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The intestinal microbiome and skeletal fitness: Connecting bugs and bones

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Abstract Recent advances have dramatically increased our understanding of how organ systems interact. This has been especially true for immunology and bone biology, where the term "osteoimmunology" was coined to capture this relationship. The importance of the microbiome to the immune system has also emerged as a driver of health and disease. It makes sense therefore to ask the question: how does the intestinal microbiome influence bone biology and does dysbiosis promote bone disease? Surprisingly, few studies have analyzed this connection. A broader interpretation of this question reveals many mechanisms whereby the microbiome may affect bone cells. These include effects of the microbiome on immune cells, including myeloid progenitors and Th17 cells, as well as steroid hormones, fatty acids, serotonin and vitamin D. As mechanistic interactions of the microbiome and skeletal system are revealed within and without the immune system, novel strategies to optimize skeletal fitness may emerge.

1. Introduction

Bone mass is the major determinant of fracture risk with aging and is regulated by a complex interplay of cellular, hormonal and metabolic pathways [1,2]. At a cellular level two cell types, the osteoblast and osteoclast, synthesize and degrade bone throughout life, respectively. A third cell, the osteocyte, is derived from osteoblasts and resides within the bone matrix to monitor biomechanical stress and coordinate osteoblast and osteoclast activities. Both adaptive and innate immune cells influence osteoblasts and osteoclasts through factors such as cytokines. The calcium/vitamin D/parathyroid hormone (PTH) axis is the most well known hormonal pathway. Decreases in serum calcium stimulate the release of PTH, which raises the serum calcium level by promoting osteoclastic bone resorption and calcium absorption in the gut while decreasing renal calcium excretion. Steroid hormones, including estrogen and glucocorticoids, also profoundly affect bone cells. Other reviews in this issue focus on the role of the microbiome on local bone diseases, such as periodontitis, rheumatoid arthritis and the spondyloarthropathies. In this review, the mechanisms by

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which the gut microbiome may affect *systemic* bone metabolism are considered.

Although direct data is limited, it is easy to envision how the microbiome could influence bone metabolism. Since bone cells are unlikely to come in direct contact with microbes outside of the oral cavity and deep seated infections, effects must be mediated indirectly by cells or soluble factors. The interaction of the microbiome with the skeletal system can be framed within one of three categories considered here (Fig. 1). These include effects of the microbiome on 1) the immune system, also known as osteoimmunology [2], 2) hormonal pathways (e.g., steroid hormones, PTH and vitamin D), and 3) the production of bacterial metabolites that could signal to bone cells. Before addressing these potential mechanisms, an overview of papers that directly address the connection between the intestinal microbiome and skeletal biology is provided.

2. The microbiome and bone — direct evidence of interactions

How manipulations of the intestinal microbiome may affect bone mass has been examined in three contexts: following the ingestion of pre- and probiotics, after treatment with broad-spectrum antibiotics and under germ-free (GF) conditions. Here, each is reviewed. Due to limited data on this topic in humans, the discussion is largely limited to murine studies.

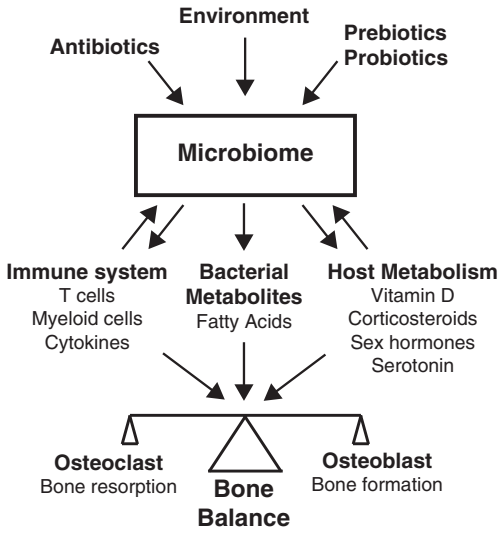

Figure 1 A conceptual framework to understand how the intestinal microbiota may regulate bone metabolism. Environmental exposures, antibiotics, and pre- and probiotics influence the composition of the intestinal microbiome. Microbes may change the relative activities of osteoclasts and osteoblasts through effects on the immune system and host metabolic pathways, as well as through the production of metabolites. The immune system and metabolic pathways may also influence the composition of the microbiome.

2.1. Prebiotics and probiotics

Prebiotics are non-digestible food constituents like dietary fiber and oligosaccharides that modulate bacterial communities in the gut with beneficial effects on the host. Inulin, oligofructose and galactooligosaccharides are the best-studied prebiotics in terms of their effects on bone (reviewed in [3]). Abrams et al. built on earlier studies to show that inulin-type fructans increased bone mineral content (BMC) and bone mineral density (BMD) in adolescents [3,4]. Similar results were obtained in animals treated with inulin type fructans. Prebiotics may increase calcium uptake thereby promoting bone mineralization by augmenting total body calcium [3]. Mechanistically, fermentation of these sugars into short chain fatty acids (SCFA) by the microbiota and acidification of the gut lumen enhance calcium solubility to increase absorption. Whether this is the sole pathway by which prebiotics increase bone mass is unclear.

Probiotics are microorganisms that after ingestion confer beneficial effects on the health of the host. The effect of probiotics and fermented food products on bone mass in animals has been reviewed [5]. Here, two recent papers are discussed. McCabe et al. treated male and female mice with *Lactobacillus reuteri* ATCC PTA 6475 three times per week for 4 weeks [6]. This strain was chosen because it suppresses tumor necrosis factor (TNF) production in monocytes through histamine [7]. Given the effect of inflammatory cytokines, like TNF, on promoting osteoclast activity and inhibiting osteoblasts [2], the authors reasoned that modulation of inflammation by *L. reuteri* 6475 may increase bone mass. Gavage with this probiotic reduced intestinal *Tnf* transcripts and increased trabecular bone mass in male but not female mice. The increase in bone mass was associated with elevated bone formation rates, without changes in a serologic biomarker of osteoclast activity [6]. Similar experiments should be done with an *L. reuteri* 6475 mutant incapable of generating histamine [7] to determine whether this pathway indeed mediates its positive effects on bone formation.

The lack of an effect of *L. reuteri* 6475 in female mice prompted the investigators to examine this probiotic in the ovariectomy model of post-menopausal osteoporosis [8]. One week after ovariectomy, mice received *L. reuteri* 6475 thrice weekly for 4 weeks. This treatment protected mice from trabecular bone loss and was associated with reduced levels of *Tnfsf11* (receptor activator of NF-κB ligand (RANKL)) and *Acp5* (TRAP5b; a marker of osteoclast number) transcripts in whole bone mRNA. *L. reuteri* 6475 induced significant changes in bacterial diversity with an increase in *Clostridiales* and a decrease in *Bacteriodes* species. Bone marrow (BM) from mice treated with *L. reuteri* 6475 contained fewer $CD4^+$ T-cells and generated fewer osteoclasts when cultured ex vivo with RANKL. It remains unclear whether *L. reuteri* 6475 prevents bone loss after ovariectomy by influencing osteoclasts or osteoblasts, or both. Taken together, these data support the notion that bacterial communities in the gut influence bone metabolism.

2.2. Effects of antibiotics on bone

Antibiotics are widely used to combat infection and promote livestock growth. Although these drugs change the microbial