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The gut-bone axis: how bacterial metabolites bridge the distance

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Introduction

Osteoporosis is a common skeletal disease that leads to bone fractures and disability stemming from insufficient skeletal development leading to low peak bone density by age 30, and/or accelerated bone loss thereafter. Skeletal involution is determined by the process of bone remodeling, which involves the continuous removal of packets of old bone by the resorptive action of osteoclasts, and their replacement by new bone formed by osteoblasts (1, 2). Menopause, aging, inflammation, and hyperparathyroidism are common causes of osteoporosis that induce progressive loss of bone mineral density (BMD) by increasing osteoclastic bone resorption, or by decreasing osteoblast activity and lifespan.

Osteoporosis often remains untreated as a result of the cost and adverse side effects of approved drugs (3–6), underscoring the critical need for the development of inexpensive and safe interventions. To this end, recent investigations have focused on establishing the role of the gut microbiome in the development of osteoporosis, and in the efficacy of probiotics or prebiotics as novel approaches for its treatment. The notion that the gut microbiome is a BMD regulator in health and disease is supported by an established correlation in humans between microbiome diversity and osteoporosis (6). Moreover, animal studies have revealed that BMD is altered by the ablation of the gut microbiome, as it occurs in mice raised in germ-free (GF) conditions (7–9), and in mice treated with antibiotics (10–12).

Probiotics are defined as viable microorganisms that confer health benefits when administered in adequate quantities (13), while prebiotics are nondigestible fermentable food ingredients that promote the growth of beneficial microbes and/or promote beneficial changes in the activity of the microbiome (14). One mechanism whereby prebiotics and probiotics elicit positive health influences is by inducing modifications in the composition of the microbiota. These modifications, such as the expansion of Clostridia and Bacteroides, result in enhanced generation of metabolites that exert critical biological activities both in the gut and systemically. Indeed, metabolites produced in the gut by the microbiota provide an essential means for the gut microbiota to regulate anatomically distant organs. The term “postbiotics” is now used to refer to such metabolites. In addition to metabolites, structural components shed by bacteria may lead to distant effects on the organs of the body. For example, constituents of the bacterial cell wall such as peptidoglycan and lipopolysaccharide stimulate bone resorption (15).

Gut-derived bacterial metabolites regulate distant organs

The gut microbiome harbors hundreds of bacterial genera that reside in the luminal stream or adhere to the gut mucosa. The intestinal microbiome affords the host enhanced energy extraction from foodstuff, regulatory effects on epithelial growth, the exclusion of colonization by pathogens, and many other benefits (16). In addition, the gut microbiome is essential for efficient immune system maturation, as well as cytoprotection against exogenous insults. The gut microbiota produces metabolites that account for anatomically distant biological effects. Indole derivatives were among the first bacterial metabolites to be described to influence intestinal immunity (17, 18). In addition, trimethylamine N-oxide (TMAO), an amine oxide present in food or generated within the human intestine by the microbiota from choline and carnitine, was found to negatively affect the vascular system and kidneys (19). 4-Ethylphenol sulfate, a metabolite produced by intestinal saprophytes, was shown to regulate human behavior and has been implicated in autism (20). Insulin-like growth factor
olites initially emerged as powerful immune cell controllers, and more recently have been recognized as pivotal regulators of bone resorption and bone formation.

Sources and mechanisms of SCFA production

Diet affects the diversity of the gut microbiota and thus by extension also influences the metabolic activity of the microbiome (26–28). A dietary element that plays a pivotal role in shaping the composition of the microbiome is vegetable fiber. This substance is regarded to be as essential as vitamins and other nutrients for organismal health. However, it is estimated that the current average consumption of fibers among adults in the United States is half the recommended amount of 30 g per day to be consumed as part of a healthy diet (29). Many of the beneficial health effects of fibers are due to metabolites generated by their digestion. Among these metabolites are SCFAs, which are derived from bacterial fermentation of complex nondigestible carbohydrates present in the diet. Amino acids and lactate catabolism also contribute to SCFA production, especially production of acetate and propionate (30,
SCFAs, the main metabolites derived from microbial fermentation of dietary fibers in the intestine, affect bone homeostasis via two routes. In addition to butyrate’s strong HDAC-inhibiting effects on osteoclasts, it directly induces metabolic reprogramming of osteoclast precursors, resulting in enhanced glycolysis at the expense of oxidative phosphorylation, thereby downregulating essential osteoclast genes such as TRAF6 and NFATc1. Indirect effects of SCFAs may account for their Treg-inducing capacity: Tregs were shown to suppress osteoclast differentiation via their secretion of antiosteoclastic cytokines as well as via a direct cell-cell contact–dependent, indoleamine 2,3-dioxygenase–inducing (IDO-inducing) mechanism. In summary, these data identify SCFAs as potent regulators of osteoclast metabolism and bone homeostasis.

SCFAs affect immune function and other biological systems

SCFAs affect the immune system by modifying gene expression profiles (68, 69), cell chemotaxis (70, 71), differentiation (54–56), proliferation (72–74), and apoptosis (75, 76). In addition, SCFAs inhibit histone deacetylases (HDACs) and stimulate histone acetyltransferase (53, 56, 77, 78) (Figure 1). Ligand binding to cell surface receptors or transmembrane transport by specialized transporters is critical for SCFA activities. SCFAs bind to four receptors, the free fatty acid receptors GPR43 and GPR41 (also known as FFAR2 and FFAR3) (79), the niacin/butyrate receptor GPR109a (also known as HCA2) (80), and the olfactory receptor Olfr78 (81). These receptors show distinct binding affinities for specific types of SCFAs, as well as different patterns of expression. An essential high-affinity SCFA transporter expressed in the intestine is Slc5a8. Accordingly, mice lacking Slc5a8 develop colitis and colon cancer (82), while GPR43 activation prevents colonic inflammation and carcinogenesis (83).

The role of SCFAs in the immune system has been extensively reviewed elsewhere (53, 71, 84); some of the main discoveries are outlined herein. The intestinal epithelium acts as a barrier to prevent the passage of intraluminal entities such as foreign antigens, microorganisms, and their toxins into subepithelial compartments. Its second function is to act as a selective filter that allows the translocation of essential dietary nutrients, electrolytes, and water from the intestinal lumen into the circulation. Disrupted intestinal barrier function is associated with development of inflammatory diseases. One cause of impaired epithelial barrier integrity is low butyrate levels, which commonly occur as a result of microbiome dysbiosis (70). In addition, efficient intestinal barrier function can be restored upon supplementation of butyrate to the diet (85). Butyrate enhances intestinal barrier function by inducing the expression of tight junction proteins such as claudin-1 (4). Furthermore, the interaction of SCFAs with intestinal epithelial cells or immune cells of the gut mucosa leads to essential anti-inflammatory and immunomodulatory effects. Among them is the enhanced production of antimicrobial peptides by intestinal mac-
The most studied immunomodulatory effect of SCFAs is their capacity to induce the differentiation and proliferation of regulatory T cells (Tregs) (56, 90, 91). These cells are essential for the maintenance of immune tolerance and a symbiotic relationship between the host and the microbiome. Tregs suppress conventional T cells through a variety of mechanisms, including the production and secretion of the immunosuppressive cytokines IL-10 and TGF-β (92, 93). Several lines of evidence support the notion that SCFAs produced by the microbiome are critical for intestinal Treg expansion, including the observation that GF mice have fewer Tregs than conventionally raised mice, and reports that nutritional supplementation with SCFAs alone is sufficient to increase Treg numbers in the intestine of GF mice (56). Even modest alterations of the microbiota composition may lead to decreased production of Tregs due to lower levels of SCFA generation, as attested by the finding of a paucity of Tregs in mice with dysbiosis as compared with mice with normal gut microbiota (94).

Butyrate and propionate are potent inducers of Tregs, whereas acetate modulates B cell function (95). In one study, propionate was more potent than acetate and butyrate, and was found to be sensed by Tregs via the fatty acid receptor GPR43 (56). Another report identified butyrate as the most potent inducer of Tregs (91). Interestingly, oral administration of SCFAs following antibiotic treatment to mice showed that a combination of propionate and butyrate expands the Treg population in the spleen, whereas a combination of propionate and acetate expands Treg numbers in the intestine (90). SCFAs support the development of peripheral Tregs in the intestine through multiple mechanisms. A key effect of SCFAs is to increase the differentiation of naive CD4+ T cells into Tregs by regulating the production of TGF-β, IL-10, and various chemokines, whereas SCFAs increased production of the antiinflammatory cytokine IL-10 (69, 83).
via intrinsic epigenetic upregulation of the Foxp3 gene in T cells. This effect, which is surface receptor–independent (87), is mediated by increased histone H3 acetylation of the promoter for Cns3 and Csn1 gene loci (91). Another effect of SCFAs is to increase the proliferation of mature Tregs (56). This effect was reported to be GRPR43-dependent in one study (56) and GRPR43-independent in another study (91). Additional effects of SCFAs, especially butyrate, on Treg maturation are mediated by dendritic cells (DCs) (90), which express both GPR109a and GRPR43 (56, 83). Since GRPR43 is expressed at high levels by myeloid cells (91), it is likely that GRPR43 mediates the effects of SCFAs on DCs. Butyrate and propionate (but not acetate) prevent the development of DCs via inhibition of histone acetyltransferase (82). Butyrate prevents DC maturation by upregulating antiinflammatory genes (96). This effect results in an increased capacity of DCs to support Treg differentiation. Butyrate and propionate induce DCs to promote the formation of IL-10–producing Tregs and inhibit the generation of IFN-γ effector T cells (83). Altogether, these reports highlight the sensitive responses of immune cells within gut mucosa and beyond to varying concentrations of SCFAs.

Effects of SCFAs on osteoclasts and bone resorption

Osteoclasts are multinucleated cells that are responsible for physiological and pathological bone resorption. SCFAs blunt osteoclast differentiation (97), and inhibition of HDAC activity is one mechanism whereby this occurs (98–100). For example, differentiation of primary bone marrow (BM) cells into osteoclasts is suppressed by butyrate and by trichostatin A, a known HDAC inhibitor (101). Treatment with the newer HDAC inhibitor dépsipeptide FR901228 confirmed the antioestoclastic properties of butyrate, suggesting a novel role for HDAC inhibitors as antiresorptive agents (102). Two further studies highlighted the antioestoclastic effects of butyrate, and to a lesser extent propionate, on osteoclast differentiation using mice and human primary cultured cells (103, 104). Suppression of osteoclast differentiation is most potent when SCFAs or HDAC inhibitors are added at early time points during osteoclast differentiation (53, 55, 101).

Mice lacking FFR1 (GPR40), a receptor that binds mid- to long-chain fatty acids, were protected against bone loss through suppression of osteoclastogenesis (105). This report led to further investigations by our laboratory on the potential of fiber-rich diets (prebiotic), bacterial transfer (probiotic), or nutritional supplementation with SCFAs (postbiotic) on bone metabolism under steady-state and bone waste–inducing conditions. We reported beneficial effects of all three approaches on bone metabolism (55). We also showed reduced osteoclast numbers in C57BL/6 mice and in osteoporotic mice following propionate and butyrate treatment (55). These observations correlated with significantly reduced markers for bone resorption.

Earlier publications highlighted the potential of Tregs to attenuate osteoporosis (106), and to increase systemic BMD by directly suppressing osteoclast differentiation (107–109) in a CTLA4/CD80/86 cell-cell contact–inducing and indoleamine 2,3-dioxygenase–inducing manner (110) (Figure 2). Considering the extensive body of literature reporting on the Treg-inducing potential of SCFAs (56, 90, 94), it was unexpected to find increased systemic bone densities in Rag1−/− (T and B cell–deficient) mice following propionate and butyrate treatment. The direct osteoclast-suppressing effects of propionate and butyrate—contrary to the indirect mechanism via expansion of Tregs—were shown to be independent of the receptors GPR41 and GRPR43, and rather occurred via a shift in cellular metabolism toward increased glycolysis at early time points during osteoclast differentiation. Blocking glycolysis during this time window rescued the anti-osteoclastogenic potential of propionate and butyrate in vitro experiments (55). To test whether these findings could be exploited as a novel strategy for the treatment of pathological bone loss, we further investigated the impact of SCFAs on ovariectomy-induced bone loss. In line with the capacity of propionate and butyrate to induce metabolic shifts toward increased glycolysis in osteoclast precursors, these SCFAs effectively prevented ovariectomy-induced bone loss. Moreover, propionate and, even more potently, butyrate prevented the ovariectomy-induced increase in osteoclast formation and bone resorption. By contrast, markers of bone formation remained unchanged. Interestingly, while high-fiber diets in steady-state wild-type mice increased bone mass, no positive effects on bone volume were observed when ovariectomized mice were fed a high-fiber diet (55). Studies are in progress to confirm the anti-osteoclastogenic activity of SCFAs in humans.

Accumulated research has revealed that the source, concentration, and amino acid balance of dietary protein are additional factors that positively contribute to the composition, structure, and function of the gut microbiome. Therefore, in a first-in-human trial registered in the German Clinical Trials Register (DRKS00017277), we combined protein supplementation with a high-fiber diet, and observed enhanced production of Tregs and decreased bone resorption in subjects receiving supplements. This dietary combination strongly improved the acceptance and willingness of patients to consume the protein supplementation. Thus, increases in SCFA levels that occur as a result of pre-, pro-, or postbiotic dietary supplementation may serve as an inexpensive, safe, and effective intervention for both the prevention and treatment of osteoporosis.

Effects of SCFAs on osteoblasts and bone formation

Physiological stimuli and pharmacological agents that increase bone formation typically act by increasing osteoblastogenesis, increasing osteoblast lifespan, or a combination of both. Activation of Wnt signaling in osteoblasts plays an essential role in increasing osteoblastogenesis and decreasing osteoblast apoptosis (111, 112). Wnt signaling is indeed critical for bone mass acquisition and skeletal involution (113). Attesting to the relevance of Wnt signaling for skeletal health and disease, activating mutations of the Wnt signaling coreceptor complex result in a high-bone mass phenotype (114), while inactivating mutations are responsible for low bone mass and the early onset of osteoporosis (115). Activation of the canonical Wnt signaling pathway results from increased production of Wnt ligands that bind to and activate the Wnt coreceptor complex, or from diminished production of Wnt signaling inhibitors such as Dkk1 (116) and sclerostin (117). Emerging reports have described skeletal effect of SCFAs, including investigations showing that butyrate promotes the osteogen-
SCFAs and PTH: mechanistic similarities and evolutionary considerations

PTH is a calcitropic hormone critical for skeletal development. Similarly to butyrate, PTH stimulates bone formation and induces bone anabolism via the Treg/Wnt10b/Wnt signaling pathway (125, 142, 143). BM CD8+ T cells respond to PTH and butyrate by releasing Wnt10b, while silencing of Wnt10b expression by CD8+ T cells blocks the capacity of PTH and butyrate to stimulate bone formation and increase bone volume (60, 136–138). Moreover, PTH and butyrate increase the production of Wnt10b expression by CD8+ T cells by expanding Tregs (60, 124).

The evolutionary advantage of the mechanistic convergence between the skeletal effects of SCFAs and those of PTH remains unknown, but it is tempting to speculate that it may be related to
energy balance during health and sickness. Immune cells depend on calcium for their activation (144). A highly activated immune system is accompanied by sickness behavior and anorexia, which renders the immune system dependent on calcium released by bone resorption rather than the calcium absorbed in the gut. A consequence of starvation is hypocalcemia that in turn leads to continuous production of PTH, which stimulates bone resorption, causing release of calcium, which then becomes available for immune cell activation (145). Food ingestion interrupts PTH secretion, causing the pattern of PTH release to change from continuous to intermittent. It is only when intermittently produced that PTH exerts a net bone anabolic activity. This activity hinges on a mechanism involving Tregs and Wnt10b. One goal of this response might be to induce calcium deposition in the skeleton, so as to create a calcium reserve for the immune system. Generation of SCFAs is an event linked to food intake. Thus, SCFA generation may signal the presence of a normal state of health, thereby activating a pathway that replenishes calcium reserve in the skeleton. Thus, SCFAs may act in concert with intermittent PTH release to expand Tregs and stimulate bone formation.

Modulation of gut-bone axis by probiotics and prebiotics

SCFA supplementation is emerging as a novel postbiotic treatment modality for optimizing postnatal skeletal development and preventing pathological bone loss. Pre- and probiotics also act, in part, by generating SCFAs that positively affect the skeleton (60, 146–148). In addition, these interventions suppress inflammation (149), regulate the immune responses in the host (41, 150), buttress a weak gut epithelial barrier (151, 152), and promote epithelial development and restitutional responses following injury (153–155). Robust evidence demonstrates that probiotics prevent the bone loss induced by ovariectomy, a model of postmenopausal osteoporosis (32–34, 36), prevent the bone loss induced by periodontal disease (156) and diabetes (135), and are beneficial for skeletal health in intact animals (35, 60, 157–159). Moreover, increasing evidence indicates that probiotics positively affect skeletal health in humans. Early trials showed that ingestion of kefir fermented milk for 6 months caused an increase in BMD in men (45), while treatment with Lactobacillus casei shirota improved distal radius fracture healing in elderly men and women (160). Another trial with a multispecies probiotic showed a significant reduction in bone turnover, but no significant changes in BMD, perhaps because the trial duration was only 6 months (42). A 1-year-long trial in older women revealed evidence of a favorable change in bone mass in response to probiotic supplementation (43), and in a study in Japanese women, the probiotic Bacillus subtilis C-3102 increased total hip bone BMD by decreasing bone resorption (44).

Prebiotics, which are predominantly nondigestible substances that act as food for the gut microbiota, are found in a variety of foodstuffs, such as artichoke, garlic, leek, dandelion greens, banana, onion, and chicory (161). Prebiotics include nondigestible oligosaccharides and soybean oligosaccharides. In many cases, a substantial amount of the food must be consumed to acquire enough prebiotic for activity, and therefore prebiotics, such as inulin, have been developed into soft chew, capsule, tablet, and shake forms (161). Prebiotics prevent ovariectomy-induced bone loss in rats (162) and increase BMD in healthy animals (37, 38, 163). In humans, prebiotics increase BMD in adolescents (164) and decrease bone turnover in postmenopausal women (165). The mechanism of action of prebiotics in bone is complex, but emerging evidence has shown that bacterial metabolic pathways, including those that function in the generation of SCFAs, are involved (148).

Conclusions and future directions

SCFAs exert complex effects in bone remodeling that suggest novel therapeutic opportunities for SCFAs in the treatment of metabolic bone disorders. In addition, nutritional supplementation with prebiotics and probiotics that increase SCFA production may represent an effective, safe, and inexpensive modality to prevent and treat osteoporosis. Additional studies will be required to identify the pre- and probiotic formulations that maximize SCFA production. While current efforts are focused on the identification of bacterial strains that provide maximal skeletal effects, it is entirely possible that the number of bacteria surviving passage through gastric acid, rather than bacterial species, will emerge as a key factor for probiotic efficacy. In animal models SCFAs have been shown to blunt osteoclastogenesis and bone resorption and stimulate bone formation. The antiresorptive activity of SCFAs is T cell–independent (55), while the bone anabolic activity of SCFAs is dependent on Tregs and CD8+ T cells (60). The factors that determine whether SCFAs act primarily as antiresorptive agents or as anabolic agents are unknown. However, the composition of the microbiota, the source and age of the treated mice, and the duration of the treatment are certainly relevant factors, highlighting the fact that it is essential to account for reciprocal host-microbiome interactions in experimental science. Efforts to understand the factors that determine the bone cell response to SCFAs will be an important subject for future research. Most of the evidence linking microbiota-produced metabolites to bone derives from animal studies. It will be critical to confirm these observations in humans and thereafter conduct clinical trials with emerging postbiotic agents.

It is estimated that metabolites of bacterial origin account for about 10% of circulating metabolites (166). We predict that rapid progress in metabolomic and other emerging technologies will lead to the discovery of several metabolites critical for the regulation of bone turnover and the maintenance of bone health. Novel immune-metabolic pathways are likely to be identified that will provide innovative therapeutic opportunities for metabolic bone diseases.

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