Published in final edited form as:

Calcif Tissue Int. 2018 April ; 102(4): 480–488. doi:10.1007/s00223-018-0403-7.

Advances in Probiotic Regulation of Bone and Mineral Metabolism

Laura R. McCabe^{1,2,3} and Narayanan Parameswaran¹

¹Department of Physiology, Michigan State University, East Lansing, Michigan

²Department of Radiology, Michigan State University, East Lansing, Michigan

³Biomedical Imaging Research Center, Michigan State University, East Lansing, Michigan

Abstract

Probiotics have been consumed by humans for thousands of years because they are beneficial for long-term storage of foods and promote the health of their host. Ingested probiotics reside in the gastrointestinal tract where they have many effects including modifying the microbiota composition, intestinal barrier function and the immune system which result in systemic benefits to the host, including bone health. Probiotics benefit bone growth, density and structure under conditions of dysbiosis, intestinal permeability and inflammation (recognized mediators of bone loss and osteoporosis). It is likely that multiple mechanisms are involved in mediating probiotic signals from the gut to the bone. Studies indicate a role for the microbiota (composition and activity), intestinal barrier function and immune cells in the signaling process. These mechanisms are not mutually exclusive, but rather, may synergize to provide benefits to the skeletal system of the host and serve as a starting point for investigation. Given that probiotics hold great promise for supporting bone health and are generally regarded as safe, future studies identifying mechanisms are warranted.

Keywords

probiotic; microbiota; osteoporosis; Lactobacillus; intestine; bone; inflammation; barrier; permeability; colon

History of Probiotics

Probiotics have been consumed by humans dating back to before 7000 BCE [1]. Probiotics are contained in many fermented products made to undergo long-term storage, such as beer, bread, wine, kefir, kumis, and cheese [2–4]. For example, probiotic *Lactobacillus* strains are found in the brine of fermented olives, pickled juices, kefir and yogurt [5]. Probiotics are also found in non-fermented foods such as meat and fruits as well as in the gastrointestinal

Laura R McCabe (mccabel@msu.edu); Orchid ID: 0000-0001-5407-162X
Narayanan Parameswaran (narap@msu.edu) ORCID ID: 0000-0002-8894-5564

SUMMARY STATEMENT OF DISCLOSURES:

Funding: This review was supported by NIH grants (RO1 DK101050 and RO1 AT007695) to LRM and NP

Conflict of Interest: LRM and NP have no conflict of interest.

tract of animals such as pigs, rats, and poultry [5]. Probiotic intake has long been associated with a longer, healthier life [1, 2, 6]. In the late 1800s, it was hypothesized that *Lactobacillus* strains produce factors that benefit gastrointestinal metabolism, the intestinal epithelium and health [1–3, 6, 7]. It was also thought that ingestion of beneficial bacteria could help treat disease through the restoration of the microbiota composition. The concept that beneficial microbes mediate health, continues to be supported today. In fact, probiotics are defined by the FAO/WHO (Food and Agricultural Organization/World Health Organization) as “live microorganisms that when administered in adequate amounts will confer a health benefit on the host”. This definition is broad without distinction between organic/inorganic, single versus multiple mediators, effects on bacterial growth, or increased resistance to infection. At the same time the definition imposes some core requirements [6, 8].

The nomenclature for bacteria, and thus probiotics, is based on a taxonomic/genetic hierarchy where bacteria are divided into phylum, class, order, family, genus, species and subspecies and/or strain. With more than 23 bacteria phyla, it is easy to see the abundance of specific probiotics and the complexity of their names. Evidence indicate that probiotic effects can be strain-specific. In addition, not all bacteria within a species act the same and/or can be regarded as a probiotic. Lactic acid producing bacteria, which include lactobacilli strains, are one of the most well-known groups of bacteria/probiotics with health benefits that have been used for centuries. They are gram-positive, obtained from fermented milk products and are usually found in carbohydrate rich environments and normal intestinal flora of many animals [3]. The genus *Lactobacillus* belongs to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae*. The most commonly isolated species are *Lactobacillus acidophilus*, *L. salivarius*, *L. casei*, *L. plantarum*, *L. fermentum*, *L. reuteri*, *L. rhamnosus*, *L. gasseri*, and *L. brevis* from human intestine. Several of these, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus reuteri* have been extensively studied and well documented. *Lactobacillus acidophilus*, can colonize the human colon, has antimicrobial effects, and is used to treat intestinal infections [9]. *Lactobacillus rhamnosus GG* or *Lactobacillus GG* (LGG) tolerates low pH environment, adheres to the gastrointestinal tract and is effective in treating diarrhea [2, 10].

Role of Probiotics in Bone Health

Bone health depends on the balance of bone resorption by osteoclasts and bone formation by osteoblasts. Hormones, immune cells and the gastrointestinal system can regulate these processes[11–13]. While the intestine is known for its key role in calcium, phosphorous and magnesium absorption (contributors to bone mineralization)[14], the intestine also produces endocrine factors that signal to bone cells, such as incretins[15] and serotonin[16]. Recent studies, including some from our lab, indicate that the intestinal microbiota and physiology can also regulate bone health [17–22]. Thus, probiotics which modify the microbiota composition and/or function and promote intestinal health can also benefit bone health. This is supported by numerous studies in a variety of animal models (Table 1). Several recent clinical studies confirm the benefits of probiotics to human skeletal health [23–29]. The mechanisms accounting for probiotic benefits on bone are not fully known, but likely involve 1) changing the intestinal microbiome, 2) modifying intestinal barrier function, and

3) impacting the immune system/immune cells (Figure 1, 2). We discuss these possible mechanisms below.

1) Probiotics modify the microbiome to regulate bone health

The human body is the host for ~100 trillion microbes comprising ~1000 species and 28 different phyla [30]. In addition to microbes outnumbering human cells, the gut microbiota also express 100-fold more genes compared to human genome[30]. Changes in microbiota composition can have beneficial or harmful consequences on human health[31]. Therefore, the microbiome-host relationship is an important target for therapeutic purposes, and probiotics may be a key therapy based on their ability to influence the intestine.

Ingested probiotics enter and populate the GI tract. Depending upon the diet, the dose of naturally ingested probiotic bacteria can range from 10^8 – 10^{12} CFU per day [32]. While bacteria are robust and hearty, the acid environment of the stomach and bile and enzyme secretion in the intestine can decrease the number of viable probiotics in the gut by 50% or greater [33]. The abundance of ingested bacteria relative to residential bacteria is much greater in the small intestine where the ratio can be 1:1, whereas in the colon the ingested probiotics are greatly outnumbered by the existing resident bacteria and may comprise only 0.0001-fold of the total bacteria [32, 34]. Ingested probiotics are maintained in the gastrointestinal tract for a few days after ingestion but are rarely maintained after one week [32, 35, 36]. Thus, probiotics likely need to be continuously ingested to obtain maximum benefits [37].

Probiotics can modify the microbiota composition simply by the increased presence and proliferation of the probiotic in the gastrointestinal tract, as well as through their ability of the probiotic to compete with other bacterial strains. Probiotics can digest complex carbohydrates and generate oligosaccharides that can be further metabolized by other bacteria that subsequently proliferate and modify the microbiota composition. Probiotic bacteria also secrete antimicrobial agents that can kill certain bacterial strains in the gastrointestinal tract. For example, *Lactobacilli* strains produce several lactic acid, bacteriocins (antimicrobial peptides) and hydrogen peroxide [38].

Changes in the composition of the microbiota affect bone parameters under a variety of conditions [39–41]. A clear link has been demonstrated between microbiota composition and bone growth. For example, the microbiota of undernourished children with stunted growth differs dramatically from healthy children [42] and when transferred to germ-free mice (mice lacking a microbiome) cause poor growth[42]. More importantly, supplementation with two bacterial strains (*Ruminococcus gnavus* and *Clostridium symbiosum*) ameliorated growth abnormalities in the mice receiving the microbiota from undernourished children[42]. Similarly, treatment with the probiotic *Lactobacillus plantarum* restored normal growth rates in undernourished mice [43]. The *L. plantarum* treatment raised serum IGF-1 and IGFBP-3 to normal levels [43] consistent with reports demonstrating the microbiota regulates the IGF-1-IGF-1R axis [44]. This is a general phenomenon since germ-free drosophila repopulated with *lactobacilli* strains regain a normal IGF axis and grow at normal rates [45, 46]. In humans, the probiotic *Bifidobacterium lactis* benefited the growth of infants born to mothers with human immunodeficiency virus[47].

Taken together, these studies demonstrate that manipulation of the microbiota with probiotics can benefit bone growth during development.

Microbiota shifts are also associated with changes in bone density. A negative association exists between bone density and intestinal dysbiosis (microbiota imbalance favoring pathogenic over beneficial bacterial strains) observed in inflammatory bowel disease [48]. There are also positive associations, whereby shifts in microbiota composition can enhance bone density. Our lab has shown that treatment of ovariectomized mice with probiotic *Lactobacillus reuteri* causes a significant change in the microbiota composition and prevents estrogen deficiency induced bone loss [19]. Shifts in microbiota composition induced by prebiotic use, also support a role for the microbiota composition in regulating bone health parameters in both mice and humans [49]. In addition, many papers that indicate the probiotic ingestion can modify bone density, though links with specific microbiota components were not determined [17, 21, 22, 50]. In the past year, studies also demonstrate the role of the microbiota in periodontal disease. Diabetes was shown to shift oral microbiota composition that was shown, by transfer to germ-free mice, to be pathogenic and promote periodontal disease and tooth loss [51]. Studies also suggest that probiotics, such as *Lactobacillus rhamnosus GG*, *Bifidobacterium* or *Lactobacillus gasseri*, may reduce periodontal disease and bone loss, though more work is needed to determine the specific mechanisms [52–54].

2) Probiotics modify intestinal barrier function to regulate bone health

The intestinal epithelium is essential for providing a selective barrier that prevents translocation of harmful substances and pathogens and their products into the blood stream. It is composed of several layers. Importantly, intestinal epithelial cells (IECs) joined together by tight junction proteins provides the key barrier support which allows only selective transport. Additionally, a mucus layer produced by goblet cells covers the surface of the epithelial cells and prevents gut bacteria and pathogen access to host epithelial cells. The intestine also secretes immunoglobulins, defensins, and other antimicrobial products that contribute to maintaining a healthy environment.

Disruption of the epithelial barrier can lead to pathogen translocation into the bloodstream causing systemic inflammation and trigger GI diseases such as inflammatory bowel disease (IBD), celiac disease, and colon cancer [55–57]. Studies from our lab and others demonstrate that GI barrier dysregulation also promotes bone loss [50, 58–61]. Therefore, dysbiosis may promote bone loss by increasing intestinal permeability and serum endotoxin levels [62–64]. The loss of estrogen is known to promote both increased intestinal permeability as well as bone loss [65]. Consistent with this, colonic paracellular permeability decreases during the oestrus phase (high levels of estrogen) of the rat when compared to the dioestrus phase (low levels of estrogen)[65]. Our lab demonstrated increased intestinal permeability as early as 1 week following ovariectomy and estrogen loss in mice [58]. *Ex vivo* Ussing chambers analyses demonstrated that the ileum had the most dynamic changes and that estrogen deficiency induces region-specific effects on intestinal permeability [58].

Similarly, while probiotic treatment can benefit dysbiosis, probiotics may mediate bone health by enhancing intestinal barrier function [66–70]. In a study causing enteropathogenic *E.coli* (EPEC)-induced dysbiosis, administration of probiotic *E.coli* Nissle 1917 increased specific claudin expression and prevented increases in intestinal permeability seen after infection with EPEC [71]. Additional studies also indicate that probiotics can rescue barrier function [72–75]. Consistent with a link between barrier function and bone health, probiotic treatment of estrogen deficient mice not only prevents intestinal permeability but also prevents bone loss [50]. Probiotic *Lactobacillus rhamnosus* GG (LGG) as well as the probiotic supplement VSL#3 reduced gut permeability, intestinal inflammation, and completely protects against bone loss induced by estrogen deficiency [50]. Together, these data highlight the role that of the gut epithelial barrier and microbiota in bone loss induced by estrogen-deficiency.

3) Role of the immune system in mediating probiotic effects on bone

The intestinal immune system, one of the largest in the body, is uniquely positioned to encounter not only dietary components but also gut microbiota and pathogens. The gut immune system must recognize and differentially respond to pathogens, dietary components and commensal and beneficial microbes [76, 77]. In addition to immune cells, paneth and goblet cells (specialized epithelial cells) play an important role in this process. Intestinal immune system dysregulation is linked to a number of diseases, including inflammatory bowel disease (IBD) and celiac disease, associated with pathologic expression of cytokines which are also important players in the regulation of bone homeostasis [78, 79]. Thus, it is not surprising that many of these intestinal conditions lead to bone loss [80, 81]. This prompted our lab and others to more closely examine the relationship of the gut and the bone in the context of changes in intestinal inflammation. In early studies, we found that induction of colitis leads to changes in the immune cell composition and cytokine expression within the bone marrow [82]. These changes in the bone marrow can signal to bone cells (stem cells, osteoblasts and osteoclasts) to significantly alter bone homeostasis. This is indeed the case in animal models of IBD, where dysregulation of cytokines (TNF α , IL1 α , IL1 β , IL-6) correlate with bone loss [83–87]. Consistent with animal models, patients with IBD have elevated levels of cytokines [83, 84] and exhibit bone loss. Thus, the role of immune system in modulating the gut-bone axis is becoming increasingly clear.

The intestinal microbiota is an important modulator of the gut immune system. Dysbiosis has been shown to increase osteoclastogenic activity including increased IL-17 producing cells and reduced bone mass [51]. Similarly, oral administration of “bad” bacteria to mice causes dysregulated intestinal inflammation and corresponding bone loss [86]. Thus, changes in intestinal microbiota can influence immune responses and bone health. Using germ-free mice Sjogren *et al*, showed that germ free mice have higher bone mass compared to conventional mice [88]. This was associated with reduced numbers of osteoclasts precursors as well as decreased frequency of CD4⁺ T cells in the bone marrow. However, the bone density findings in germ free mice have been variable showing more, less or no change [43, 89–91]; the differing compositions of the microbiota used to populate the conventionalized mice mostly likely contributes to the differing responses.

Previous studies indicate that probiotics can modulate immune function [92, 93]. Because probiotics are orally ingested, they can readily interact with the gut immune components which consequently influence local and distant organ functions. Identification of the dysregulated inflammatory pathways in dysbiosis-induced bone loss prompted us (and others) to examine the role of immune system in mediating the probiotic benefits on bone health. Consistent with its anti-TNF α activity, *L. reuteri* treatment decreases intestinal TNF α expression in male mice and correspondingly increases bone density [94]. Although healthy female mice did not respond to *L. reuteri* treatment in terms of bone density, induction of mild inflammatory state is sufficient to increase bone density following probiotic administration [95]. These results suggest that *L. reuteri* effects on bone are somehow linked to the inflammatory state of the organism. The precise immunological mechanisms by which *L. reuteri* affects bone density is still under active investigation. Interestingly however, *L. reuteri* reversed ovariectomy-induced bone loss and this was associated with corresponding changes in bone marrow CD4⁺ T cells [96]. This was associated with anti-osteoclastogenic activity of *L. reuteri*. This activity is suggested to be in *L. reuteri* conditioned media which inhibits the differentiation of monocytic-macrophages to osteoclasts [96]. Earlier studies indicate that, histamine secreted by *L. reuteri*, is involved in suppressing TNF α production from human monocytic cells [97]. Thus, the *L. reuteri* effects on bone may involve anti-inflammatory effects on the gut and potentially direct effects on bone cells. Whether these factors influence gut immune or bone immune components *in vivo* is still under active investigation.

Similar to our findings with *L. reuteri*, other groups have shown that LGG and VSL#3 administration reduces TNF α , IL-17 and RANKL expression in the gut and bone marrow of ovariectomized mice [89]. Ohlsson *et al* [98] demonstrated that suppression of bone marrow T-regulatory cells by ovariectomy is partially reversed by probiotic supplementation. Consistent with regulation of anti-inflammatory T-cells, bone TNF α and other pro-inflammatory cytokines were reduced by probiotic supplementation in the ovariectomized mice, and enhanced TGF β 1 expression (associated with increased T-reg) was observed. Wang *et al* showed that the probiotic *L. casei* alters macrophage phenotype. Specifically, wear debris generated from hip implantation activates macrophages leading to a pro-inflammatory state and osteolysis [99]; however, *L. casei* treatment inhibited osteolysis and the pro-inflammatory state of the macrophages. Such anti-inflammatory effects of probiotics in periodontal models have also been demonstrated [54]. Other studies have indicated that probiotics can beneficially affect hematopoiesis in the bone marrow further suggesting that orally administered probiotics can significantly affect immune cells in the bone marrow that can consequently affect bone health [100]. Taken together, while these studies suggest that probiotics can significantly influence immune system and bone, further definitive studies are necessary to identify specific mechanisms.

Conclusions

The above studies indicate that probiotics hold great promise for supporting bone health. It is likely that multiple mechanisms are involved in linking probiotic signals from the gut to the bone. Here, we discussed studies that indicate a role for the microbiota (composition and activity), intestinal barrier function and immune cells in the signaling process. These

mechanisms are not mutually exclusive, but rather, may synergize to provide benefits to the skeletal system of the host and serve as a starting point for investigation. Given that probiotics are generally regarded as safe, future studies identifying mechanisms are warranted.

Acknowledgments

The authors would like to acknowledge funding from the National Institute of Health, grants RO1 DK101050 and RO1 AT007695.

References

1. Gasbarrini, G., Bonvicini, F., Gramenzi, A. Probiotics History. J Clin Gastroenterol; Proceedings from the 8th Probiotics, Prebiotics & New Foods for Microbiota and Human Health meeting; Rome, Italy. September 13–15, 2015; 2016. p. S116-S119.
2. Gogineni V. Probiotics:History and Evolution. J Anc Dis Prev Remedies. 2013; 1:1–7.
3. Azizpour K, et al. History and basic of probiotics. Research Journal of Biological Sciences. 2009; 4:409–426.
4. Ozen M, Dinleyici EC. The history of probiotics: the untold story. Benef Microbes. 2015; 6(2):159–65. [PubMed: 25576593]
5. Fontana L, et al. Sources, isolation, characterisation and evaluation of probiotics. Br J Nutr. 2013; 109(Suppl 2):S35–50. [PubMed: 23360880]
6. Anukam KC, Reid G. Organisms associated with bacterial vaginosis in Nigerian women as determined by PCR-DGGE and 16S rRNA gene sequence. Afr Health Sci. 2007; 7(2):68–72. [PubMed: 17594282]
7. Calatayud GA, Suarez JE. A new contribution to the history of probiotics. Benef Microbes. 2017; 8(2):323–325. [PubMed: 28403650]
8. Hill C, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014; 11(8):506–14. [PubMed: 24912386]
9. McFarland LV. From yaks to yogurt: the history, development, and current use of probiotics. Clin Infect Dis. 2015; 60(Suppl 2):S85–90. [PubMed: 25922406]
10. Gorbach SL. Probiotics and gastrointestinal health. Am J Gastroenterol. 2000; 95(1 Suppl):S2–4. [PubMed: 10634218]
11. Cenci S, et al. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. J Clin Invest. 2000; 106(10):1229–37. [PubMed: 11086024]
12. Hock JM, et al. Human parathyroid hormone-(1–34) increases bone mass in ovariectomized and orchidectomized rats. Endocrinology. 1988; 122(6):2899–904. [PubMed: 3371266]
13. Yamada C. Role of incretins in the regulation of bone metabolism. Nihon Rinsho. 2011; 69(5): 842–7. [PubMed: 21595269]
14. Christakos S, et al. Intestinal Regulation of Calcium: Vitamin D and Bone Physiology. Adv Exp Med Biol. 2017; 1033:3–12. [PubMed: 29101648]
15. Ramsey W, Isales CM. Intestinal Incretins and the Regulation of Bone Physiology. Adv Exp Med Biol. 2017; 1033:13–33. [PubMed: 29101649]
16. Lavoie B, Lian JB, Mawe GM. Regulation of Bone Metabolism by Serotonin. Adv Exp Med Biol. 2017; 1033:35–46. [PubMed: 29101650]
17. Ohlsson C, et al. Probiotics protect mice from ovariectomy-induced cortical bone loss. PLoS One. 2014; 9(3):e92368. [PubMed: 24637895]
18. Parvaneh K, et al. Probiotics (Bifidobacterium longum) Increase Bone Mass Density and Upregulate Sparc and Bmp-2 Genes in Rats with Bone Loss Resulting from Ovariectomy. Biomed Res Int. 2015; 2015:897639. [PubMed: 26366421]

19. Britton RA, et al. Probiotic *L. reuteri* Treatment Prevents Bone Loss in a Menopausal Ovariectomized Mouse Model. *J Cell Physiol.* 2014
20. Collins FL, et al. *Lactobacillus reuteri* 6475 Increases Bone Density in Intact Females Only under an Inflammatory Setting. *PLoS One.* 2016; 11(4):e0153180. [PubMed: 27058036]
21. Zhang J, et al. Loss of Bone and Wnt10b Expression in Male Type 1 Diabetic Mice Is Blocked by the Probiotic *Lactobacillus reuteri*. *Endocrinology.* 2015; 156(9):3169–82. [PubMed: 26135835]
22. McCabe LR, et al. Probiotic use decreases intestinal inflammation and increases bone density in healthy male but not female mice. *Journal of Cellular Physiology.* 2013; 228(8):1793–8. [PubMed: 23389860]
23. Sommer F, Bäckhed F. The gut microbiota — masters of host development and physiology. *Nat Rev Microbiol.* 2013; 11:227–238. [PubMed: 23435359]
24. Blanton LV, Charbonneau MR, Salih T, Barratt MJ, Venkatesh S, Ilkaveya O, Subramanian S, Manary MJ, Trehan I, Jorgensen JM, Fan Y-M, Henrissat B, Leyn SA, Rodionov DA, Osterman AL, Maleta KM, Newgard CB, Ashorn P, Dewey KG, Gordon JI. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science.* 2016 Feb 19; 351(6275)
25. Schwarzer M, Makki K, Storelli G, Machuca-Gayet I, Srutkova D, Hermanova P, Martino ME, Balmand S, Hudcovic T, Heddi A, Rieusset J, Kozakova H, Vidal H, Leulier F. *Lactobacillus plantarum* strain maintains growth of infant mice during chronic undernutrition. *Science.* 2016 Feb 19; 351(6275):854–7. [PubMed: 26912894]
26. Steenhout PG, Rochat F, Hager C. The Effect of *Bifidobacterium lactis* on the Growth of Infants: A Pooled Analysis of Randomized Controlled Studies. *Ann Nutr Metab.* 2009; 55:334–340. [PubMed: 19844090]
27. Lei M, Hua L-M, Wang D-W. The effect of probiotic treatment on elderly patients with distal radius fracture: a prospective double-blind, placebo-controlled randomised clinical trial. *Benef Microbes.* 2016; 7:631–637. [PubMed: 27633174]
28. Tu M-Y, Chen H-L, Tung Y-T, Kao C-C, Hu F-C, Chen C-M. Short-Term Effects of Kefir-Fermented Milk Consumption on Bone Mineral Density and Bone Metabolism in a Randomized Clinical Trial of Osteoporotic Patients. *PLoS One.* 2015; 10:e0144231. [PubMed: 26655888]
29. Lambert, et al. Combined bioavailable isoflavones and probiotics improve bone status and estrogen metabolism in postmenopausal osteopenic women: a randomized controlled trial. *Am J Clin Nutr.* 2017; 106:909–20. [PubMed: 28768651]
30. Fukuda S, Ohno H. Gut microbiome and metabolic diseases. *Semin Immunopathol.* 2014; 36(1): 103–14. [PubMed: 24196453]
31. Ley RE, et al. Microbial ecology: human gut microbes associated with obesity. *Nature.* 2006; 444(7122):1022–3. [PubMed: 17183309]
32. Derrien M, van Hylckama Vlieg JE. Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends Microbiol.* 2015; 23(6):354–66. [PubMed: 25840765]
33. Oozeer R, et al. Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk. *Appl Environ Microbiol.* 2006; 72(8):5615–7. [PubMed: 16885316]
34. Loh G, Blaut M. Role of commensal gut bacteria in inflammatory bowel diseases. *Gut Microbes.* 2012; 3(6):544–55. [PubMed: 23060017]
35. Firmesse O, et al. *Lactobacillus rhamnosus* R11 consumed in a food supplement survived human digestive transit without modifying microbiota equilibrium as assessed by real-time polymerase chain reaction. *J Mol Microbiol Biotechnol.* 2008; 14(1–3):90–9. [PubMed: 17957115]
36. Fujimoto J, et al. Identification and quantification of *Lactobacillus casei* strain Shirota in human feces with strain-specific primers derived from randomly amplified polymorphic DNA. *Int J Food Microbiol.* 2008; 126(1–2):210–5. [PubMed: 18573558]
37. Bezkorovainy A. Probiotics: determinants of survival and growth in the gut. *Am J Clin Nutr.* 2001; 73(2 Suppl):399S–405S. [PubMed: 11157348]
38. Lebeer S, Vanderleyden J, De Keersmaecker SC. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev.* 2008; 72(4):728–64. Table of Contents. [PubMed: 19052326]

39. McCabe L, Britton RA, Parameswaran N. Prebiotic and Probiotic Regulation of Bone Health: Role of the Intestine and its Microbiome. *Curr Osteoporos Rep.* 2015; 13(6):363–71. [PubMed: 26419466]
40. Quach D, Britton RA. Gut Microbiota and Bone Health. *Adv Exp Med Biol.* 2017; 1033:47–58. [PubMed: 29101651]
41. Pacifici R. Bone Remodeling and the Microbiome. *Cold Spring Harb Perspect Med.* 2017
42. Blanton LV, et al. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science.* 2016; 351(6275)
43. Schwarzer M, et al. *Lactobacillus plantarum* strain maintains growth of infant mice during chronic undernutrition. *Science.* 2016; 351(6275):854–7. [PubMed: 26912894]
44. Yan J, et al. Gut microbiota induce IGF-1 and promote bone formation and growth. *Proc Natl Acad Sci U S A.* 2016; 113(47):E7554–E7563. [PubMed: 27821775]
45. Storelli G, et al. *Lactobacillus plantarum* promotes *Drosophila* systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab.* 2011; 14(3):403–14. [PubMed: 21907145]
46. Hyun S. Body size regulation and insulin-like growth factor signaling. *Cell Mol Life Sci.* 2013; 70(13):2351–65. [PubMed: 23508807]
47. Steenhout PG, Rochat F, Hager C. The effect of *Bifidobacterium lactis* on the growth of infants: a pooled analysis of randomized controlled studies. *Ann Nutr Metab.* 2009; 55(4):334–40. [PubMed: 19844090]
48. Sylvester FA. Inflammatory Bowel Disease: Effects on Bone and Mechanisms. *Adv Exp Med Biol.* 2017; 1033:133–150. [PubMed: 29101654]
49. Whisner CM, Weaver CM. Prebiotics and Bone. *Adv Exp Med Biol.* 2017; 1033:201–224. [PubMed: 29101657]
50. Li JY, et al. Sex steroid deficiency-associated bone loss is microbiota dependent and prevented by probiotics. *J Clin Invest.* 2016; 126(6):2049–63. [PubMed: 27111232]
51. Xiao E, et al. Diabetes Enhances IL-17 Expression and Alters the Oral Microbiome to Increase Its Pathogenicity. *Cell Host Microbe.* 2017; 22(1):120–128 e4. [PubMed: 28704648]
52. Gatej SM, et al. Probiotic *Lactobacillus rhamnosus* GG prevents alveolar bone loss in a mouse model of experimental periodontitis. *J Clin Periodontol.* 2017
53. Ricoldi MST, et al. Effects of the probiotic *Bifidobacterium animalis* subsp. *lactis* on the non-surgical treatment of periodontitis. A histomorphometric, microtomographic and immunohistochemical study in rats. *PLoS One.* 2017; 12(6):e0179946. [PubMed: 28662142]
54. Kobayashi R, et al. Oral administration of *Lactobacillus gasseri* SBT2055 is effective in preventing *Porphyromonas gingivalis*-accelerated periodontal disease. *Sci Rep.* 2017; 7(1):545. [PubMed: 28373699]
55. France MM, Turner JR. The mucosal barrier at a glance. *J Cell Sci.* 2017; 130(2):307–314. [PubMed: 28062847]
56. Konig J, et al. Human Intestinal Barrier Function in Health and Disease. *Clin Transl Gastroenterol.* 2016; 7(10):e196. [PubMed: 27763627]
57. Nagao-Kitamoto H, et al. Pathogenic role of the gut microbiota in gastrointestinal diseases. *Intest Res.* 2016; 14(2):127–38. [PubMed: 27175113]
58. Collins F, et al. Temporal and Regional Intestinal Change in Permeability, Tight Junction and Cytokine Gene Expression Following Ovariectomy-induced Estrogen Deficiency. *Physiological Reports.* 2017 In press.
59. Irwin R, et al. Intestinal inflammation without weight loss decreases bone density and growth. *Am J Physiol Regul Integr Comp Physiol.* 2016; 311(6):R1149–R1157. [PubMed: 27733383]
60. Irwin R, et al. Colitis-induced bone loss is gender dependent and associated with increased inflammation. *Inflamm Bowel Dis.* 2013; 19(8):1586–97. [PubMed: 23702805]
61. Harris L, et al. Inflammatory bowel disease causes reversible suppression of osteoblast and chondrocyte function in mice. *Am J Physiol Gastrointest Liver Physiol.* 2009; 296(5):G1020–9. [PubMed: 19299577]

62. Tremellen K, Pearce K. Dysbiosis of Gut Microbiota (DOGMA)--a novel theory for the development of Polycystic Ovarian Syndrome. *Med Hypotheses*. 2012; 79(1):104–12. [PubMed: 22543078]
63. Maruyama K, Sano G, Matsuo K. Murine osteoblasts respond to LPS and IFN-gamma similarly to macrophages. *J Bone Miner Metab*. 2006; 24(6):454–60. [PubMed: 17072737]
64. Moriyama H, Ukai T, Hara Y. Interferon-gamma production changes in parallel with bacterial lipopolysaccharide induced bone resorption in mice: an immunohistometrical study. *Calcif Tissue Int*. 2002; 71(1):53–8. [PubMed: 12043012]
65. Braniste V, et al. Oestradiol decreases colonic permeability through oestrogen receptor beta-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells. *J Physiol*. 2009; 587(Pt 13):3317–28. [PubMed: 19433574]
66. Rosenfeldt V, et al. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. *J Pediatr*. 2004; 145(5):612–6. [PubMed: 15520759]
67. Stratiki Z, et al. The effect of a bifidobacter supplemented bovine milk on intestinal permeability of preterm infants. *Early Hum Dev*. 2007; 83(9):575–9. [PubMed: 17229535]
68. Madsen K, et al. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology*. 2001; 121(3):580–91. [PubMed: 11522742]
69. Zareie M, et al. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut*. 2006; 55(11):1553–60. [PubMed: 16638791]
70. Bron PA, et al. Can probiotics modulate human disease by impacting intestinal barrier function? *Br J Nutr*. 2017; 117(1):93–107. [PubMed: 28102115]
71. Zyrek AA, et al. Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. *Cell Microbiol*. 2007; 9(3):804–16. [PubMed: 17087734]
72. Anderson RC, et al. *Lactobacillus plantarum* DSM 2648 is a potential probiotic that enhances intestinal barrier function. *FEMS Microbiol Lett*. 2010; 309(2):184–92. [PubMed: 20618863]
73. Resta-Lenert S, Barrett KE. Probiotics and commensals reverse TNF-alpha- and IFN-gamma-induced dysfunction in human intestinal epithelial cells. *Gastroenterology*. 2006; 130(3):731–46. [PubMed: 16530515]
74. Qin H, et al. *L. plantarum* prevents enteroinvasive *Escherichia coli*-induced tight junction proteins changes in intestinal epithelial cells. *BMC Microbiol*. 2009; 9:63. [PubMed: 19331693]
75. Moorthy G, Murali MR, Devaraj SN. Lactobacilli facilitate maintenance of intestinal membrane integrity during *Shigella dysenteriae* 1 infection in rats. *Nutrition*. 2009; 25(3):350–8. [PubMed: 19036564]
76. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. *Nat Rev Immunol*. 2014; 14(10):667–85. [PubMed: 25234148]
77. Kundu P, et al. Our Gut Microbiome: The Evolving Inner Self. *Cell*. 2017; 171(7):1481–1493. [PubMed: 29245010]
78. Neurath MF. Cytokines in inflammatory bowel disease. *Nature reviews Immunology*. 2014; 14:329–42.
79. Strober W I, Fuss J. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*. 2011; 140:1756–67. [PubMed: 21530742]
80. Bjarnason I, et al. Reduced bone density in patients with inflammatory bowel disease. *Gut*. 1997; 40:228–33. [PubMed: 9071937]
81. Zanchetta MB, Longobardi V, Bai JC. Bone and Celiac Disease. *Current Osteoporosis Reports*. 2016; 14:43–48. [PubMed: 26875096]
82. Trottier MD, et al. Enhanced production of early lineages of monocytic and granulocytic cells in mice with colitis. *Proc Natl Acad Sci U S A*. 2012; 109(41):16594–9. [PubMed: 23012474]
83. Ali T, et al. Osteoporosis in inflammatory bowel disease. *The American journal of medicine*. 2009; 122:599–604. [PubMed: 19559158]
84. Ciucci T, et al. Bone marrow Th17 TNF α cells induce osteoclast differentiation, and link bone destruction to IBD. *Gut*. 2015; 64:1072–81. [PubMed: 25298539]

85. Harris L, et al. Inflammatory bowel disease causes reversible suppression of osteoblast and chondrocyte function in mice. *American journal of physiology Gastrointestinal and liver physiology*. 2009; 296:G1020–G1029. [PubMed: 19299577]
86. Irwin R, et al. Colitis induced bone loss is gender dependent and associated with increased inflammation. *Inflammatory bowel diseases*. 2013; 19:1586. [PubMed: 23702805]
87. Metzger CE, et al. Inflammatory Bowel Disease in a Rodent Model Alters Osteocyte Protein Levels Controlling Bone Turnover. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 2017; 32:802–813.
88. Sjogren K, et al. The gut microbiota regulates bone mass in mice. *J Bone Miner Res*. 2012; 27:1357–1367. [PubMed: 22407806]
89. Li JY, et al. Sex steroid deficiency-associated bone loss is microbiota dependent and prevented by probiotics. *The Journal of clinical investigation*. 2016; 126:2049–63. [PubMed: 27111232]
90. Yan J, et al. Gut microbiota induce IGF-1 and promote bone formation and growth. *Proceedings of the National Academy of Sciences*. 2016; 113:E7554–E7563.
91. Quach D, et al. Microbiota reconstitution does not cause bone loss in germ-free mice. *mSphere*. 2018 In Press.
92. Klaenhammer TR, et al. The impact of probiotics and prebiotics on the immune system. *Nat Rev Immunol*. 2012; 12(10):728–34. [PubMed: 23007572]
93. Frei R, Akdis M, O'Mahony L. Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence. *Curr Opin Gastroenterol*. 2015; 31(2):153–8. [PubMed: 25594887]
94. McCabe LR, et al. Probiotic use decreases intestinal inflammation and increases bone density in healthy male but not female mice. *J Cell Physiol*. 2013; 228:1793–1798. [PubMed: 23389860]
95. Collins FL, et al. *Lactobacillus reuteri* 6475 Increases Bone Density in Intact Females Only under an Inflammatory Setting. *PLoS One*. 2016; 11:e0153180. [PubMed: 27058036]
96. Britton RA, et al. Probiotic *L. reuteri* treatment prevents bone loss in a menopausal ovariectomized mouse model. *J Cell Physiol*. 2014; 229:1822–1830. [PubMed: 24677054]
97. Thomas CM, et al. Histamine derived from probiotic *Lactobacillus reuteri* suppresses TNF via modulation of PKA and ERK signaling. *PloS one*. 2012; 7:e31951. [PubMed: 22384111]
98. Ohlsson C, et al. Probiotics protect mice from ovariectomy-induced cortical bone loss. *PloS one*. 2014; 9:e92368. [PubMed: 24637895]
99. Wang Z, et al. Probiotics protect mice from CoCrMo particles-induced osteolysis. *Int J Nanomedicine*. 2017; 12:5387–5397. [PubMed: 28794630]
100. Salva S, et al. Dietary supplementation with probiotics improves hematopoiesis in malnourished mice. *PLoS One*. 2012; 7(2):e31171. [PubMed: 22347448]
101. Chiang SS, Pan TM. Antiosteoporotic effects of lactobacillus-fermented soy skim milk on bone mineral density and the microstructure of femoral bone in ovariectomized mice. *J Agric Food Chem*. 2011; 59:7734–7742. DOI: 10.1021/jf2013716 [PubMed: 21668014]
102. Maekawa T, Hajishengallis G. Topical treatment with probiotic *Lactobacillus brevis* CD2 inhibits experimental periodontal inflammation and bone loss. *J Periodontol Res*. 2014; 49:785–791. DOI: 10.1111/jre.12164 [PubMed: 24483135]
103. Messori MR, Oliveira LFF, Foureaux RC, et al. Probiotic Therapy Reduces Periodontal Tissue Destruction and Improves the Intestinal Morphology in Rats With Ligature-Induced Periodontitis. *J Periodontol*. 2013; 84:1818–1826. DOI: 10.1902/jop.2013.120644 [PubMed: 23327675]
104. Oliveira LFF, Salvador SL, Silva PHF, et al. Benefits of *Bifidobacterium Animalis* Subsp *Lactis* Probiotic in Experimental Periodontitis. *J Periodontol*. 2016; :1–20. DOI: 10.1902/jop.2016.160217
105. Ghanem KZ, Badawy IH, ABDEL-SALAM AM. Influence of yoghurt and probiotic yoghurt on the absorption of calcium, magnesium, iron and bone mineralization in rats. *Milchwissenschaft*. 2004; 59:472–475.
106. Tomofuji T, Ekuni D, Azuma T, et al. Supplementation of broccoli or *Bifidobacterium longum*-fermented broccoli suppresses serum lipid peroxidation and osteoclast differentiation on alveolar

- bone surface in rats fed a high-cholesterol diet. *Nutr Res.* 2012; 32:301–307. DOI: 10.1016/j.nutres.2012.03.006 [PubMed: 22575044]
107. Rodrigues FC, Castro ASB, Rodrigues VC, et al. Yacon flour and *Bifidobacterium longum* modulate bone health in rats. *J Med Food.* 2012; 15:664–70. DOI: 10.1089/jmf.2011.0296 [PubMed: 22510044]
108. Kruger MC, Fear A, Chua W-H, et al. The effect of *Lactobacillus rhamnosus* HN001 on mineral absorption and bone health in growing male and ovariectomised female rats. *Dairy Sci Technol.* 2009; 89:219–231. DOI: 10.1051/dst/2009012
109. Kim JG, Lee E, Kim SH, et al. Effects of a *Lactobacillus casei* 393 fermented milk product on bone metabolism in ovariectomised rats. *Int Dairy J.* 2009; 19:690–695. DOI: 10.1016/j.idairyj.2009.06.009
110. Narva M, Collin M, Lamberg-Allardt C, et al. Effects of long-term intervention with *Lactobacillus helveticus*-fermented milk on bone mineral density and bone mineral content in growing rats. *Ann Nutr Metab.* 2004; 48:228–234. DOI: 10.1159/000080455 [PubMed: 15334032]
111. Amdekar S, Kumar A, Sharma P, et al. *Lactobacillus* protected bone damage and maintained the antioxidant status of liver and kidney homogenates in female wistar rats. *Mol Cell Biochem.* 2012; 368:155–165. DOI: 10.1007/s11010-012-1354-3 [PubMed: 22661381]
112. Rovenský J, Švík K, Mat’ha V, et al. The Effects of *Enterococcus faecium* and Selenium on Methotrexate Treatment in Rat Adjuvant-induced Arthritis. *Clin Dev Immunol.* 2004; 11:267–273. DOI: 10.1080/17402520400001660 [PubMed: 15559373]
113. Mutu R, Kocabagli N, Alp M, et al. The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. *Poult Sci.* 2006; 85:1621–5. [PubMed: 16977848]
114. Plavnik I, Scott ML. Effects of additional vitamins, minerals, or brewer’s yeast upon leg weaknesses in broiler chickens. *Poult Sci.* 1980; 59:459–67. [PubMed: 7413573]
115. Nahashon SN, Nakaue HS, Mirosh LW. Production variables and nutrient retention in single comb White Leghorn laying pullets fed diets supplemented with direct-fed microbials. *Poult Sci.* 1994; 73:1699–711. [PubMed: 7862610]

Figure 1. Probiotic ingestion alters the gastrointestinal microbiota composition, permeability and local and systemic immune cells

Probiotic induced changes in microbiota composition can beneficially influence intestinal barrier function and inflammation. Similarly, increasing intestinal barrier function can benefit the immune and possibly microbiota composition. Finally, a strong immune system is likely to benefit intestinal barrier function and microbiota composition.

Figure 2. Probiotic ingestion promotes healthy microbiota composition, barrier integrity and reduces inflammation, all of which bone health (growth, density, architecture).

Table 1

Strain	Species (and model)	Sex	Treatment time	Analysis method	Bone effects	Reference
<i>Lactobacillus reuteri</i> (ATCC 6475)	Mice	Male	4 weeks	µCT	↑ BV/TV ↑ Tb.N. ↑ Tb.Th. ↑ Osteocalcin ↑ BFR	[22]
<i>Lactobacillus reuteri</i> (ATCC 6475)	Mice (OVX)	Female	4 weeks	µCT	↑ BV/TV ↓ RANKL mRNA ↓ TRAP5 mRNA	[19]
<i>Lactobacillus reuteri</i> (ATCC 6475)	Mice (Dorsal Surgery)	Female	8 weeks	µCT	↑ BV/TV ↑ MAR ↓ RANKL mRNA ↑ OPG mRNA ↑ IL-10 mRNA	[20]
<i>Lactobacillus reuteri</i> (ATCC 6475)	Mice (STZ-induced Type 1 Diabetes)	Male	4 weeks	µCT	↑ BV/TV ↑ MAR ↑ Osteocalcin ↑ Wnt10b mRNA	[21]
<i>Lactobacillus rhamnosus</i> (HN001)	Mice (OVX)	Female	4 weeks	µCT	↑ BV/TV ↑ Osteocalcin ↓ RANKL mRNA ↓ TNFα mRNA ↓ IL-17 mRNA	[50]
<i>L. paracasei</i> (NTU 101) or <i>L. plantarum</i> (NTU 102)- fermented soy milk	Mice (OVX)	Female	8 weeks	µCT SEM	↑ BV/TV ↑ Tb.N.	[101]
<i>L. paracasei</i> or <i>L. paracasei</i> and <i>L. plantarum</i>	Mice (OVX)	Female	6 weeks	µCT	↑ Cortical BMC ↑ Cortical area ↑ OPG mRNA	[17]
<i>L. Casei</i>	Mice	Unknown	10 weeks	µCT	Prevents wear-debris-induced osteolysis	[99]
<i>L. rhamnosus</i> GG	Mice (periodontitis)	Female	6 weeks	µCT	↓ Bone loss ↓ Inflammation ↓ Osteoclasts	[52]
<i>L. gasseri</i> SBT2055	Mice (periodontitis)	Female	5 weeks	Histomorphometry	↓ Bone loss ↓ Inflammation	[54]
<i>L. brevis</i> CD2	Mice (periodontitis)	Male	<1 week	Histomorphometry	↓ Bone loss ↓ Inflammation	[102]
<i>Bacillus subtilis</i>	Rats (periodontitis)	Male	6 weeks	Histomorphometry	↓ Bone loss ↓ Inflammation	[103]
<i>Bifidobacterium animalis lactis</i>	Rats (periodontitis)	Male	2 weeks	µCT	↓ Bone loss ↓ Inflammation	[104]

Strain	Species (and model)	Sex	Treatment time	Analysis method	Bone effects	Reference
<i>Lactobacillus casei</i> , <i>L. reuteri</i> and <i>L. gasseri</i>	Rats				↑ BMC ↑ Ca absorption	[105]
<i>Bifidobacterium longum</i> – fermented broccoli	Wistar Rats	Male	12 weeks	Histomorphometry	↓ TRAP ⁺ osteoclasts	[106]
<i>Bifidobacterium longum</i> (ATCC 15707)	Wistar Rats	Male	28 days	Texture analyzer Plasma emission spectrophotometry	↑ Tibial Ca, P and Mg content ↑ Fracture strength	[107]
<i>Lactobacillus rhamnosus</i> (HN001)	Sprague-Dawley Rats	Male	3 weeks	DEXA	↑ Ca and Mg retention	[108]
<i>Lactobacillus rhamnosus</i> (HN001)	Sprague-Dawley Rats (OVX)	Female	3 months	DEXA	↓ Bone loss	[108]
<i>L. casei</i> 393-fermented milk	Sprague-Dawley Rats (OVX)	Female	6 weeks	DEXA Texture Analyzer Plasma emission spectrophotometry	↑ BMD ↑ Bone strength ↑ Ca content ↓ TRAP activity	[109]
<i>L. helveticus</i> -fermented milk	Spontaneously hypertensive rats	Male	14 weeks	DEXA Plasma emission spectrophotometry	↑ BMD ↑ BMC	[110]
<i>L. casei</i> and <i>L. acidophilus</i>	Wistar rat (adjuvant-induced arthritis)	Female	12 days	X-ray	↓ Bone loss	[111]
<i>Enterococcus faecium</i> (with methotrexate)	Lewis rat (adjuvant-induced arthritis)	Male	50 days	X-ray	↓ Arthritic score	[112]
<i>Bacillus licheniformis</i> and <i>Bacillus subtilis</i>	Broiler		6 weeks	Measuring Calipers	↑ Tibia lateral and medial wall thickness	[113]
<i>Brewer's yeast</i>	Broiler		9 weeks	Visual	↓ Tibial dyschondroplasia	[114]
<i>Lactobacillus</i>	Broiler		28 days	Atomic absorption spectrophotometry Phosphomolybdic Acid method	↑ Ca and P retention	[115]