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Gut Microbiome and Bone: To Build, Destroy or Both?

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Abstract

Purpose of the Review—The gut microbiota can be considered a hidden organ that plays essential roles in host homeostasis. Exploration of the effects of microbiota on bone have just begun. Complimentary studies using germ-free mice, antibiotic and probiotic treatments reveal a complicated relationship between microbiota and bone. Here we review recent reports addressing the effect of gut microbiota on bone health, discuss potential reasons for discrepant findings, and explore potential mechanisms for these effects.

Recent findings—Manipulation of microbiota by colonization of germ free mice, antibiotics or probiotic supplementation significantly alters bone remodeling, bone development and growth, as well as bone mechanical strength. Different experimental models reveal context dependent effects of gut microbiota on bone.

Summary—By examining phenotypic effects, experimental context and proposed mechanisms, revealed by recent reports, we hope to provide comprehensive and fresh insights into the many facets of microbiota and bone interactions.

Keywords

microbiome; bone; germ-free; antibiotics; probiotics; I GF-1 SCFA

Introduction

The gut microbiome is composed of trillions of microorganisms that reside in the gastrointestinal tract and encode 150-fold more genes than the human genome. In the last decade, there have been significant advances in our understanding of how this diverse set of gene products shapes physiology, including well-documented effects on host systemic immune function and metabolic parameters, including body weight and systemic insulin resistance[123]. Increasingly, evidence suggests that bone health is also impacted by gut microbiota[4]. Investigation of the impact of gut microbiota on bone physiology primarily involves studies performed in mice, utilizing either germ-free (GF) mice or manipulation of

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Compliance with Ethical Guidelines

Conflict of Interest

Julia Charles and Jing Yan declare no conflict of interest.

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This article does not contain any studies with human or animal subjects performed by any of the authors.

the gut microbiota in conventionally raised mice. Data on the connection between the human gut microbiome and bone health is very limited.

The gut microbiota can be altered by any of several methods, alone or in combination. Antibiotics can be used to broadly deplete microorganisms or specific microorganisms believed to confer beneficial effects on the host (probiotics) can be introduced by oral administration. Non-digestible dietary fiber and oligosaccharides (prebiotics) also alter gut microbial communities by preferential expansion of some species, as well as influencing the production of microbial metabolites. The effect of prebiotics on bone has recently been reviewed in depth and will not be covered in this review[5]. A variety of study designs have been used to interrogate the impact of the gut microbiome on bone, and the conclusions reached have varied substantially. Here, we review recent studies addressing the connection between microbiome and bone and discuss potential causes for discrepancies in findings and proposed mechanisms by which the microbiome may impact bone. Lastly, we consider future directions for this emerging field.

Studying the effects of microbiota on bone using GF mice

GF animals are powerful tools to study the effects of microbiota on bone. Germ-free mice are raised in sterile isolators and have never been exposed to microbiota. Therefore, GF mice can be used as a “test tube” to examine the effects of specific microbes or communities of microbes[6]. The effect of microbiota on bone has been interrogated by contrasting GF mice to a variety of comparator groups. As summarized in Table 1 and discussed below, GF mice have been compared to conventionally raised mice, to GF mice colonized with conventional microbiota, and to GF mice monoassociated with specific microorganisms. As more data become available a more comprehensive view of the effects of microbiota on bone and an appreciation of the complexity of the microbial-host interaction is beginning to emerge.

By comparing GF mice with either conventionally raised mice or GF mice colonized with normal microbiota at weaning (3 weeks of age), Sjogren et al. found that the presence of microbiota lead to lower trabecular and cortical bone mass. The lower bone mass in conventionally raised mice was associated with increased osteoclast number both in vivo and in vitro, suggesting more active bone resorption.[7] Consistent with this, our group colonized GF mice with conventional microbiota after sexual maturity and found that colonized mice have lower trabecular bone mass and significant increases in the bone resorption marker CTX-I one month after colonization[8,9]. Using a similar strategy, Li et al found decreased cortical bone 4 months after colonization, with a less pronounced impact on trabecular bone and no significant increase in CTX-I or osteoclast numbers by histomorphometry[8]. These studies differ in both the age at and duration of colonization, both of which may alter the impact of microbiota on bone.

Support for duration dependent effects of colonization come from the comparison of GF mice colonized for 1 versus 8 months. We found that 8 months after colonization, trabecular bone mass was comparable between colonized mice and GF mice, and no difference in CTX-I levels was observed, suggesting that the reduced bone mass and increased bone resorption after colonization is transient[9]. Therefore, the duration of colonization should be

taken into consideration when evaluating the effect of microbiota on bone mass. Similarly, the age at colonization may be critical in determining the magnitude of effect on bone.

In contrast to the above observations, Schwarzer et al. found that femur length is longer, and trabecular and cortical bone mass is significantly higher in conventionally raised mice compare to GF mice, without affecting the bone mineral density (BMD) [10]. Many explanations for this discrepancy in the bone mass phenotype of GF mice are possible. Schwarzer et al. is the only group that used male BALB/C mice and differences in either genetic background and/or sex may explain the discrepancy. Sex-specific effects of microbiota on bone have been observed in antibiotic-treated mice. Antibiotic-treated male mice had reduced bone mineral content, whereas female mice showed improved bone mineral content compared with controls[11]. It is possible that differences in either microbiota composition or host response to microbiota are driven by sex hormones or other sex specific factors. Moreover, gut microbial communities can vary widely between facilities[12], and bacterial composition is likely to contribute to variations between the findings of different research groups. For example, Schwarzer et al. showed that mice from a colony monoassociated with one *Lactobacillus plantarum* strain had femur length comparable to conventionally raised mice, whereas mice monoassociated with a different *L. plantarum* strain more closely resembled GF mice.

The results of Schwarzer et al. suggest that gut microbiota have an anabolic effect on bone. By comparing GF mice with conventionally raised mice, they demonstrated increased femur length as well as bone mass in conventional mice[10]. Using littermate-controlled studies, our findings that the bone formation marker P1NP and bone formation rate are significantly higher in colonized compared to GF mice, supporting the concept that microbiota can promote bone formation. Unlike in humans, the mouse growth plate remains open after sexual maturity and longitudinal growth continues, thus allowing us to study the effect of microbiota on growth plate after the robust development window. We found that the growth plate of colonized mice was significantly thicker, as measured by both micro-CT and by histology, and had more hypertrophic chondrocytes. In addition, the secondary ossification center displayed qualitatively more active mineral incorporation. These data suggest that there is more active endochondral ossification in the colonized mice, which might alter longitudinal growth of long bones. Indeed, longer femurs and L5 vertebrae were observed in the long-term colonized animals. In addition, the radial growth of the long bone is also affected by microbiota, with an enlarged periosteal area and endosteal area in long-term colonized mice.[9] Since cortical bone is the major determinant of fracture risk, the effect of microbiota on cortical bone indicates a critical role for microbiota in bone health.

Studying the effects of microbiota on bone using antibiotics

Although GF animals are powerful tools to study the effects of microbiota, GF individuals do not exist outside the laboratory. A number of caveats apply to studies done with GF mice. First, the immune system has been shown to impact bone physiology through numerous mechanisms, and animals raised in GF isolators do not undergo normal immune system maturation[13]. Second, microbiota exposure directly impacts gut function through effects on barrier function, gut vascularity and expression of receptors for some microbial metabolites,

any of which could conceivably influence microbial-host interactions[14,15]. Thus, antibiotic treatment may be a more physiologically relevant model for studying the impact of microbiota on bone[16,17]. The gut microbiome is relatively stable in the absence of perturbation[18], but can be either largely depleted with broad-spectrum antibiotics[19] or disrupted by using lower dose or more narrow spectrum antibiotic treatment[20]. Therefore, antibiotic treatment has been used by some investigators to study the effects of microbiota on bone.[21]

To test whether endogenous microbiota contribute to the regulation of bone remodeling under homeostatic conditions in adulthood, our group treated female SPF mice with broad-spectrum antibiotics for 1 month, depleting over 99% of resident bacteria in the gut. Analogous to the higher bone mass observed in GF mice compared to mice colonized for one month, antibiotic treatment increased trabecular bone mass compared to control-treated SPF mice. This increase in bone mass was associated with both decreased pro-osteoclastogenic cytokine production and increased bone formation, as reflected by the serum marker P1NP. Thus, the results from antibiotic treatment and short-term colonization experiments concurred and suggest that microbiota impact both bone resorption and formation. Interestingly, mice treated with oral vancomycin, which is poorly systemically absorbed, was sufficient to increase bone mass and decrease P1NP, suggesting that Gram-positive resident bacteria may play important roles in regulation of bone remodeling by microbiota[9].

Several studies investigated the effects of microbiota perturbation by antibiotic treatment on bone growth in early postnatal development in mice. Cho et al. investigated the effects of low dose antibiotics, which have been used to promote growth in livestock by farmers, in regulating the metabolic phenotype in mouse model. They showed that low dose penicillin (LDP), chlortetracycline, or vancomycin started at weaning increased BMD at 3 weeks of age but not at 7 weeks[20]. Cox et al. tested whether even earlier exposure might have more substantial effects by exposing pregnant mice to LDP shortly before pup's birth and through weaning and comparing the effects to mice exposed to LDP post-weaning. In male mice, BMD decreased in mice exposed to antibiotic either before or after birth, while in female mice exposure to antibiotics at either time point lead to significantly elevated BMD. In both genders, the later the antibiotic was administered, the less strong the effects of antibiotic on body composition was, suggesting greater host vulnerability to microbiota disruption in infancy[11]. In another study, Nobel et al. test the impact of therapeutic-dose pulsed antibiotic treatment (PAT) on microbiota diversity and host growth in mice, with the goal of mimicking microbiota perturbation by intermittent antibiotic usage, which is common in children. PAT mice developed larger bones than controls, with increased bone mineral content, although the strength of the effect varied with the specific antibiotic used[22].

One recent paper examined whether disruption of microbiota by antibiotic treatment or genetic modulation alter bone biomechanical properties. WT (C57BL/6) and TLR5 KO mice, which are known to have altered gut microbiota, were compared. TLR5 KO mice were noted to have decreased bone strength as tested by three-point bending tests on femurs, despite a larger total cross-sectional area. After treatment with ampicillin and neomycin from age 4 to 16 weeks, bone strength of both WT and TLR5 KO mice was decreased

compared to untreated animals, after considering the differences in bone cross-sectional geometry.[23] Further studies are needed to examine whether microbiota alter bone matrix properties, explaining the difference in the bone strength noted in this report.

These studies are summarized in Table 2 and together provide strong evidence that microbiota regulate bone remodeling, bone development and growth, as well as bone mechanical strength. The effect of microbiota perturbation with antibiotics appears to vary with age of treatment, sex, and specific antibiotic regimen and duration. A deeper understanding of the effect and mechanism of antibiotic treatment effects on bone health will likely require a much more nuanced understanding of bacterial community composition and how this is altered by antibiotics, age, and sex.

Manipulation of the microbiota with probiotics: effects on bone in health and disease

Humans constantly ingest probiotics in the form of fermented foods. Probiotic capsules consisting of defined microbial strains or strain mixtures are readily available online and over the counter and are taken for a wide variety of reasons. Frequently used probiotic strains include species of *Lactobacillus*, *Bifidobacteria*, *Streptococcus*, and species of the yeast *Saccharomyces*, though others have been used as well. Furthermore, there is increasing interest in using manipulation of the gut microbiota with specific probiotics to modulate the immune system to treat rheumatic autoimmune conditions and inflammatory bowel disease[24]. Given the prevalence of probiotic use, the effects of probiotics on bone health is of significant interest. Mccabe et al. have shown that prolonged administration of probiotics such as *Lactobacillus reuteri* decrease intestinal inflammation and increase trabecular and vertebral bone mineral density and mass in healthy male mice, but affect female mice only under inflammatory setting.[25,26] The impact of other probiotic species or combinations of species on bone in healthy animals is not known, but several studies examined the bone effects of probiotics in a variety of disease models.

Estrogen-deficiency induced bone loss models

The influence of microbiota and probiotic treatment has been studied in hormone deficiency-induced osteoporosis models. Hormone deficiency can be induced either by surgical ovariectomy (Ovx) or sex hormone inhibition. Britton et al. showed that *Lactobacillus reuteri* treatment significantly protected mice from bone loss after Ovx in association with reduced levels of a bone resorption marker and decreased osteoclastogenesis. *L. reuteri* suppressed Ovx-induced pro-osteoclastogenic bone marrow CD4 T-lymphocytes and directly suppressed osteoclastogenesis *in vitro*[27]. Similarly, Ohlsson et al. found that treating mice with either the single *Lactobacillus* (*L*) strain, *L. paracasei* DSM13434 (*L. para*) or a mixture of three strains, *L. paracasei* DSM13434, *L. plantarum* DSM 15312 and DSM 15313 (*L.* mix) protected mice from Ovx-induced cortical bone loss and bone resorption. This protection was associated with altered pro-osteoclastogenic cytokines[28]. In contrast, in a rat Ovx model, Parvaneh et al. showed that *Bifidobacterium longum* supplementation increased BMD, but rather than decreasing bone resorption markers they observed an increase in bone formation[29,30].

In a recent study, Li et al. used a model of Lupron induced sex steroid deficiency to investigate the role of microbiota in bone loss. Interestingly, trabecular bone loss after Lupron treatment required the presence of gut microbiota, as bone mass was preserved in GF mice. Sex steroid deprivation was shown to increase intestinal permeability and induce a signature cytokine profile associated with osteoclastogenesis and osteoporosis. Interestingly, the effect of sex steroid deficiency on cortical bone was microbiota independent. Treating conventional mice with either *Lactobacillus rhamnosus* GG (LGG) or the commercially available probiotic supplement VSL#3 (but not *E. coli*) reduced gut permeability, dampened intestinal and bone marrow inflammation, and completely protected against bone loss after sex steroid deprivation and both decreased bone resorption marker and increased bone formation markers[8]. Cumulatively, these data suggest that the observed effect of probiotic treatment may depend on the individual specie(s) contained in the probiotic, the regimen and duration of treatment, the bone compartment examined, as well as the estrogen-deficiency model used.

Low bone mass in type 1 diabetes models

Type 1 diabetes (T1D) is associated with low bone mass, and the effect of probiotics in T1D induced osteoporosis was investigated. Administration of *L. reuteri* prevented T1D-induced bone loss and marrow adiposity in mice. *L. reuteri* has been reported to inhibit TNF, and these investigators proposed that *L. reuteri* treatment prevented the suppression of Wnt10b in diabetic bone by decreasing TNF[31].

Microbiota, malnutrition and bone

Microbiota also have a profound effect on nutrient absorption and caloric uptake, and thus may affect bone growth in conditions of undernutrition. Selected *Lactobacilli* strains promote *Drosophila* juvenile growth in the setting of malnutrition[32] and in a recent study this group further investigated the influence of the microbiota on the somatotropic axis during undernutrition. Juvenile mice from colonies monoassociated with either *Lactobacillus plantarum* (*Lp*) WJL strain or NIZO2877 strain, or maintained GF, were weaned to either breeding or a nutritionally depleted diet. Both Lp^{WJL} and $Lp^{NIZO2877}$ monoassociated juveniles gained more weight and body growth compare to GF juveniles, with Lp^{WJL} having a significantly stronger effect. In the setting of undernutrition, Lp^{WJL}-colonized animals showed a 2-fold increase in weight gain, body length gain, and femur length gain compare to $Lp^{NIZO2877}$-associated animals. Interestingly, the quantitative difference between the two strains did not result from differences in food and calorie intake, as these indexes were similar relative to body weight[10]. Thus, growth benefits of *Lactobacillus plantarum* appear to be strain specific, further complicating our understanding of the impact of probiotics on microbiota and bone.

Chronic undernutrition itself has been shown to modify the gut microbiome and is associated with impaired bone growth during adolescence. In a recent study, microbiota from healthy and undernourished children (6 to 18 months of age) were transplanted into young GF mice. Five weeks after microbiota transplantation, mice receiving gut resident microbes from healthy children saw more rapid increases in body weight and lean mass than those receiving microbiota from undernourished individuals. Paradoxically, animals

receiving microbiota from undernourished donors showed increased femoral cortical bone volume and bone mineral density compared with animals receiving microbiota from healthy donors[33]. Nevertheless, these data demonstrate that in cases of nutritional deficiency, changes in the microbiota may contribute to bone growth and development.

Mechanisms underlying microbiota-mediated effects on bone

There is no simple explanation for the reported effects of gut microbiota on bone. Several mechanisms have been proposed to explain how the gut microbiome might affect bone at a distance. While short chain fatty acids (SCFA), metabolites produced by the microbiota during fermentation of dietary fibre, have been reported to have direct effects on osteoclasts and osteoblasts,[34] it is not clear that the concentration of these metabolites proximal to bone cells is adequate to explain the effect of microbiota on bone[35]. Several mechanisms by which microbiota may indirectly regulate bone are discussed below, including regulation of immune cells and inflammatory cytokine or hormones and growth factors, and regulation of gut epithelial barrier function and nutritional uptake.

Immune mediated mechanisms

Osteoclast differentiation is regulated by pro-osteoclastogenic cytokines, including RANKL, TNF, IL-6, IL-1, and IL-17[36]. Microbiota can profoundly affect immune system maturation, inflammatory cytokine production, and T helper cell differentiation. For example, symbiont species, such as segmented filamentous bacteria and *Bifidobacterium adolescentis,* elicit a pro-inflammatory immune response by promoting the differentiation of Th17 cells, which have been shown to play a role in rheumatoid arthritis and inflammatory bowel disease (IBD) induced bone loss[37,38]. On the other hand, *Bacteroides fragilis* and *Clostridia* species belonging to clusters IV and XIVa elicit an anti-inflammatory response by inducing Treg cells locally in the lamina propria and also in the circulation.[39–41] Thus, it is possible that microbiota composition can impact bone health by influencing T cell differentiation. Indeed, compared to GF mice both conventionally raised and colonized mice have more bone marrow $CD4^+$ T cells and higher expression of RANKL, TNF, IL-6 and IL-1 locally in the gut, as well as distally in the bone marrow. This correlated with elevated bone resorption marker levels[8,9]. Microbiota effects on pro-osteoclastogenic cytokine production may also be mediated by gut endothelial barrier function. Li et al. found that supplementation with either of the probiotics LGG or VSL#3 tightened intestinal barrier integrity and decreased permeability after Ovx, thus decreasing inflammatory, pro-osteoclastogenic cytokine production[8]. Therefore, gut microbiota can impact bone through alterations in systemic and bone marrow immune status, which in turn regulates osteoclastogenesis. Moreover, microbiota could alter bone resorption through effects on B-cell production of the osteoclast inhibitor osteoprotegerin, as microbiota are known to affect B-cell development[42].

Growth factor and hormone mediated mechanisms

Another proposed mechanism by which gut microbiota may affect bone is through modulation of growth factor and hormone production. The intestinal microbiome has been recognized as a virtual “endocrine organ” both because it influences host hormone levels and because some microbes can produce and secret hormones, including serotonin and

dopamine. Hormones such as sex-steroids, serotonins, cortisol, and exogenous glucocorticoids have a plethora effects on bone, thus microbiota may regulate skeletal remodeling through affecting hormone levels[4]. A recent study by Schwarzer et al. showed that growth hormone and IGF-1 levels are higher in conventionally raised neonatal mice compared to GF controls[10]. Our group also reported that colonization significantly increase IGF-1 levels in adult mice. In contrast, either broad-spectrum antibiotics or vancomycin were sufficient to decrease IGF-1 levels in SPF mice[9]. IGF-1 is a pluripotent growth factor that promotes osteoclast, osteoblast and chondrocyte differentiation through endocrine, autocrine and paracrine actions. Thus, modulation of IGF-1 could contribute to microbiota effects on osteoclasts, osteoblasts and growth plate. Indeed, treating GF neonates with recombinant IGF-1 was sufficient to mimic the effects of colonization on skeletal growth, whereas an IGF-1 receptor inhibitor inhibited postnatal bone growth in colonized animals[10]. To investigate the mechanism for microbiota-mediated increases in IGF-1, we administered SCFA to antibiotic-treated mice. SCFA supplementation reversed the changes in serum IGF-1 levels that were observed in antibiotic-treated mice, indicating that microbiota-derived SCFAs are sufficient to mediate the observed changes in IGF-1 levels in the host[9]. Whether SCFA affect IGF-1 through their specific G-protein coupled receptors or by acting as histone deacetylase inhibitors, and what the target organ of SCFA action is are not known.

Microbiota and sex hormones exhibit reciprocal interactions. Antibiotic treatment lead to lower estrogen levels and a correlation between estrogen levels and fecal microbiota composition and richness has been found. On the other hand, a triangular link between the microbiota, hormones and immunity has been proposed. For example, sex steroid deficiency promotes intestinal permeability, thus creating a chronic inflammatory state that increase bone resorption[8]. GF mice have exaggerated cortisol release[43], and cortisol and exogenous glucocorticoids are known to negatively regulate bone health by decreasing calcium absorption, promoting osteocyte and osteoblast apoptosis, and increasing osteoclast mediated resorption[44]. Therefore, it's possible that microbiota affect bone via modulating the cortisol pathway. Serotonin is another possible mediator of the connection between microbiota and bone. Gut is the major site of serotonin production, through the actions of the enzyme tryptophan hydroxylase (TPH1). Some microbes produce serotonin and several studies demonstrate that gut microbiota induce host serotonin production in the gut[7,45], however the majority of reports suggest that the absence of gut serotonin production in *Tph1*$^{-/-}$ mice has little effect on bone physiology[46–48]. Therefore, whether induction of serotonin is involved in microbiota modulation of bone phenotype still needs further investigation.

Nutrition mediated mechanisms

Nutrient uptake could be an important mechanism for effects of microbiota on bone remodeling[49,50]. Gut microbiota plays a pivotal role in food digestion and energy recovery, as well as supplying and regulating the production and/or absorption of vitamins. Studies comparing GF and conventionalized mice revealed that the microbiota promotes absorption of monosaccharides from the gut and induce energy harvest from the diet and energy storage in the host[35]. Moreover, gut microbiota synthesize vitamin K and B-group vitamins[51], therefore helping to ensure sufficient vitamin intake, especially under conditions of poor

nutrition as a result of insufficient food intake or poor eating habits. Gut microbiota also regulates calcium absorption, which may be mediated by changing luminal pH and increasing calcium solubility[5]. Therefore, beneficial effects of gut microbiota on nutrition uptake and energy harvest could promote host bone health.

Future directions

The effect of the gut resident microbiota on human skeletal health is unknown. The translational promise of research into how microbiota impact bone health is the potential for manipulating the microbiome or its metabolites to optimize bone health and growth. The microbiome of a healthy adult contains approximately 160 bacterial species on average.[52] Although microbial species composition varies widely, the gene products and functionalities (the metagenome) represented by these species is more consistent among individuals.[53] A clearer understanding of the specific bone effects and mechanisms by which gut microbiota impact bone growth, turnover and mechanical strength may come from analysis of alterations in the metagenome and metabolomics profile induced by specific manipulations of the microbiome.

Perturbations in gut bacterial communities, termed dysbiosis, have been observed in a variety of chronic inflammatory conditions, including aging, obesity, metabolic syndrome, inflammatory bowel diseases and rheumatoid arthritis. Whether dysbiosis in these conditions is associated with bone loss, for example, age related bone loss, is an area of active investigation. Furthermore, as the links between intestinal dysbiosis and disease strengthen, there has been an explosion of interest in manipulation of composition of the resident gut microbiota as a therapeutic modality. More than a dozen randomized clinical trials have been completed assessing microbiome based therapies in a variety of disease states.[54] With the rising popularity of probiotics and medical use of probiotics and fecal microbiota transplant (FMT) to treat disease, understanding whether these manipulations have unintended consequences for bone health is increasingly important.

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Table 1

Summary of studies using GF models to study the effects of microbiota on bone

Source	Strain and vendor	Gender	Treatment age	Treatment duration	Groups	Microbiota source/ composition	Bone measurement	Findings
Sjogren et al.	C57Bl6/J	Female	3-week-old	4 weeks	GF vs. conventional; GF vs. colonized	Cecal content from C57BL6/J donor mice	Micro-CT, histomorphometry, pQCT	Lower bone mass in conventional and colonized mice
Schwarzer et al.	BALB/c mice	Male	3-week-old	5 weeks	GF vs. conventional; GF vs. Lactobacillus monocolonized mice	*Lactobacillus plantarum*^{WJL}, *Lactobacillus plantarum*^{NIZO2877}	Micro-CT	Reduced femur length, cortical thickness, cortical bone fraction, and the trabecular fraction in GF animals, cortical BMD unaffected
Li et al.	C57BL6/J from Taconic	Female	10-week-old	10 weeks	GF vs. conventional; GF vs. colonized	colon and cecal contents of 10-week old Conv.R mice	Micro-CT, histomophometry, bone turnover markers	Trend towards lower bone mass in conventional and colonized mice
Yan et al.	CB6F1 from NIA (Charles River)	Female, Male	2-month-old	1 month, 8 months	GF vs. colonized	Fecal material from 3 month old NIA mice	Micro-CT, histomophometry, bone turnover markers	Reduced bone mass, increase bone resorption, increase bone formation, thicken growth plate and 2nd ossification center after short-term colonization; Increased bone length, periosteal area, and endosteal area after long-term colonization

Table 2

Summary of studies using antibiotic-treated models to study the effects of microbiota on bone

Source	Strain	Gender	Treatment age	Treatment duration	Groups	Antibiotic used	Bone measurement	Findings
Cho et al.	C57BL/6J	Male, Female	4-week-old	3 weeks, 7 weeks	Control vs. antibiotics	low-dose penicillin, chlortetracycline, vancomycin	DEXA	Increased BMD at 3 week but not at 7 weeks
Cox et al.	C57BL/6J	Male, Female	Born, 4-week-old	Until 20-week-old	Control vs. antibiotic administered before born or weaning	low-dose penicillin	DEXA	Decrease bone mineral content in male; increase mineral content in female
Nobel et al.	C57BL/6J	Female	10-day-old	3 intermittent treatment in 30-day period	Control vs. antibiotic	Therapeutic dose of Amoxicillin and tylosin alone or combination	DEXA	developed larger bones than controls, increases in bone area and mineral content were most pronounced in the amoxicillin group
Yan et al.	BALB/c	Female	2-month-old	1 month	SPF vs. antibiotic mixture; SPF vs. vancomycin	Cocktail of ampicillin, vancomycin, metronidazole, and neomycin; Cocktail of gentamicin, ciprofloxacin, streptomycin, and bacitracin; Vancomycin alone	microCT, bone turnover markers	Increased bone mass, reduced bone formation marker in antibiotic treated mice
Guss et al.	C57BL/6J, WT and TLR5 KO		Born, 4-week-old	Until 16-week-old	WT vs. TLR5 KO; SPF vs. antibiotics	Ampicillin and neomycin	microCT, mechanic test	Wider and shorter femur, and less whole bone strength in TLR5 KO; Less bending strength in both WT and KO mice treated with antibiotics

Abbreviations: BMD: Bone mineral density; DEXA: dual energy X-ray absorptiometry; KO: knockout; WT: wildtype