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Review Article

Gut Microbiome and Osteo Integrity in Osteoporosis Management

The Unequivocal Dyad of Gut Microbiome and Osteo Integrity- Dynamic Cross-Talk and Implications in Osteoporosis Management

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Abstract: The gut microbiome is the consortia of microorganisms, viruses, fungi, bacteria and protozoa, that inhabit the gastrointestinal human tract. Many nonmodifiable and modifiable factors regulate the gut microbiota composition, which includes age, sex, medications, stress, environmental triggers and diet. The symbiotic relationship between the gut microbiome and the human host is crucial for mutual survival. The dysbiosis of gut microbiota is the underlying causative factor in the development of many inflammatory diseases. Bone is a dynamic organ, that continuously remodels throughout the entire life, maintaining an equilibrium between osteoblastic bone formation and osteoclastic bone resorption. The term "osteo microbiology" is rapid in emerging research that shows the roles of gut microbes in bone mineral density and skeletal integrity. The gut is a major source of water-soluble and lipid-soluble vitamins like B and K and it directly or indirectly influences bone remodelling and mineralization of the bone matrix. The gut microbiota like butyrate released metabolites to influence the actions of T regulatory cells, exerting an indirect effect on osteoblast proliferation and bone formation. In addition, the microbial metabolites generated by gut microbiota can inhibit osteoclastic activity and prevent bone loss. Thus, the gut microbiota can modulate bone remodelling equilibrium and protect the bone against metabolic diseases like osteoporosis (OP). The present review discusses the importance of the gut bone axis, modulation of this axis by active cross-talk between the gut microbiota and bone cascades, thereby enhancing the positive effects of skeletal integrity, bone health and OP management.

Keywords: Gut microbiome; Bone; Skeletal integrity; Osteoblasts; Osteoclasts; Remodelling.

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1. INTRODUCTION

The gut comprises a dynamic organ, that shelters a rich diversity of microbial flora, which include bacteria, fungi, archaea, protozoa and viruses. '*The gut microbiome'* is referred to as the collective consortia of microbes. Many *gut microbes* live-in harmony with the human host, thereby making this symbiotic association a crucial element in mutual survival. The human host plays a beneficial role by offering secondary metabolites important to sustain life from microbes, the lone sources for such metabolites. The gut microbiota significantly influences innate and acquired immunity as well as controlling the production of immunological intermediates, which include several cytokines, that determine the outcome of proinflammatory or anti-inflammatory responses. The gut interacts with proximal and distant organs, evident from the existence of gut-brain, gut-bone, gut-lung, gut-heart and other biologically important loops[1-4]. However, alterations in microbial diversity of the gut (dysbiosis) contribute to the pathogenesis of many diseases. In addition, alterations in the cross-talk between gut microbiomes and other intermediates constitute complex interactions that contribute to the pathogenesis of many diseases, which include cancer, Inflammatory Bowel Diseases (IBD), diabetes, neurodegenerative and autoimmune disease [5-9]. Understanding these complex interactions to modulate biological processes in the human host is crucial in the management of diseases, especially progressive diseases of which, no permanent cure prevails. Bone is a tissue that undergoes regular remodelling in the entire life. Bone remodelling is the process where old and worn-out bones are replaced with new healthy bones. Bone remodelling repairs micro-damaged bones and regulates calcium homeostasis, brought about by finely orchestrated actions of the bone builders-osteoblasts and bone resorbing-osteoclasts [10]. A delicate equilibrium between osteoblasts and osteoclasts facilitates bone remodelling as well as being responsible for the shaping, growing and maintenance of bones. Any disruption or alteration of this equilibrium will affect the skeletal system, forming the basis of several diseases [11]. The bone homeostasis regulation encompasses the intercellular cross-talk among osteoblast, osteoclast, osteocyte and chondrocyte. Factors involving intricate cell signalling networks regulate this coordination [12]. In recent years, the positive link between the gastrointestinal tract and bone metabolism is well established in recent years. Osteoporosis (OP) is a debilitating skeletal disease, progressively manifested through reduction of bone mass, osteo micro architectural deterioration and augmented brittleness of bones. OP causes bone fragility, compromises bone strength and predisposes individuals to face a high risk of developing fractures. Contrary to decades ago when OP was mostly observed in elderly people, the current scenario is alarming in many young productive people, in their active period of life showing the early onset of the disease [13]. Postmenopausal osteoporosis, the most prevalent type of OP, is developed from estrogen deficiency. Hence, estrogen replacement therapy is proven a gold standard treatment for OP. However, estrogen therapy increases the risk of an individual developing breast and endometrial cancers [14,15]. Other preferred lines of OP treatment are by using the calcitonin, selective estrogen receptor modulators (bazedoxifene, raloxifene), a monoclonal antibody to RANK ligand (denosumab), bisphosphonates (ibandronate, alendronate, risedronate and zoledronic acid), and the analogue of parathyroid hormone (teriparatide) [16-21]. The OP treatment options are challenging, because the treatment should be carried out during the acute and mild phases to avert future fractures.

1.1. Primary and secondary osteoporosis

Primary osteoporosis refers to decline or loss of bone mass related to aging and reduced gonadal function. This is not caused due to existing illness or chronic medical conditions. Two distinct types of primary osteoporosis have been reported referred to as Type I and Type II primary osteoporosis. Type I primary osteoporosis is prevalent in postmenopausal women with decreased levels of estrogen [22]. Estrogen deficiency induces increased production of proinflammatory cytokines IL-1 and IL-6 which stimulate the osteoclasts that eventually resorb bone resulting in accelerated loss of bone mass [23]. Type II osteoporosis also referred to as Senile osteoporosis unlike Type I occurs in aged population of both sexes. The pathogenicity of this type of osteoporosis and the resultant bone loss is not completely understood and remains obscure. It is also reported to differ from Type I primary osteoporosis in that, it is not essentially due to increased osteoclastic activity. [24] Secondary osteoporosis is caused due to chronic illness or existing medical conditions like hyperparathyroidism, hyperthyroidism or leukemia. Secondary osteoporosis could also be caused by long term treatment with certain medications. For example, corticosteroids, thyroid replacement therapy, treatment with aromatase inhibitors could all predispose an individual for the risk of developing secondary osteoporosis [24]. These conditions/medications which induce significant bone loss may superimpose upon the primary risk factors which culminate in osteoporosis thereby exacerbating bone loss in affected individuals. Idiopathic osteoporosis is another type of osteoporosis where the pathogenesis is of unknown origin. This is widely prevalent in both children and adolescents and usually manifests between 8-14 years of age. The disease usually goes into remission at the age of puberty accompanied by resumption in the normal growth of bone. Although osteoporosis is a preventable, treatable and manageable disease, there is no defined cure for the disease. Intervention is mainly aimed at preventing further bone loss in affected individuals through medication, hormone replacement, lifestyle modifications, alterations in diet regime and prevention of falls. Although, women are at more risk for the development of osteoporosis, post menopause, recent statistics imply that, men are also frequently affected with the disease owing to andropause caused by decreased testosterone levels with advanced age.

1.2. Osteoblasts and osteoclasts-the builders and destructors of bone

Bone being a dynamic tissue undergoes remodelling which is a continuous process of resorption during, which the old bones will be digested and removed followed by formation during which bone is renewed to maintain strength and mineral homeostasis. The remodelling cycle comprises the following phases; activation, resorption, reversal, formation and quiescent phases. The integrity of the skeletal system depends on the coordinated actions of two important cell types which are controlled in an orchestrated fashion resulting in a sequel of events that build or break the bone [25]. The cells of the bone which are involved in formation of the bone are referred to as osteoblasts and the cells which are involved in breakdown of the skeleton are referred to as osteoclasts. Both osteoblasts and osteoclasts act in tandem

with each other contributing to the formation and resorption respectively at regular intervals thereby maintaining bone homeostasis and bone remodelling equilibrium. Any shift in this delicate equilibrium that exists between the action of osteoblasts and osteoclasts will, adversely influence bone homeostasis culminating in debilitating diseases like osteoporosis . Activated osteoclasts mediate bone resorption through dissolution of minerals and degradation of organic matrix components of the bone in the localized environment under the ruffled borders of the cell. Bone formation is a complex sequence of events that involves proliferation of mesenchymal stem cells (MSC) and their differentiation into osteoblast precursors, formation of both organic and inorganic components of the bone matrix and finally mineralization. Activated osteoblasts secrete bone matrix proteins such as collagen I and several non-collagenous proteins including osteocalcin, osteopontin, osteonectin and bone sialoprotein II. The principal function of osteoblasts is to synthesize the proteins of the bone matrix and to bring about the process of calcification [26].

1.3. Risk factors for osteoporosis

Lack of exercise, sedentary lifestyle, calcium deficient diet, Vitamin D deficiency, Hyperparathyroidism, decreased calcitonin levels and genetic predisposition could all make an individual vulnerable for the development of osteoporosis [27]. Vitamin D deficiency and secondary hyperparathyroidism could not only culminate in rapid bone loss and enhanced bone fragility, but also induce neuromuscular impairment that can predispose an individual to the risk of falls [28]. The OP (primary or secondary) and other ailments such as gastrointestinal complications, CVDs, cancer risk and age are some of the key factors that influence the pathogenesis of the disease based on which, it is desirable to determine the best anti-osteoporotic regime for a patient, considering the serious adverse effects of some antiosteoporotic drugs. Modifiable and unmodifiable risk factors like genetic predisposition, environmental triggers, dietary habits, sex, age and comorbidity can make an individual susceptible to OP [29-36.] However, dietary interventions can prevent or be used to manage OP [37]. Recently, the significance of the gut microbiome has been understood, serving as a potential modulator to control "*on and off switch"* for many human diseases. The gut bone axis exhibits complex crosstalk that determines the outcomes of osseointegration and skeletal health, thereby preventing and managing diseases that affect the skeletal system. Understanding the mechanisms of the gut microbiome on bone metabolism has opened promising avenues to alleviate fracture risk and improve bone mineral density in osteoporosis patients.

1.4. Gut bone axis and bone metabolism

The gut microbiome modulates homeostasis through intestinal and extraintestinal effects. Metabolites generated from the microbes have an influential role on host organs that circulate from the gut into the systemic circulation [38]. The gut microbiota-bone axis is the effect of the microbial community associated with gut or the metabolites for regulating skeletal homeostasis and achieving bone integrity[39]. The emerging trends in osteo microbiology implicate that, gut microbes alter bone mineral density. The key endocrine modulators are calcitriol, calcitonin and Parathyroid hormone (PTH)[40]. Calcium is another proven key element in the gut-bone signalling axis, that functions via the calcium-sensing receptors (CaSR) [41]. In the human body, the CaSR are in various tissues of the stomach, intestine, parathyroid glands and kidneys [42]. Hence, its utmost importance in human health is tight control of calcium homeostasis through dynamic interactions between these organs [43]. As the gut is a major source of water-soluble and lipid-soluble vitamins like B and K, the organs directly or indirectly remodel bone and mineralization of the bone matrix [44]. Osteocalcin is a non-collagenous protein that is abundant in the bone matrix [45, 46]. Vitamin K is vital for the activation of osteocalcin, also called bone Gla-protein. Vitamin K mediates the carboxylation of osteocalcin. This carboxylation is crucial for the binding of osteocalcin to bone minerals during bone formation. Although vitamin K is obtained through dietary sources, intestinal production of vitamin K through gut microbiome and microbial metabolism constitutes an important vitamin source. Reduced vitamin K production owing to dysbiosis of gut microbiota could cause inadequacy in vitamin K levels, culminating in elevating concentrations of uncarboxylated osteocalcin in systemic circulation. The absence of bound carboxylated osteocalcin in the bone matrix weakens the bone matrix and makes the bone tissue highly brittle and vulnerable to fracture. Metabolites released from the gut microbiota like butyrate can influence the T regulatory cells' actions, exerting an indirect effect on osteoblast proliferation and bone formation[47]. In addition, these microbial metabolites generated by gut microbiota can inhibit osteoclastic activity and prevent bone loss [48]. Gut bacterial growth influences mineral bone density [49.] Stefano et al. [50] reported that bone loss at the femur neck and lumbar spine was associated with intestinal bacterial overgrowth, implicating that intestinal bacterial overgrowth is a predisposing factor in osteoporosis. Gut bacteria overgrowth can lead to malabsorption, thereby having serious repercussions on the metabolism of calcium, Vitamin K, Vitamin B and carbohydrates, required for controlling key osteogenic events [51].

1.5. Management of osteoporosis

Osteoporosis is a progressive skeletal disease and there is no cure for it. But it is a preventable and manageable disease. High risk populations who are predisposed to develop osteoporosis at a later stage in life could be closely monitored and evaluated for essential parameters, that reflect bone health. This includes imaging studies involving measurement of Bone Mineral Density (BMD) and evaluation of blood markers like Serum calcium and Vitamin D levels. Postmenopausal osteoporosis can be managed with Estrogen Replacement Therapy (ERT), although it has a risk which predisposes the recipient to increase in incidence of breast and endometrial cancer [52]. Treatment with recombinant parathyroid hormone is the only proven and effective drug available in the market that helps in bone formation. Most of the other antiosteoporotic drugs act either by inhibiting or slowing down bone resorption. Bisphosphonates and selective estrogen receptor modulators like Raloxifene, bazedoxifene are among the other first line drugs currently used for the management of osteoporosis [53].

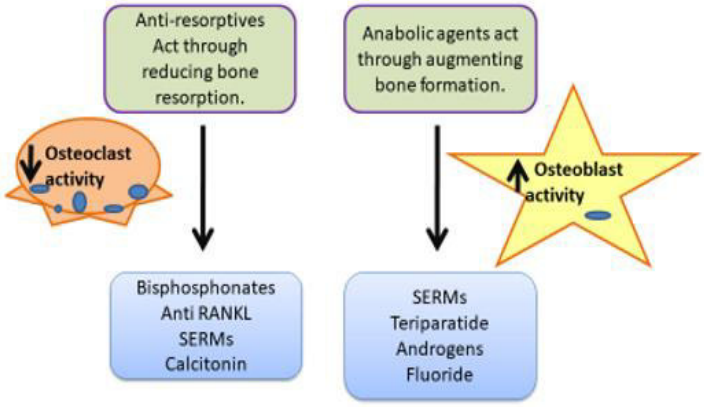

Fig 1: Schematic illustration of commonly used antiosteoporotic drugs.

Osteoclasts are bone-resorbing cells, which are targets for antiresorptive drugs. Osteoblasts are bone-forming cells, which are the targets for anabolic agents. Treatment options aim to minimize fracture risk by reducing bone loss and improving bone mass.

1.6. Lifestyle modifications

Lifestyle modifications can considerably help to slow down the rate of progression of the disease. Avoiding a sedentary lifestyle, indulging in regular exercises like walking, weight bearing exercise and other non strenuous exercises, avoiding or restricting the use of alcohol and tobacco smoking can help reduce the severity of the disease. In addition to this, avoiding episodes of falls can appreciably reduce the risk of fatal fractures (hip region, wrist and vertebrae) commonly seen in osteoporotic patients [54, 55]. All these lifestyle modifications could greatly improve the quality of life in osteoporotic patients and considerably reduce the morbidity associated with the disease.

1.7. Nutritional and nutraceutical support

Consumption of a healthy balanced diet rich in green leafy vegetables, fruits and dairy products, regular intake of calcium and vitamin D supplements could all help in preventing further damage due to the disease. Polymorphisms in Vitamin D Receptor gene (VDR) gene is one of the well known risk factor, that predispose an individual not only to osteoporosis but also to breast cancer [56]. Micronutrients including Vitamin C and zinc could help modulate the immune system, influence the production of proinflammatory cytokines and hence could help to reduce the rate of osteoclastogenesis. A diet rich in probiotics could also be potential adjuncts in the management of osteoporosis as they are proposed to have an array of health benefits [57], including maintenance of skeletal integrity. Melatonin is a pineal hormone, that controls the circadian rhythm and sleep-wake cycle in humans. Recently, it has been proven, that melatonin rich foods like tomatoes, bananas and others can greatly influence the production of cytokines and the outcome of immune response and controls the bone remodelling process by slowing down the rate of bone resorption [58].

2. Probiotics –The Benefactors of Bone

Different research groups have reported the beneficial effects of probiotic administration in preventing bone loss and preserving bone mineral density [59-60]. The probiotics Lactobacillus spp (*L. reuteri, L. rhamnosus* GG, *L .paracasei* and *L. plantarum*), *Bifidobacterium longum*, prebiotics mixtures such as *L. paracasei* and *L. plantarum*; VSL# (combination of probiotics) were shown to exert protective effects against bone loss in several *in vivo* and *in vitro* models. Britton et al.,[61] showed that probiotic *(L .reuteri)* treatment in ovariectomized mice culminated in attenuation of bone loss. Immunomodulatory factors secreted by *L. reuteri* confer protection against bone loss. Reports indicate that known bone resorption activators like TRAP 5 and RANKL remarkably reduced upon treatment with probiotics. [62] The findings support that osteoclastogenesis was reduced in mice treated with *L. reuteri*, in agreement with the reports that *L. reuteri* inhibited osteoclastogenesis in the *in vitro* models. A considerable increase in the helper T cells ($CD4^+$) population was observed in ovariectomised mice in comparison with control. This demonstrates that *L. reuteri* modulates bone metabolism through osteoclastogenesis suppression, thereby inhibiting osteoclast triggering signals from T-cells .[62] McCabe et al. [63] reported that male mice, femoral and vertebral bone mass were enhanced during treatment with the oral probiotic, *Lactobacillus reuteri.* The authors examined the RNA profiles of jejunal and ileal samples, demonstrating that *L. reuteri* could inhibit the basal TNFα mRNA in male mice. The study showed that *Lactobacillus reuteri* reduced intestinal inflammation through anti- TNFα activity *Bifidobacterium longum* administration elevated serum osteocalcin levels and favoured bone formation parameters. Parvaneh et al. [64] reported reduced serum C-terminal telopeptide levels and bone resorption parameters. The supplementation with *B. longum* shows the femur microstructure modifications and increases bone mineral density in ovariectomised rats. Augmenting bone formation and decreasing its breakdown by upregulating SPARC and BMP-2 genes [65] is crucial for human health. Recent evidence showed that, *Lactobacillus rhamnosus* exhibited an immunomodulatory effect to promote bone health in ovariectomised mice, as this was evident from decreased osteoclastogenic cytokines (Interleukins 6 and 17, TNF-α), concomitant with an increase in anti-osteoclastogenic cytokines (Interleukins 4 and 10, IFN-γ) in the treated-group. *Lactobacillus rhamnosus* suppresses osteoclastogenesis and regulates Treg-Th17 differentiation and distorts Treg-Th17 cell balance, thereby influencing bone metabolism [66] Ovariectomised mice treated with Lactobacillus species (*plantarum* GKM3 and *paracasei* GKS6) diminished bone resorption by modulating Bone Morphogenetic Proteins (BMP) and RANKL pathways, eventually improve osteoblast differentiation and suppresses osteoclast formation [67]. Recent reports implicate that, the

probiotics beneficial effects on human hosts are primarily due to commensal microbes stimulation, which enhances butyrate production [68]. VSL#3 is the commercially available probiotic supplement, which is a consortium of bacteria comprising *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus, Lactobacillus paracasei* and *Streptococcus thermophilus.* This probiotic supplement protects bone health and prevents bone loss through modulation of increased intestinal permeability and diminution of inflammation in the intestines and bone marrow [69].

2.1. The protective effects of probiotics on bone

Bone remodelling is a continuous but complex process that is associated with integration of signals derived not only from various bone cells but also emanating from other systems like endocrine system, immune system and the nervous system. The close nexus between maintenance of bone homeostasis and the gastrointestinal system through regulation of calcium absorption is well understood. Recently, lot of research attention has been paid to understand the emerging role of gut microbiota in regulating the dynamic bone remodelling process. Augmentation of gut microbiota through ingestion of prebiotics/probiotics has been identified as a novel and effective strategy to regulate the deranged bone remodelling process induced due to a number of pathologic conditions that accelerate bone loss, weakens the bone and lead to systemic skeletal diseases like osteoporosis [70]. One of the early reports implicating the effects of gut microbiota on bone was by Sjogren et al [71]. It was reported that, probiotics can influence gut health by regulating the pH of the intestinal lumen, production of antimicrobial peptides, potentiating the barrier function of intestines through enhanced mucus production and modulating the immune efficiency of the host. In a previous report involving the usage of Lactobacillus reuteri 4 weeks of oral administration of probiotics in male mice and not female mice was found to result in a significant increase in Bone Mineral Content (BMC), Bone Mineral Density (BMD), trabecular number, trabecular thickness, and trabecular density of the vertebra and femora. This enhanced bone density was reported to be due to an increase in bone formation mediated by osteoblasts. Although the mechanism of action underlying this effect could not be fully understood, it is reported that supplementation with the probiotic bacteria was shown to decrease the expression of cytokine TNF-α in the small intestines. [71] Another study reported that rats treated with yogurt loaded with *Lactobacillus casei, Lactobacillus reuteri* and *Lactobacillus gasseri* as probiotic supplement enhanced the absorption of calcium, that in turn resulted in significant increases in Bone Mineral Content (BMC) as compared to control animals that did not receive the supplement. Male rats supplemented with *Bifidobacterium longum* for 28 days exhibited elevated levels of calcium, magnesium and phosphorus in the tibia than the untreated animals. All these reports indicate the relevance of probiotic supplements rich in gut microbiota with bone health and integrity [72].

2.2. Mechanism of action of probiotics

Although not completely understood, Some of the mechanisms through which probiotics exert their beneficial effects are 1. Competitive exclusion of pathogens 2. Bacteriocin production 3. Enzymatic modulation of activities on the gut lumen 4. Enhanced concentrations of short chain fatty acids such as butyrate, acetate and propionate. 5. Immunomodulatory properties 6. Antiinflammatory effects on the intestines. Competitive exclusion is the active competition between two bacterial species for a receptor in the intestinal tract and in the competition, one overtakes the other and thereby prevents binding of pathogenic bacteria in the intestines [73]. The major regulatory mechanisms and key signaling pathways that control this need to be investigated further. Bacteriocins are antimicrobial peptides produced by Bifidobacteria and lactobacilli which inhibit the proliferation of pathogenic bacteria. Increased bacteriocin production could contribute a mechanism for the antibacterial properties of probiotics against pathogenic bacteria. Also, strong cell adhesion property and increased mucin production are other factors that contribute for the antagonistic effects of probiotics against many pathogenic bacteria. Short Chain Fatty Acids (SCFA) have multifaceted beneficial roles in many tissues, including brain, liver, intestines, muscle, adipose tissue etc., It was reported that SCFA acetate production by *Bifidobacterium* can enhance epithelial cell mediated defense in the intestinal tissue and thereby defends the host against infections which could otherwise be lethal. Finally, the major mechanism of action of probiotics appears to be mediated through excellent fine tuning of the immune system through the production of immunomodulatory and anti-inflammatory molecules. This helps to regulate the host immune response.[74] Probiotics actively stimulate the production of sIGA by intestinal B cells. This improves innate immune defences majorly through enhancing the barrier function. Also probiotics reduce inflammation in the intestines through downregulated expression of TLRs and modulation in the activity of Gut Associated Lymphoid Tissue (GALT) [75]. In addition to all these mechanisms, epigenetic alterations which could probably confer potential anticarcinogenic effects may account for the beneficial effects and sense of well being acquired due to probiotic consumption. This particular area of research warrants further detailed investigation, especially on human subjects.

3. Regulation of Bone Metabolism by Gut Microbiota

3.1. *Microbiome of the gut and its role in mineral uptake in bone*

Bone metabolism influences the presence and availability of calcium. Calcium hydroxyapatite (99% out of the overall calcium) is the most prevalent form of calcium, distributed in the teeth and bones to confer strength to these hard tissues. Bone tissue is an important calcium reservoir for various vital metabolic functions mediated through intra- and extracellular calcium pools [76]. Dietary calcium proceeds through the walls of transcellular and paracellular mechanisms at the anterior of the small intestine[77]. Gut microbiota facilitate the absorption of calcium and vitamin D. Prebiotics are nutritional health supplements, providing multiple benefits, believed to influence the actions of probiotics for the gut microorganisms. Prebiotics control bone metabolism by changing the microenvironment of the gut. They feed the intestinal microbiota, and the active metabolites generated include short-chain fatty acids (SCFA), released into the systemic circulation, thereby exerting beneficial effects on the gastrointestinal tract and other organs [78]. SCFA interaction with proteins is responsible for calcium absorption and modulating bone metabolism by enhancing TRPV6 transcriptional levels and calbindin-D9k. SCFA reduces the

pH of the intestinal lumen. Thus, calcium absorption is enhanced owing to the repression of calcium complex generation[79]. Despite the underlying mechanisms, modifications in the gut microbiome for calcium absorption and other bone linked minerals are the strategy to reduce osteoporosis development. The calcium is absorbed through the transcellular active transport process in the mammalian small intestine. Vitamin D in calcium homeostasis facilitated diffusion and passive absorption via a paracellular chemical diffusion gradient over the intestine. Thus, augmenting calcium absorption through modulation of gut microbiota is a novel therapeutic approach for osteoporosis prevention. Treatment with prebiotic fibres could be an alternative strategy to favour calcium absorption and provide a favourable microenvironment for the bone[80]. Xenograft models revealed that any alterations in the gut microbiome during the rapid bone acquisition stage in the early stage of life (1-4 months) might compromise bone strength owing to impairment in function and loss of quality of materials of bone tissue [81]. The key factors that modulate the microbiota and regulate bone metabolism and influence pathogenesis of osteoporosis has been illustrated in Figure 1.

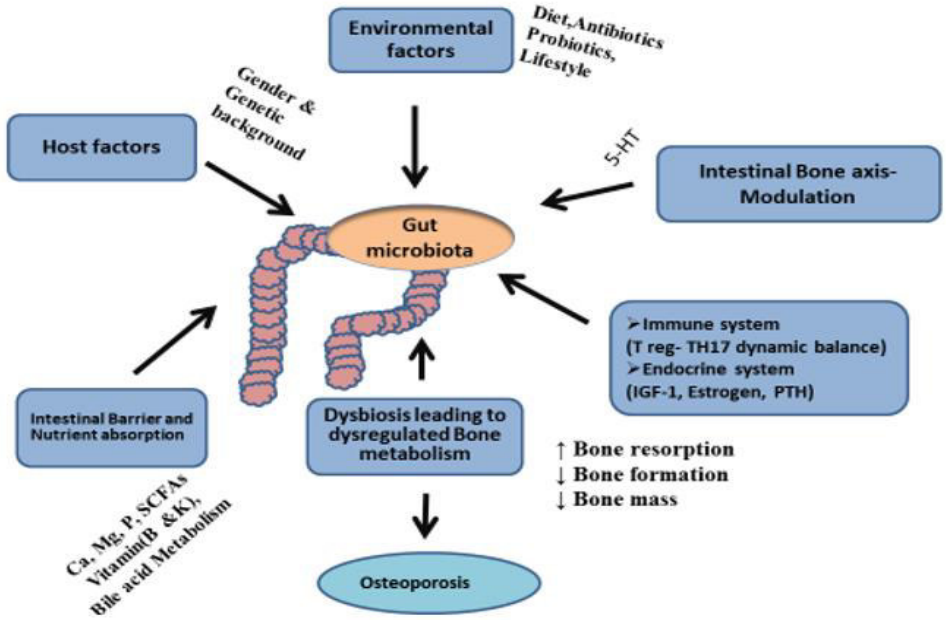

Fig 2: Key factors that regulate bone metabolism and the modulation by microbiota in osteoporosis.

The gut microbiome is predominantly influenced by diet, antibiotics and probiotics via its action on bone mass using multiple mechanisms such as 1) influencing beneficial bacteria, enhancing estrogen bioavailability and managing bone mass with the support of prebiotics. 2) Other mechanisms are augmenting the immune system by modulating the expression of inflammatory cytokines, 3) generating metabolites of the gut microbiome, for example, short-chain fatty acids. Other mechanisms to influence the gut microbiome are 4) Alteration of intestinal permeability and enhancing effects of vitamin D on bone mineral absorption, and 5) effects on the gut-brain axis and the intensity of hormones: 5-HT- 5-hydroxytryptamine; IGF-insulin like growth factor, SCFA, short-chain fatty acid, PTH-Parathyroid hormone.

3.2. Gut microbiome against bone loss –regulation bone metabolism of the immune system

Many studies reveal the process, in which the gut microbiome regulates osteoclast and osteoblast activities [82]**.** The immune system and the skeletal system form an intricate and complex network, assisting in making the body respond to invading pathogens [82]. CD4+ and CD8+ T cells play a crucial role in regulating bone health [83]. The diverse immune cells regulate bone remodelling, activate the intestinal lining and translocate bone tissue. The subset of T lymphocytes T reg cells stimulated at the intestinal lining and moved to the bone marrow, where they influenced bone remodelling in the trabecular and endosteal sites[83]. Numerous cytokines control osteo metabolism. The RANK-RANK Ligand interactions and the resultant downstream signalling offer extensive functions in the bone remodelling mechanisms [84,85] Interleukins (1, 6, 8, 11, 15, 1, 32) and tumour necrosis factor (TNF) are the major cytokines triggering osteoclastogenesis. However, Interferons (α, β, γ), and Interleukins (4, 10, 13, 18, 33) are the cytokines that possess inhibitory effects on osteoclastogenesis [85]. Depletion and deficiency of sex steroid estrogen are linked to bone loss. The gut microbiota regulates inflammatory responses and impacts estrogen levels. *In vivo* studies indicated that estrogen deficiency accelerated and augmented gut permeability and influenced higher Th17 cells. The impact of sex steroid deficiency was a trabecular bone loss, demonstrated in germ-free (GF) mice owing to enhanced osteoclastogenic cytokine production. This finding demonstrates the nexus between estrogen and gut microbiota, which has a direct implication on gut health and skeletal integrity. Treatment with *Lactobacillus rhamnosus* diminished gut permeability, reduced inflammation of intestines and bone marrow, providing a promising positive effect against bone loss. Thus, probiotic therapy reduces gut permeability and inflammatory pathways in sex steroid-deficient mice, reflecting the probiotics protective role against postmenopausal osteoporosis.[86] Some microorganisms that inhabit the gut and confer protective roles on the bone have been listed in Table 1.

Table 1: Gut micro microorganisms and their beneficial effects on bone growth and metabolism

S.No	Name of the microorganism	Effect on bone growth and metabolism
1	*Lactobacillus reuteri*	• Secretes immunomodulatory factors that suppresses TNF signaling and prevents bone loss • Suppresses RANKL and TRAP 5 and inhibits osteoclastogenesis
2	*Bifidobacterium longum*	• Elevates osteocalcin and enhances bone formation • Reduces bone resorption parameters • Upregulates Sparc and BMP-2 genes and increases bone formation
3	*Lactobacillus rhamnosus*	• Decreases osteoclastogenic cytokines IL-6, IL-17 and TNF-α • Increases antiosteoclastogenic cytokines IL-4, IL-10 and IFN-γ • Regulates Treg-Th17 differentiation and modulates bone metabolism
4	*Lactobacillus plantarum* & *Lactobacillus paracasei*	• Modulates Bone morphogenetic proteins(BMP) • Modulates RANKL pathways

3.3. The gut microbiota as an "endocrine organ"

Calcium-regulating hormones play a crucial role in producing healthy bone growth and remodelling. Numerous systemic and local hormones regulate bone metabolism [87]. It is confirmed that the gut microbiome supplies a net anabolic incentive to the skeleton, for which the insulin-like growth factor- (denoted as IGF-1) plays a crucial regulatory role. Juvenile growth in mammals is a pattern of longitudinal bone growth, which occurs at the ossification of the growth plates. This process is dependent on IGF1 via the endocrine and paracrine mechanisms. The interplay of the host with the gut microbiota influences the production and insulin/insulin-like growth factor, signalling the bone metabolism [88]. Parathyroid hormone (PTH) is crucial for postnatal skeletal development[89]. For PTH to exert its action, the metabolite generated by microbiota was pivotal to stimulate bone formation and enhance bone mass [90]. Gonadal steroids (androgen, estrogen) have dominant effects on the skeleton, growth, size and shape, playing a pivotal role in maintaining skeletal homeostasis during adulthood [91]. The gut microbiota modulates and regulates estrogen levels by secreting β-glucuronidase- the major enzyme that functions via conjugated estrogens into their biologically active forms. A highly imbalanced gut microbiota decreases microbial diversity and attenuates circulating estrogens [92]. *In vivo* studies in mice show that estrogen deficiency culminates osteopenia [93]. Interestingly, estrogen depletion did not induce appreciable bone loss if gut microbiota is absent [94]. Continuous PTH doses induce osteopenia and increase calcium mobilization. However, PTH doses can increase bone mass when administered intermittently. Nevertheless, the administration of parathyroid hormone did not influence bone mass in the absence of gut microbiota. Serotonin (also known as 5-hydroxytryptamine or 5-HT), the neurotransmitter, exerts its action by influencing skeletal metabolism via its transporter gene, serotonin transporter or 5-HTT [95]. Phytoestrogens are compounds that possess structural similarities with endogenous estrogens. They act as natural selective estrogen receptor modulators, and their activity is influenced by the gut microbiome. One of the well-known phytoestrogens, daidzein (soy-isoflavone), is actively converted into metabolite equol *in vivo* post-consumption. This bacterial metabolite of daidzein was proven to provide stronger estrogenic action on the bones [96-98].

3.4. *The triad of Microbiota, miRNAs and osteoporosis.*

About 18-25 necleutides. These molecules regulate and control gene expression through complementarity in bases between miRNA and target mRNA. Role of miRNAs have been implicated in the pathogenesis of many diseases including inflammatory bowel diseases (IBD), asthma and cancer. Several reports also implicate the involvement of miRNAs in the onset and progression of osteoporosis primarily through modulation of bone remodelling equilibrium. This has also been confirmed through bioinformatics based approach in which correlation between expression patterns of miRNA and post menopausal osteoporosis (PMO) has been identified. It was also reported that, five miRNAs were increased in the serum and bone of patients with osteoporotic fractures as compared to patients with non-osteoporotic fractures thereby providing a strong evidence for the nexus between miRNA expression and pathogenesis of osteoporosis. It is reported that the target of these miRNAs are crucial signaling pathways that control osteoporosis like androgen receptor signaling pathway, JAK-STAT signaling, Wnt signaling and TGF β signaling pathway. miR-195, miR-1-3p, miR 125a-5p, miR-100-5p, miR 338, miR 483-5p,miR194-5p, miR33-3p, miR33a, miR-497-5p, miR-181c-5p are few miRNAs that are closely associated with control of onset, progression and determining the outcomes of osteoporosis [99]. These miRNA control diverse processes that influence osteoporosis including modulating osteoclast function controlling osteoblast differentiation, reduced adipogenesis and enhanced osteogenesis of mesenchymal stem cells. Osteoporotic animals exhibited decreased Bone morphogenetic protein (BMP) and bone tension and enhanced production of miR in osteoclasts. Downregulated expression of miR-155 is associated with a reduction in TRAP, Il-1 Beta, M-CSF, RANK and a concomitant increase in leptin receptor with reduced cell proliferation and resorption of bone by osteoclasts. Hence one of the novel approaches for the therapy of osteoporosis could be pharmacological blocking of miR-155 expression [100]. Several reports demonstrated that miRNAs could be modified by microbiota, which modulate and regulate miRNA expression. Hence, a diet rich in probiotics not only influences microbiota composition of the gut but also significantly influences the expression of several crucial miRNAs in the host. Other than the host miRNAs, several exogenous miRNA derived from food have been reported. This strongly indicates that probiotics and other components that influence gut microbiota could be a potential source of miRNA that regulate homeostasis and modulate the pathogenesis of many bone diseases including osteoporosis. Inflamma-miRNAs primarily derived from food could exert anti-inflammatory

actions majorly by varying the configuration of microbiota in an interaction which is bidirectional. Fine tuning the expression of miRNAs by modifying the microbiota could be a good strategy in the therapy of osteoporosis [101, 102].

3.5. *Modulation of intestinal barrier integrity and gut homeostasis by exogenous agents*

The presence and composition of gut microbiota is greatly influenced by physical activity, medications and diet. The existence of different diet patterns in different geographical locations have shown great variability in the consortia of microbiota of the gut. Treatment with antidiabetic agents like insulin, metformin or dapagliflozin have been reported to alter the gut microbiota following administration. Several probiotic supplements containing bacterial species like Bifidobacterium and Lactobacillus species were reported to increase the production of Short Chain Fatty Acids (SCFA) in the gut. SCFA are associated with the tight regulation and maintenance of intestinal barrier and any defect in intestinal SCFA due to gut microbiota dysbiosis could result in the leakage of harmful pathogenic bacteria from the gut into the systemic circulation [103] . On the contrary Lipopolysaccharides (LPS) which is a major component of cell wall of gram negative bacteria play a role in enhancing the breakage of intestinal barrier and promote the leakage of harmful bacteria into the blood. Dysbiosis is reported to be associated with increased LPS and decreased SCFA, thereby affecting homeostasis in the gut [104]. The presence of these bacterial species in the probiotics was found to augment the growth of other beneficial organisms that were known to produce SCFAs. In these cases the probiotic administration was found to inhibit osteoclastic bone resorption and bring about a three fold increase in Bone Mineral Density (BMD) as compared with the control group [105]. Multiple mechanisms involved in the protective effects of probiotics and prebiotics against osteoporosis have been illustrated in Figure 3.

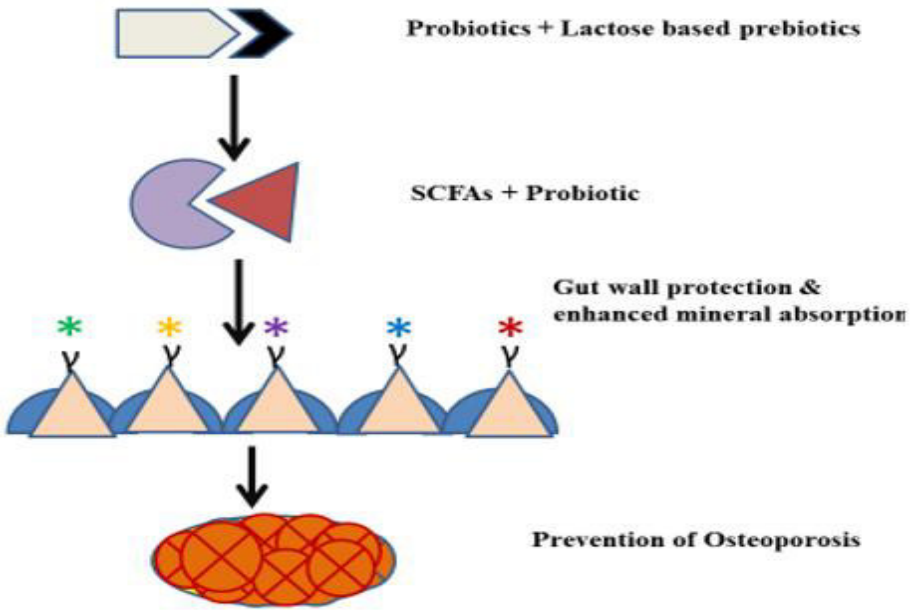

Fig 3: A schematic diagram for multiple mechanisms by lactose-based prebiotics and probiotics in preventing osteoporosis

Lactose-based prebiotics is transformed into short-chain fatty acids such as acetic acid, propionic acid and butyric acid. They also transform into lactic acid and gases (carbon dioxide, methane, and hydrogen) using gut microbiota. Lactose-based prebiotics exhibit unique functional values by (a) converting insoluble inorganic salts to soluble salts and boosting the absorption of these molecules to the gut wall, (b) preserving mineral absorption surface in the intestine, (c) enhancing bone remodelling (modulate the activity of osteoclasts and osteoblasts) and (d) degrading phytic acid.

3.6. *Dysbiosis and implications on host Immuno competence in osteoporosis*

The dysbiosis of gut microbiota and resultant compromises in intestinal barrier integrity is one of the key features that predisposes the individual to the development of bone related disorders like osteopathy, osteoporosis and osteoarthritis. Although human studies implicating the effect of gut microbiota and relevance of the immune system is scanty, animal studies have proven beyond doubt, the close association and cross talk between the two and the resultant outcome of immune response. Owing to the fact that osteoporosis is a systemic skeletal disease, there is a persistent low grade inflammation observed in the patients. This is also supported by data obtained from animal studies wherein bone loss is associated with elevated levels of proinflammatory markers like TNFα, IL-1, LPS and IL-17. The levels of these proinflammatory markers were significantly reduced in animals supplemented with probiotics or treated with short chain fatty acids. This indicates that dysbiosis of gut microbiota could have a definitive impact on the innate and adaptive immune defences influencing immune homeostasis also [106]. Recent reports indicate the benefits of biologically significant secondary metabolites produced by gut microbiota (detected in the faeces and plasma of the host) as potential markers to evaluate the progression of the disease in animal models of osteoporosis. The variations in these metabolites could influence the crucial endocrine signaling (like GLP-1and estrogen signaling) that can be pivotal in determining the outcomes of the disease. Restoring the equilibrium in the gut microbiota through nutritional interventions either in the form of probiotics or administration of natural anti-inflammatory agents could

restore the altered immune homeostasis and thereby have a beneficial effect on the immunocompetence of the host [107].

3.7. Role of human gut Microbiota on bone remodelling and osteoporosis

A number of studies involving animal models of osteoporosis have time and again shown the benefits of gut microbiota in reducing bone loss and resorption. Most of such studies were carried out with probiotic bacteria belonging to different species of *Bifidobacterium* and *Lactobacillus*. One study showed that administration of *L. reuteri* to osteoporotic mice models could completely protect against bone loss and could restore bone volume/total volume values to levels comparable to the control animals. In addition, significant enhancement in trabecular Bone Mineral Content and Bone Mineral Density was observed in the ovariectomized osteoporotic animals given *L. reuteri* as compared to untreated groups of animals. The mechanisms involving these protective effects were found to be associated with downregulated expression of mRNAs for TRAP 5 and RANKL both of which were found to influence osteoclastic activity [108]. Interestingly it was observed that treatment with L. reuteri did not significantly influence the bone formation process mediated by osteoblasts. There are also several reports indicating that administration of *Lactobacillus paracasei, Streptococcus salivarius, Lactobacillus rhamnosus, Bifidobacterium, thermophilus*, or commercially available probiotic supplement VSL# 3 to osteoporotic animal models could significantly decrease bone loss in the osteoporotic animals as compared to untreated controls. The protective effects observed were attributed to inhibition of osteoclastic activity rather than stimulation of osteoblastic activity. The results of these studies were found to be in agreement with the results of other studies obtained from osteoporotic rats models. The impact of gut microbiome on the pathogenesis of osteoporosis is mainly mediated by the immune system. Clostridium species in the gut promote the accumulation of T reg cells which induce a suppressive effect on the proliferation/differentiation of osteoclasts. Hence lack of clostridium induced a reduction in Foxp3 T reg cells which is in turn associated with increased bone loss. Dysbiosis in the gut could also reduce folic acid absorption in the jejunum, leading to hyperhomocysteinemia that eventually enhanced bone matrix degradation and decreased bone mineral density. In addition to this, production of signaling molecule nitric oxide could be modulated by gut microbiota which could have implications on osteoblastic or osteoclastic activity . Modulatory influence of estrogen and probiotics on gut microbiome and resultant effects on immune homeostasis, inflammation and gut membrane integrity is depicted in Figure 4.

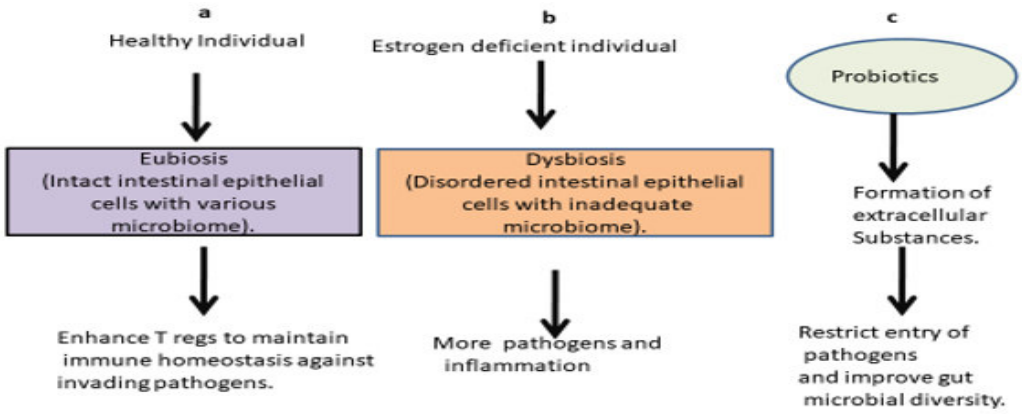

Fig 4: Gut microbiome is modulated through estrogen and probiotics

(a) In healthy individuals, gut microbial diversity is maintained through beneficial bacteria to activate the immune system against invading pathogens. (b) During estrogen-deficient conditions or in the PMO, estrogen deficiency alleviates gut microbial diversity and healthy bacteria causing inflammation via the generation of more pathogens. (c) Probiotics improve gut health by restricting pathogen entry and enhancing gut microbial diversity through the generation of extracellular substances.

4. Gut Microbiome-Targeted Strategies in Postmenopausal Osteoporosis

Healthy and sufficient estrogen levels are pivotal to maintaining intestinal microbial diversity. The epithelial barrier of the gut is regulated by the gut microbiome, composed of bacteroidetes, firmicutes, actinobacteria and proteobacteria [110] .The firmicutes/bacteroidetes (F/B) ratio is associated with gut microbiome homeostasis[111]. Dysbiosis is characterised by a decline in microbial diversity and an imbalance in gut microbiota. An increased F/B ratio is regarded as dysbiosis, which triggers inflammation. Resident gut microbiota elicited immune responses, a critical determinant in the pathogenesis of osteoporosis during postmenopausal conditions. In a healthy environment, a dynamic nexus prevails between the host immune system, gut microbiota and the intestinal mucosal epithelial barrier. This restricts the survival of pathogenic microorganisms in the intestines, maintains musculoskeletal balance and upregulates homeostatic conditions. During diseased conditions, pathogens in the host gut penetrate the epithelial barrier of the intestinal mucosa and provoke an immune response, subsequently triggering bone resorption by osteoclasts. This culminates in bone loss and bone fragility during post-menopausal conditions. [112]. These perturbances in the gut microbiome are remarkable in the pathogenesis of postmenopausal osteoporosis (PMO). Alternatively, probiotics reduce the bone resorption rate and mitigate damage by restraining immunologic triggers and reinstating the balance between the host and gut microbiota [113]. Estrogen is important in regulating the gut microenvironment and exerting action through the increased epithelial thickness, increased glycogen levels and mucus secretion. Many phytoestrogens exert anti estrogenic or pro estrogenic effects based on the target tissue, which are selective modulators of estrogen receptors. For this reason, phytoestrogens are used as therapeutic interventions for hyperestrogenic diseases[114]. Hence, phytoestrogens and endogenous estrogens can exhibit reversible effects on the reproductive tract of females. Intestinal bacteria produce equol, and mice that produce equol has thinner vaginal

epithelial and lower uterine weight as against one lacking the equol. The tissues of the intestine, adipose and brain express estrogens influence varied physiological responses during neural development [115].Estrobolome is the microbiome gene repertoire in estrogen-gut metabolism. Thus, beta-glucuronidase from microbes metabolize estrogens and phytoestrogens based on their actions on estrogen receptor alpha and beta. When estrogens bind to estrogen receptors, they trigger downstream signalling pathways, which cause physiological changes and exert effects on target organs [116]. Modern sophisticated techniques like high-throughput sequencing (denoted as HTS) have emphasised the significance of the gut microbiome in programming skeletal metabolism, through mechanisms not fully explored and understood. A combination of various host mechanisms modulates bone metabolism, such as mineral absorption, immunomodulation and hormonal control [117]. Vital control of the gut microbiome on bone mass regulation is exerted through a range of mechanisms such as influencing the growth of beneficial bacteria, boosting the estrogen bioavailability and modulating bone mass through prebiotics [117] . Osteo Microbiology is an emerging field of research which focuses on the dynamic interactions of bone and the microbes. The epigenetic control of pathogenesis of osteoporosis has become increasingly evident in recent times which has led to the development of epigenetics oriented therapies for osteoporosis. Such therapies could involve exogenous agents that modify the expression of non coding genetic material like miRNAs or long noncoding RNAs (lncRNAs) [118]. A combination of lifestyle modifications with pharmacological interventions and effective nutritional supplementation could considerably reduce the detrimental effects of osteoporosis on the host and provide a good quality of life especially in the elderly.

5. CONCLUSION AND FUTURE PERSPECTIVES

Dysbiosis, the disruption of gut microbiota, is reported to be the cause for the pathogenesis of many diseases, including bone diseases like osteoporosis. Recent scientific studies implicate that reinstating the balance in gut microbiome through administration of probiotics or prebiotics is a promising strategy in osteoporosis management. The dynamic cross-talks between the consortia of microbes inhabiting the intestine, immunomodulators like cytokines, osteogenic factors and sex hormone estrogen is crucial to skeletal health and bone homeostasis. Future research should investigate the influence of cross-talk on molecular targets and downstream effects on osteoporosis therapy.

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7. AUTHORS CONTRIBUTION STATEMENT

Dr. M. Sreepriya, corresponding author of the manuscript conceptualized the theme of the manuscript and was involved in designing, structuring, language editing, hypothesizing the mechanisms, compiling and presenting the information in the right sequel in the manuscript. Dr. Soumya Krishnan was actively involved in literature survey, writing the rough draft of the manuscript, representation of information in the form of tables and figures, plagiarism checking, reference writing (using endnote) and other essential features of the manuscript.

8. ABBREVIATIONS

BMD, bone mineral density; BMP-2, bone morphogenetic protein 2; CaSR, calcium-sensing receptors; GM, gut microbiome; IBD, inflammatory bowel disease; IFN-γ, interferon-gamma; IGF, insulin-like growth factor; OC, osteocalcin; OP, osteoporosis; OVX, ovariectomized; PMO, postmenopausal osteoporosis; PTH, parathyroid hormone; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor kappa-B ligand; SERMs, selective estrogen receptor modulators; SPARC, secreted protein acidic and rich in cysteine; TNF, tumor necrosis factor; TRAP 5, tartrate-resistant acid phosphatase type 5; TRPV6, transient receptor potential cation channel subfamily V member 6.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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