studies suggest that ERT has the

capacity to change the profile of gut microbiota as well, which could impact the usefulness of ERT and health in general. Hases et al. [45] researched the outcomes of estrogen supplementation in OVX (ovariectomized) rats. Their findings revealed that it was through the modulation of gut microbiota that estrogen caused a variety of changes, especially the rise of beneficial strains, such as *Lactobacillus* and *Bifidobacterium*. On the other hand, such modification of gut microbiota had been connected with pronounced intestinal barrier function, shown especially by elevated levels of tight junction proteins [105, 106]. The authors linked these findings to the hypothesis that estrogen may impact either the gut epithelium directly or function through the modulation of immune system dynamics, and thereby influence the gut microbiota dynamics and eventually affect the intestinal barrier permeability. Meng et al. [40], on the other hand, focused on the mechanistic details of how β -estradiol impacted on intestinal functionality and gut microbiota as well. According to the results of their research, β -estradiol treatment changed the gut microbiota composition, which in turn was associated with a variety of gene expressions with regard to the intestines' function and metabolism. The research indicated that the estrogen receptor pathway together with the GPR30 receptor augment the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin complex' effect, which further influences the gut microbiome and its associated features. On one side, they are performing functional studies that investigate the potential effect of estrogen replacement therapy on gut microbiome. Two scientists, Chen et al. [42], revealed this fact that reduction of estrogen levels is linked to gut microbial alteration; however, the application of estrogen can restore this condition.

Gastrointestinal cells are affected by estrogen through the modulation of mucus secretion, which creates favorable conditions for the growth of a diverse bacterial community dominantly consisting of beneficial species. A potential improvement in gut health and indirectly a bone health occurs upon restoration of estrogen levels due to the Gut-Bone Axis. The changes in gut microbiome could influence the metabolism of bone by hormone therapy, therefore, the existence of beneficial bacteria that help to reduce inflammation and promote bone density could be increased [107]. Nevertheless, estrogen is used for a long time, and this will increase the likelihood of the development of breast cancer and cardiovascular complications. Therefore, it is important to review the advantages as well as the downsides before taking this kind of treatment [108]. Especially with a significant disparity of ages, lifestyle, and baseline gut microbiota composition, the implications of estrogen supplementation in gut bacteria and bone strength vary widely. The connection between estrogen therapy and gut bacteria is highly associated with the health status of bones, mainly for those

with osteoporosis [109, 110]. There is no doubt that this relationship will also help to develop an effective approach to addressing the health issues of postmenopausal females. Further studies are needed to detect the detailed mechanism through which estrogen treatment influences the gut bacteria and patterns of bone strength. These efforts aim to adjust lifestyle habits in order to establish osteoporosis prevention and treatment regimens based on personalized approaches. Upcoming investigations, in the meantime, will be looking at alternative ways of providing equivalent benefits that are not at risk of side effects.

Conclusion

Summary of key findings

Estrogen is a vital hormone that maintains bone strength and health by controlling the density of bones within the body. It works by directly interacting with osteoblasts (cells that build up bone) and osteoclasts (cells that break down bone) to keep a fine balance between the two, thus fine-tuning the microenvironment of bones. The drop in estrogen levels leads to an imbalance in the process of bone turnover, further contributing to the increased risk of developing osteoporosis. In the matter of microbiota, the gut plays a crucial role in the health of the bones. Microbial metabolites, where these products are formed by gut microbes, exert their influence on bone cells, impacting their metabolic activities. Disruption of gut microbial balance can trigger inflammation of the bones and subsequently enhance the risk of development of this disease. Particularly, the axis of the gut-bone connection demonstrates how critical the diverse and healthy gut microbiota is for preserving and improving function of the bones. Here, microbial metabolites act as important managers, communicating with immune and skeletal cells when necessary, besides helping in the maintenance of the intestinal barrier, which is key to healthy bone environment and immune homeostasis.

Gut microbiota, bone microenvironment, and immunity form inter-related complex relationships. Under the influence of such disturbance, the gastrointestinal system's normal communication between the skeletal system and immune cells can be disrupted, which leads to the systems' inflammation reaction. The aforementioned interference is a key factor in the development of osteoporosis. In the advances of osteoporosis, immune cells have a dual role: not only they are involved in the bone remodeling process, but they also play a central part by initiating and accelerating the condition. It is crucial to have a detailed comprehension of their actions, which will provide the basis for developing the most optimal treatment options. This relation is rather dynamic, mainly caused by the change in estrogen levels and the gut microbiota, hence affecting bone health through sophisticated mechanisms in many different ways.

Lowered estrogen levels may target gut microbiota and bring about notable shifts in the composition, which can lead to both altered estrogen metabolism and availability, consequently, impacting bone health. Altogether, these aspects shape the bone health status of an individual, each pointing to their connections.

Future research directions

Estrogen and bone health research, particularly in osteoporosis, needs to target specific aspects. There is a need to explain the role of estrogen in both gut microbiota and bone health, which includes understanding the metagenomic and metabolomic profiles of gut bacteria to estrogen. Determining the bacterial strains that specifically affect bone health and elucidating the mechanisms by which they interact with bones are extremely important tasks. They must also be studied to identify the specific bioactive compounds produced by gut bacteria that modulate immune responses and cellular processes concerning the bones. Further studies along these lines are warranted to evaluate whether gut microbial modulation can influence immune mechanisms and to examine the possibility of utilizing genetic modifiers as adequate therapeutic targets. Personalized pharmacological strategies in the context of immune system modulation and the further optimization of mesenchymal stem cell therapies as a backbone for successful treatment approaches are central to their implementation. In particular, it is critical to sponsor additional research on the effects of probiotics, prebiotics, and synbiotics on the gut microbiota composition. In concert with such an accumulation of knowledge, novel mechanisms and biomarkers discovery using state-of-the-art technologies will be required to progress in communicating with our patients. Understanding the interactions among diet, exercise, and stress with gut microbial community and bone strength can tremendously help in improving osteoporosis and other related conditions' management.

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Authors' contributions

Maohua Chen, Jiaxu Wang, Yuanhang Yang, Yingge He, and Liqi Li wrote the main manuscript text and prepared figures. All authors reviewed the manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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