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Are Probiotics the New Calcium and Vitamin D for Bone Health?

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Abstract

Purpose of Review Calcium and vitamin D supplementation is recommended for patients at high risk of fracture and/or for those receiving pharmacological osteoporosis treatments. Probiotics are micro-organisms conferring a health benefit on the host when administered in adequate amounts, likely by influencing gut microbiota (GM) composition and/or function. GM has been shown to influence various determinants of bone health.

Recent Findings In animal models, probiotics prevent bone loss associated with estrogen deficiency, diabetes, or glucocorticoid treatments, by modulating both bone resorption by osteoclasts and bone formation by osteoblast. In humans, they interfere with 25-hydroxyvitamin D levels, and calcium intake and absorption, and slightly decrease bone loss in elderly postmenopausal women, in a quite similar magnitude as observed with calcium ± vitamin D supplements. A dietary source of probiotics is fermented dairy products which can improve calcium balance, prevent secondary hyperparathyroidism, and attenuate age-related increase of bone resorption and bone loss.

Summary Additional studies are required to determine whether probiotics or any other interventions targeting GM and its metabolites may be adjuvant treatment to calcium and vitamin D or anti-osteoporotic drugs in the general management of patients with bone fragility.

Keywords Bone turnover · Bone mineral density (BMD) · Osteoporosis · Gut microbiota · Nutrition · Dairy products · Intestinal absorption

Introduction

Supplements of vitamin D ± calcium are recommended for osteoporotic patients with low calcium intake or absorption, vitamin D insufficiency, or under pharmacological treatment for osteoporosis [1]. Intakes of 800–1000 mg/day of calcium and 800 IU of vitamin D are recommended in the general management of patients with osteoporosis [2•]. However, the efficacy of calcium and vitamin D treatment on fracture risk reduction and hence its role in osteoporosis treatment have been challenged over the last decade. Calcium supplements associated with vitamin D treatment, not calcium supplementation alone, are associated with a modest reduction in fracture risk. Adverse events of calcium supplementation include mainly gastrointestinal symptoms and renal stones. Higher cardiovascular risk resulting from calcium supplementation at appropriate doses has not been confirmed by current evidence. In addition, high loading dose of vitamin D may increase the risk of fall and fracture and is no more recommended [3–5].

Probiotics are live micro-organisms which, when given in adequate amounts, meaning able to trigger the targeted effect, confer a health benefit to the host [6••]. Their adequacy and strength depend on food processing and matrix, strain specificity, and the targeted effect. Probiotics are available as yogurt, milk-based foods, powder, capsules, or solutions like ice cream and beer. The probiotic bacteria concentration for every gram is approximately 10e7 to 10e8, with a serving size of 100 to 200 mg. Probiotics usually administered include

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Lactobacillus, *Bifidobacterium*, *Escherichia*, *Enterococcus*, and *Bacillus subtilis*, as well as yeast like *Saccharomyces*. Gut microbiota (GM) is more and more recognized as an important determinant of bone health. Its composition changes in relation with age [7, 8], sex [9], diet [10, 11], living conditions, geography [8, 12], diseases requiring or not antibiotics treatment, and various drugs [13, 14]. Within dietary intakes, pre- and probiotics are also major determinants of GM composition and function.

In this paper, the contribution of probiotics to bone health and how they may interfere in osteoporosis management are discussed.

Evidence for a Role of Gut Microbiota in Bone Metabolism

Germ-Free Animals

Germ-free (GF) mice with a C57BL/6 genetic background, characterized by the absence of GM, have higher bone mass and better microstructure, with higher relative bone volume, cortical area, and trabecular number. When these mice are recolonized with a normal GM by 3 weeks of age, trabecular BMD and cortical area are lower than in GF mice controls. These differences in the bone phenotype are associated with change in osteoclasts number and activity, since osteoclast number is reduced while bone formation rate is maintained in GF animals. In contrast, osteoclast precursors are increased in GF mice recolonized with a normal GM. These data indicate that in the absence of GM, bone mass and microstructure are better in relation with a decreased bone resorption [15]. Normal GM also supports growth and bone development in Balb/c and CB6F1 mice [16, 17], indicating that the mouse genetic background influences how the GM affects bone physiology.

Antibiotics

Low doses of antibiotics in early life change fecal microbiome and the expression of genes implicated in carbohydrate metabolism, with increased short-chain fatty acid production and in hepatic lipid metabolism [18]. They have been used as growth promoters in poultry and cattle industry [19]. Subtherapeutic doses of various antibiotics, such as low dose penicillin from birth on or from weaning, modulate BMD in female mice, suggesting that intestinal microbiota alterations during a critical development window exert lasting metabolic consequences [20]. Tetracycline administration is also associated with higher bone strength [21] and has been shown to prevent OVX-induced bone loss [22].

Effects of Probiotics Administration on Bone

The effect on bone health of the direct administration of some bacteria to the gastrointestinal tract, i.e., probiotics use, has been tested in intervention studies in animal and human.

In animal models (Table 1)

OVX-induced bone loss in mice is prevented by various probiotics including *Lactobacillus reuteri* [24], *Lactobacillus paracasei* prevent OVX-mediated bone loss [27, 31], and *Lactobacillus helveticus* fermented milk [30]. *Bifidobacterium longum* partially prevent OVX-induced bone loss in rats, without significantly affecting bone strength [32]. In these models, the decrease of osteoclastic bone resorption with probiotics is associated with a decrease of pro-inflammatory cytokines (TNFα, IL-1β) and RANKL expression osteoclastic bone resorption [24, 31]. Other models showed a benefit of probiotics on inflammation observed in various conditions in bone. In a rat model of collagen-induced arthritis, *Lactobacillus casei* decreases both TNF-alpha and IL6 production, and increases the anti-inflammatory cytokine IL-10 [34]. In rodents, *Bacillus subtilis* reduces periodontitis-stimulated bone loss [35]. *Saccharomyces cerevisiae* blunts alveolar bone loss, by decreasing the expression of IL-1β, TNFα, and IL-10 [36].

The effects of probiotics administration on bone were not only observed in high-bone turnover conditions. *Bacillus longum* combined with the prebiotic yacon flour increases bone mineral content in rats [33]. *Lactobacillus reuteri* also increases vertebral and femoral BMD in male mice, but not in female mice [23], and prevents bone loss in type-1 diabetes [25] and trabecular bone loss in glucocorticoid-treated mice [26]. No benefit of *Lactobacillus rhamnosus* on glucocorticoid-induced bone loss was observed in the same study [26]. In diabetic mice and glucocorticoid-treated mice, two models characterized by low bone turnover pattern with low bone formation, *Lactobacillus reuteri* treatment prevented the TNFα suppression of Wnt10b in bone which has been implicated in the decrease of osteoblast activity and osteoporosis activity in these two conditions [25, 26].

In humans

Oral supplementation with bile salt hydrolase-active *Lactobacillus reuteri* increases circulating 25-hydroxyvitamin D levels, without effect any on other fat-soluble vitamins, and reduces cholesterol and the absorption of non-cholesterol sterols in hypercholesterolemic adults [37]. Probiotics also improve vitamin D levels in women with gestational diabetes [38] or after bariatric surgery to a higher extent than those observed in controls in parallel to weight loss [39, 40]. The mechanisms of this effect on vitamin D remain unclear, and may involve increased intestinal production of lactic acid,

Table 1 Effects of probiotics on bone metabolism in mammalian experimental animals

Author	Year	Experimental model	Probiotics	Bone mass/density	Biochemistry
McCabe [23]	2013	Healthy male and female mice	*Lactobacillus reuteri*, 3×/week, 4 weeks	Increased femur and vertebral vBMD, TbN and TrTh, in males but NOT in females	Increased BFR, decreased gut TNFα expression
Britton [24]	2014	OVX mice	*Lactobacillus reuteri*, 3×/week, 4 weeks	Prevention of femur and vertebral trabecular bone loss	Decreased RANKL expression and osteoclast number. Decreased CD4+ T cells.
Zhang [25]	2016	T1D mice	*Lactobacillus reuteri*, 3×/week., 4 weeks	Increased BMD, TbN and TbTh, incr. OB surfaces (in controls too), incr. MOI	Prevention of Wnt10b suppression in bone
Schepper [26]	2019	GC-treated mice	*Lactobacillus reuteri*, 8 weeks *Lactobacillus rhamnosus* GG (LGG)	Prevention of femur and vertebral trabecular bone loss with *Lactobacillus reuteri*, but not with *Lactobacillus rhamnosus* GG.	*Lactobacillus reuteri* maintained the beneficial immunosuppressive effects of GCs (GC-Tx suppression of CD4+ T-lymphocytes)
Chiang [27]	2011	OVX mice	*Lactobacillus paracasei* and *Lactobacillus plantarum* fermented soy skim milk, 8 weeks	Prevention of femur trabecular bone loss	
Kimoto-Nira [28]	2007	Senescent intact mice	Heat killed *Lactococcus lactis*	Decreased bone loss with heat killed but not with living bacteria fermented milk	
Li [29]	2016	E2-depleted mice	*Lactobacillus rhamnosus* or VLS3 (8 strains), 4 weeks	Full prevention of vertebral BV/TV decrease Increase in BV/TV in sham animals	
Narva [30]	2007	OVX rats	*Lactobacillus helveticus* fermented milk, 12 weeks	Prevention of bone loss	Decreased bone turnover
Ohlsson [31]	2014	OVX mice	*Lactobacillus paracasei* or *Lactobacillus paracasei* and *Lactobacillus plantarum* 2 + 3 daily	Prevention of cortical bone loss	Decreased cortical bone TNFα, IL-1β, RANKL/OPG expression Increased TGFβ expression in bone marrow
Parvaneh [32]	2015	OVX rats	*Bifidobacterium longum*, daily, 16 weeks	Attenuation of decreased BMD	Increased osteocalcin, decreased CTX, Increased BMP2 and Sparc bone expression, increased fecal bifidobacteria
Rodriguez [33]	2012	Intact rats	*Bifidobacterium longum* and yacon flour (FOS)	Increased tibia Ca and Pi content	

T1D, type 1 diabetes; *MOI*, cross-sectional moment of inertia; *OVX*, ovariectomized: *GC*, glucocorticoids

synthesis of 7-dehydrocholesterol, and higher expression and activity of vitamin D receptors.

In a 6-month randomized controlled trial, healthy 1- to 6-year old children receiving milk fortified with 5 × 10e8 *Lactobacillus reuteri* have a greater weight and height monthly gain [41].

Five randomized placebo-controlled trials have assessed the effects of probiotics on bone metabolism in healthy postmenopausal women (Table 2). Different amounts of various strains were administered for 6 or 12 months. Some decrease in bone resorption markers was observed in 3 of the trials, and a benefit on BMD in 4 of them. In one study, lactic acid bacteria were combined with isoflavone, so that the specific contribution of probiotic on the benefit on lumbar spine, femoral neck, and trochanter BMD (1.2 to 2.1% positive difference as compared with the placebo group) is difficult to individualize [43]. The 3 other studies used various probiotic strains (*Lactobacillus reuteri*, *Bacillus subtilis*, or a combination of 3 *Lactobacillus* strains) which prevented bone loss at the distal tibia, at the lumbar spine, or at the hip, respectively [44, 45, 47]. The magnitude of the effect (≤ 1 percentage point difference versus placebo at 12 months) was however much lower than those observed with anti-resorptive drugs used in osteoporosis treatment, but of the same order compared with calcium ± vitamin D (Fig. 1) [48]. None of these studies was designed to test the effect on incident fractures.

In a double-blind placebo-controlled clinical trial including 417 elderly patients with an acute distal radius fracture, *Lactobacillus casei* Shirota accelerated functional recovery, with treatment outcomes of patients receiving probiotic at month 4 at comparable levels with those of patients receiving

Table 2 Effects of probiotics on bone mineral density and bone turnover markers in randomized controlled trials

Author	Year	Population	No	Age (years)	Duration (months)	Probiotics dose	Control	BMD	BTM
Jafarnejad [42]	2017	Postmenopausal women with osteopenia	50	58	6	7 probiotic bacteria species 1 caps/day + Ca 500 mg + vitamin D 200UI/day	Placebo + Ca 500 mg + vitamin D 200UI/day	NS	Lower sCTX, BAP, PTH and TNFα
Lambert [43]	2017	Postmenopausal women with osteopenia	78	61.8	12	60 mg isoflavone aglycones/day and probiotic lactic acid bacteria + calcium 1200 mg/day, magnesium 550 mg/day, calcitriol 0.25 μg/day)	Placebo + calcium 1200 mg/day, magnesium 550 mg/day, calcitriol 0.25 μg/day)	Attenuated BMD loss (Δ%points: LS, 1.2; FN, 2.0; Tr, 2.1)	Lower sCTX
Nilsson [44]	2018	Postmenopausal women with osteopenia	90	76.3	12	*L. reuteri* 2 × 5 × 10e9 CFU/day	Placebo	Lower reduction (mean difference 95% CI) in: - tibia total vBMD: ITT 1.02% (0.02–2.03), PP 0.93% (0.21–1.65). - trabecular bone volume fraction: PP 0.80% (0.13–1.46)	NS
Takimoto [45]	2018	Non-osteoporotic postmenopausal women	61	57.6	6	*B. subtilis* 3.4 × 10e9 CFU/day	3.4 × 10e9 CFU/day	Increased hip BMD: Δ%points: Hip, 1.7; LS, 0.9 (NS)	Lower uNTX
Jansson [46]	2019	Early postmenopausal women	249	58.6	12	Three *Lactobacillus* strains (*Lactobacillus paracasei* DSM 13434, *Lactobacillus plantarum* DSM 15312, and *Lactobacillus plantarum* DSM 15313) 1 × 10e10 CFU/day	Placebo	Attenuated BMD loss LS: mean difference 95% CI: 0.71%, (0.06–1.35)	NS

No, number of subjects (intervention + placebo); *BMD*, bone mineral density; *BTM*, bone turnover markers; *CFU*, colony-forming units; *NS*, non-significant; *LS*, lumbar spine; *FN*, femoral neck; *Tr*, femoral trochanter; *ITT*, intention-to-treat; *PP*, per protocol analysis

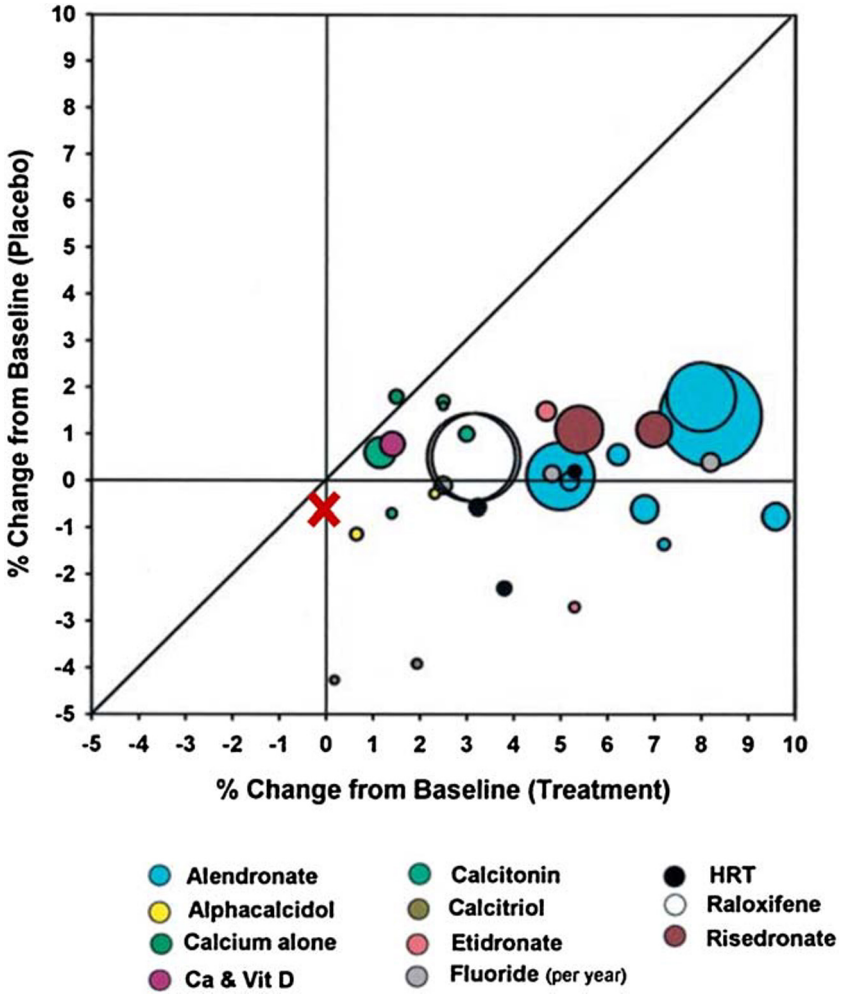

Fig. 1 The effects of probiotics (*Lactobacillus paracasei* DSM 13434, *Lactobacillus plantarum* DSM 15312, and *Lactobacillus plantarum* DSM 15313) on changes in spine BMD relative to placebo in the study of Jansson et al. [46] (X), compared with the effect of various agents tested in other studies (O). Adapted from Hauselmann and Rizzoli [48], with permission from the publisher. The size of the symbols (O) is proportional to the number of patients evaluated at the end of the studies. Displacement of the dot on the right and below the equality line reflects the magnitude of treatment effects

placebo at month 6, suggesting that probiotic may accelerate the fracture healing process [49].

Additional studies are required to confirm these data and to optimize the choice of probiotic strains, usually based for the clinical interventions reported above on preclinical works in animal models, and the dose of these strains to maximize the benefit on bone. It is likely that the benefit on bone loss prevention may depend of time since menopause and bone loss rate, as suggested in the largest intervention study showing that the protective effect of *Lactobacillus* treatment was significant for participants below, but not above the median time since menopause [46]. A major limitation is certainly the amount of bacteria ingested. For instance, in adult monozygotic tweens, 2 servings per day of fermented milk products containing 5 different species of bacteria did not modify the large intestine GM composition. In contrast, when the same fermented milk products were given to gnotobiotic mice by gavage, there was a rapid change (within 24 h) in microbiome-encoded enzymes affecting carbohydrate metabolism [50].

Fermented Dairy Products

In humans, fermented dairy products are the primary source of probiotics [51•]. However, the specific effects of probiotics on bone as compared with calcium, protein, phosphorus, or zinc, as well as prebiotics as also provided by dairy products, are difficult to specifically identify (Fig. 2). Furthermore, the problem remains as to whether a sufficient amount of bacteria is capable of reaching the distal part of the gastrointestinal tract. Some data suggest that when yogurt is consumed on a regular basis, it influences the composition and metabolism of the human intestinal microbiota. Indeed, yogurt consumers have lower level of Enterobacteriaceae and higher beta-galactosidase activity in their GM. Beta-galactosidase activity (and *Bifidobacterium* population) is positively correlated to the quantity of fermented products ingested [52].

Some observational studies have examined the associations between bone traits and fermented milk products. In an Irish cohort of 4310 community dwelling older adults (> 60 years), each unit increase in yogurt intake was associated with a 39% lower risk in women, and 52% lower risk of osteoporosis in

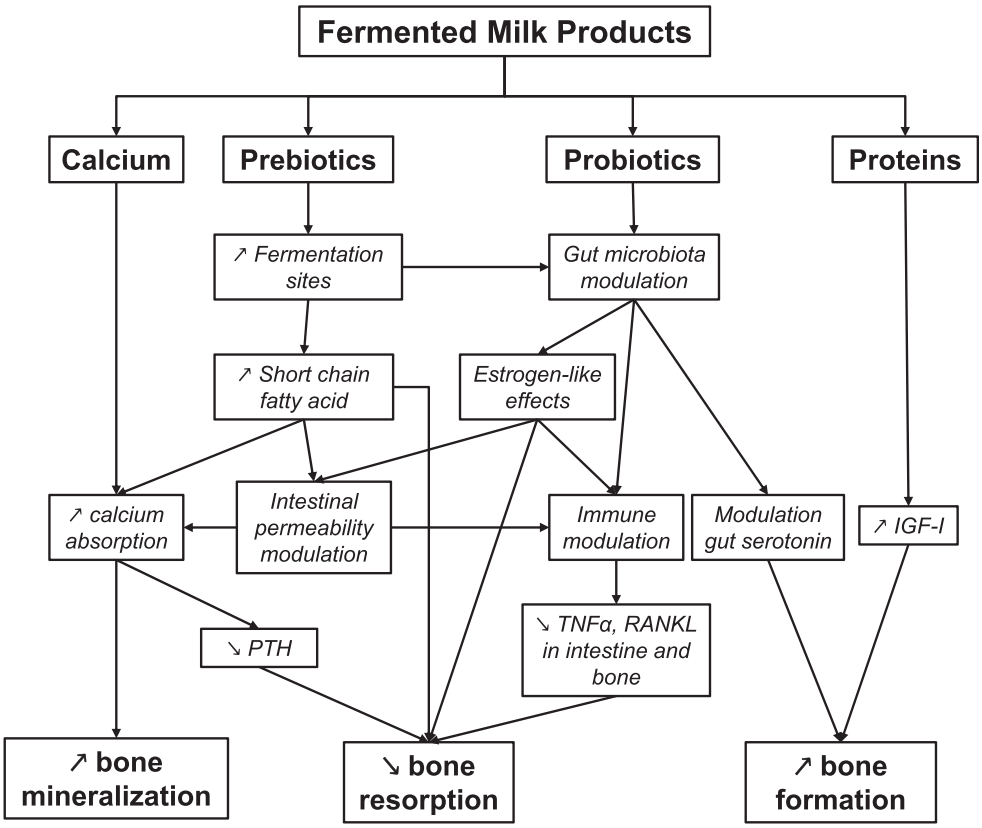

Fig. 2 Effects of fermented dairy products on bone mass and metabolism. Adapted from Rizzoli and Biver [51•], with permission from Springer Nature

men [53]. The associations between yogurt and bone were of higher magnitude than with milk intake. In a cross-sectional and longitudinal study in older women (65 years of age) from the Geneva Retirees cohort, yogurt consumers had larger bone size at the distal tibia and radius. Compared with non-consumers, cortical bone loss at the radius was attenuated in these subjects, not in milk or ripened cheeses consumers, independently of total calcium, protein, and energy intakes [54]. In a 12-year follow-up of the Framingham Offspring Study, dairy product intake, including yogurts, was associated with lower trochanter BMD loss, with a weak protective trend for hip fracture, while there was no significant association with other dairy groups [55]. In a long-term follow-up of Swedish postmenopausal women, mortality and fracture rate were lower in the women with a high compared with low intake of cheese or fermented milk products. For each serving, the rates of mortality and of hip fracture were lower by 10–15% ($P < 0.001$) [56].

Intervention studies using fermented dairy products, fortified or not, to promote bone health have been reviewed previously [51•]. They increase IGF-I and are effective to promote bone mineral accretion during growth, whereas in adults, a dairy improves calcium balance and prevents secondary hyperparathyroidism, and age-related increases in bone resorption and bone loss.

Mechanisms of Interaction Between Probiotics and Bone Health

GM Metabolites and Intestinal Wall Permeability

GM produces various metabolites, either from endogenous compounds that are generated by the microorganisms themselves and their hosts or from the fermentation of undigested dietary components that reach the colon. They are key regulators of the integrity of the gut epithelium. These metabolites can also translocate from the gut across a disrupted intestinal barrier to modulate multiple inflammatory or metabolic processes [57]. A compromised gut permeability may contribute to multiple chronic diseases including osteoporosis by promoting the absorption of toxins and pathogens and decreasing nutrient bioavailability. In sex hormone deficiency, intestinal wall permeability is increased in relation with a reduction in gap junction protein transcripts [58]. The administration of probiotics prevents this increase in gut permeability and lowers the production of osteoclastogenic cytokines [29]. In addition, treatment with a non-absorbable mucus supplement that enhances intestinal barrier function prevents glucocorticoid-mediated osteoblast and osteocyte apoptosis in mice or *Salmonella*-induced bone loss in broiler chickens [26, 59].

Interaction with Diet and Prebiotics

Prebiotics are non-digestible fiber saccharides that pass undigested the upper part of the gastro-intestinal tract. By acting as substrate for GM, they stimulate the growth and/or metabolism of bacteria of the large bowel [60]. Various prebiotics such as galactooligosaccharides, fructooligosaccharides, fiber dextrin, inulin, and agarve fructans, at doses up to 20 g/day in human, increase the number of *bifidobacteria* and *lactobacilli*, and decrease that of coliforms. Prebiotics may also have direct effects on the immune system without being metabolized [61]. The fiber content of the diet markedly influences GM composition and metabolism [62], as does the ingestion of probiotics [63], and may interfere in the pathogenesis of several chronic diseases. For instance, an animal-based diet increases fat and protein intakes and leads to inter-individual differences in GM composition and microbial gene expression which may support the link between dietary fat, bile acids, and the outgrowth of microorganisms capable of triggering inflammatory bowel disease [10]. A Mediterranean diet, which is rich in fiber, fermented dairy products, and polyphenols, is associated with changes in the GM and various health benefits, including a lower hip fracture risk [64–66].

Both experimental animal models and intervention studies in human suggest that prebiotics influence calcium absorption and retention, and bone mineral density (for review see [60]). The evidence on BMD in human is however limited with few studies of relatively low quality (low number of participants and heterogeneity of the populations, not at risk of low calcium diet/absorption which may have minimize the effects) [67–71].

Fermentation of prebiotic fibers by saccharolytic microbes within the large intestine leads to a reduction in intestinal pH and to the synthesis of short-chain fatty acids (SCFAs), including acetate, propionate, valerate, isovalerate, butyrate, and isobutyrate [60, 62, 72, 73]. These microbial metabolites have emerged as key bone regulatory factors produced by the GM and diffusing into the circulation [74]. In addition to their influence on limiting intestinal wall permeability [75], SCFAs may indeed not only inhibit osteoclast number and activity, and thereby bone resorption, but also stimulate bone formation by promoting Wnt10b signaling in bone marrow stromal cells, leading to their proliferation and differentiation into osteoblasts. Both mechanisms involve interactions of SCFAs with Treg cells. These data indicate that probiotics, prebiotics, and diet may influence bone remodeling. This is supported by studies showing that SCFAs have direct effects on bone metabolism and bone mass. For instance, when mice are given SCFAs (acetate, propionate, or butyrate) in the drinking water, there are an increase in trabecular bone volume, and a reduction in osteoclast number and biochemical markers of bone resorption [76]. Furthermore, propionate or butyrate prevents OVX-induced as well as inflammation-dependent bone loss by inhibiting osteoclast differentiation and bone resorption [76]. Butyrate increases osteoblast differentiation [77] and bone formation, and is associated with higher bone sialoprotein and osteoprotegerin production [78].

Interaction with the Immune System and Inflammation

There is a close interplay between the immune and bone systems, and it is well established that chronic inflammatory conditions are associated with osteoporosis [79]. GM can modulate the immune system development, since in GF animals, hence lacking GM, mucosal and spleen immune systems are immature [80]. The beneficial effect of probiotics on BMD involves, as reported above for SCFAs, the contribution of the immune system. In male mice, lymphocytes are critical for the beneficial effects of *L. reuteri* on BMD and experiments using *L. reuteri* supernatants demonstrated that the regulation of T-lymphocytes is mediated, at least partially, by factors secreted by the probiotic strain [81]. OVX-induced bone loss is not observed in OVX mice depleted of T cells or lacking the T cell costimulatory molecule CD40 ligand [82]. In OVX germ-free mice, there is no increase of TNFα+ T cells in the bone marrow, contrary to what is observed in control OVX mice [83]. Since TNFalpha is a central cytokine involved in bone loss induced by estrogen deficiency, GM may thus be necessary to present the antigens stimulating TNFα production by T cells [84]. In addition, aging, which is one of the main risk factor of osteoporosis, is associated with modifications of the GM characterized by the increase of the proportion of opportunistic pro-inflammatory bacteria, a reduction in genes involved in pathways responsible for the production of SCFAs, and an increase in bacterial genes involved in tryptophan metabolism pathways [85]. The amount of gut pro-inflammatory bacteria is correlated with plasma levels of cytokines such as IL-6 and IL-8, and therefore with systemic low-grade inflammation [86]. Multiple additional factors (place of residence, frailty, comorbidities, drugs, markers of inflammation, and nutritional status…), including well-established risk factors of bone fragility, contribute to GM composition and its greater inter-individual variations in older people compared with younger adults [7, 8].

Interaction with Estrogens

The interaction of sex steroids with GM and its impact on bone metabolism have been shown in hypogonadal mice. Sex hormone deficiency (ovariectomy, OVX) is associated with attenuated cortical and trabecular bone loss in germ-free animals compared with controls, in relation with a lower bone resorption [29]. In these models, estrogen deficiency is associated with increased intestinal permeability, possibly due to the reduction in gap junction protein transcripts [29, 58]. In

addition, OVX increases GM diversity and number of Bacteroidetes phylum, and reduces short-chain fatty acid production [87]. Interestingly, probiotics supplementation prevents sex steroid deficiency–associated bone loss in these mice [29]. These data suggest that GM composition and its metabolites may modulate postmenopausal bone loss. It remains unknown how it may interfere with menopausal hormone therapy or antiresorptive drugs used in the treatment of postmenopausal osteoporosis.

Interaction with Vitamin D

Preclinical studies demonstrated that vitamin D receptor plays a critical role in mucosal barrier homeostasis by preserving the integrity of junction complexes of the colonic epithelium [88]. In addition, lack of VDR induces dysbiosis since cecal content and stools of VDR knock-out animals are depleted in lactobacillus and enriched in clostridium and bacteroides [89]. In human, GM composition and circulating levels of lipopolysaccharide, an endotoxin from the outer membrane of most Gram-negative bacteria known to promote low-grade inflammation, vary according to vitamin D intake or circulating calcifediol levels [90–92]. These data suggest that vitamin D deficiency may compromise the mucosal barrier, leading to increased intestinal permeability and potentially chronic low-grade inflammation. The modulation of gut microbiome with vitamin D3 supplementation seems to predominate in the upper gastrointestinal tract [93]. In addition, probiotic strains such as *Lactobacillus rhamnosus* and *Lactobacillus plantarum* increase VDR expression in both mouse and human intestinal epithelial cells [94]. A protection of *Salmonella*-induced colitis by these probiotics is observed in VDR+/+, but not in VDR−/− mice, indicating that vitamin D pathways are required for probiotic protection in colitis [94].

Interaction with Calcium

GM is associated with the digestion and availability for absorption of various ingested nutrients including dietary carbohydrates, proteins, plant polyphenols, bile acids, and vitamins, and is therefore a key factor in shaping the biochemical profile of the diet [95]. This interaction between GM and prebiotics promotes calcium absorption via various mechanisms: first, the reduction in bowel content pH increases calcium bioavailability [73, 96]. Second, calcium surface absorption is increased, since cellular uptake of SCFAs increases intestinal cell proliferation resulting in increased intestinal crypt depth and greater cell density and blood flow in the villi [73, 97]. Last, SCFAs may signal for greater gene expression of the intracellular calcium transporters [60]. Higher calcium absorption decreases parathyroid hormone (PTH) production and may thereby lower bone resorption [98].

In addition, studies in animals suggest that calcium from the diet or supplements might interfere with gut microbiota, and partly explains the beneficial effects of calcium on body weight/fat loss [99]. Calcium supplementation in dietary obese animals has a prebiotic-like effect which modulates GM composition in favor of potentially beneficial bacteria in the gut, and in turn may modulate systemic low-grade inflammation, as demonstrated by the lower plasma endotoxin LPS in host animals receiving calcium supplements compared with controls [100].

Interaction with Other Bone Regulatory Pathways

Interactions between GM and several bone regulatory pathways, including PTH, IGF-I, or serotonin, have also been reported. Bone loss associated with primary hyperparathyroidism involves microbial-dependent expansion of intestinal TNFα+ T cells and Th17 cells [101]. In addition, butyrate production by gut luminal microbiota is required for the bone anabolic activity of PTH [102]. Serum IGF-I levels in mice are increased in response to microbial colonization, while it decreases after antibiotic treatment. Supplementation of antibiotic-treated mice with SCFAs restores IGF-I concentrations and bone mass to levels observed in control mice [17]. In GF animals, serotonin, known to reduce bone formation secretion, is decreased in relation to lower tryptophan hydroxylase-1 expression in the large intestine [103].

Conclusion

There is compelling evidence supporting that probiotics may improve bone health. In animal models, probiotics prevent bone loss associated with estrogen deficiency, diabetes or glucocorticoid treatments, by modulating both bone resorption by osteoclasts and bone formation by osteoblast in relation with GM composition and metabolism. In humans, they interfere with 25-hydroxyvitamin D levels, and calcium intake and absorption, and slightly decrease bone loss in elderly postmenopausal women, in a quite similar magnitude as observed with calcium and vitamin D supplements. A dietary source of probiotics is fermented dairy products which benefits calcium balance and prevents secondary hyperparathyroidism, age-related increase of bone resorption, and age-related bone loss. However, some important issues remain to be elucidated. In some models, the response to probiotics is sex specific and seems more readily detectable in subjects with high bone turnover like children or adolescents or early postmenopausal women [23, 46]. The types and doses of probiotics, in terms of efficacy and tolerance, the time and duration of administration, and the offset of the effects upon probiotics discontinuation need still to be defined. Finally, there is currently no data demonstrating whether probiotics may reduce fracture risk.

With this respect, genetic background, sex, immune status, age, diet, living conditions, geography, and drugs are likely important confounding factors in evaluating the effects of probiotics on bone health. Additional studies are required to determine whether probiotics or any other interventions targeting GM and its metabolites such as prebiotics may be adjuvant treatment to calcium and vitamin D supplements, anti-osteoporotic drugs and also to promotion of a balance diet and regular physical activity in the general management of patients with bone fragility.

Compliance with Ethical Standards

Conflict of Interest Dr. Rizzoli reports personal fees from Abiogen, Amgen, EuropeanMilkForum, Danone, Echolight, Mylan, Radius Health, Nestlé, Rejuvenate, Sandoz, and Theramex, outside the submitted work.

Dr. Biver reports fees from Nestle paid to the institution.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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