Review

CellPress

# Effects of the gut microbiota on bone mass

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**The gut microbiota (GM), the commensal bacteria living in our intestine, performs numerous useful functions, including modulating host metabolism and immune status. Recent studies demonstrate that the GM is also a regulator of bone mass and it is proposed that the effect of the GM on bone mass is mediated via effects on the immune system, which in turn regulates osteoclastogenesis. Under normal conditions, the skeleton is constantly remodeled by bone-forming osteoblasts (OBs) and bone-resorbing osteoclasts (OCLs), and imbalances in this process may lead to osteoporosis. Here we review current knowledge on the possible role for the GM in the regulation of bone metabolism and propose that the GM might be a novel therapeutic target for osteoporosis and fracture prevention.**

## Osteoporosis, a disease of fragile bones caused by hereditary and environmental factors

Fractures caused by osteoporosis constitute a major health concern and result in a huge economic burden on health care systems. The lifetime risk of any osteoporotic fracture is high in the western world (around 50% for women and 20% for men), and fractures are associated with significant mortality and morbidity in the elderly [1]. Osteoporosis is characterized by enhanced skeletal fragility due to reduction in bone quantity and/or quality. Bone strength cannot be directly measured *in vivo*, but bone mineral density (BMD; see Glossary) is highly correlated with bone strength and is commonly used in the clinic to predict fracture risk [2]. The risk of osteoporosis depends both on how much bone is acquired during skeletal growth and development until peak bone mass is reached at 20–30 years of age, and on the rate of the subsequent age-dependent bone loss. Twin and family studies have shown that between 50% and 85% of the variance in peak bone mass is genetically determined, and that there is a heritable component also for age-related bone loss, but environmental factors seem to play a relatively more pronounced role in this latter process [3].

In recent years, the importance of the GM for both health and disease has been intensively studied. The GM constitutes trillions of bacteria, which collectively contain 150-fold more genes than our human genome. It is acquired at birth and, although a distinct entity, it has clearly coevolved with the human genome and can be considered a multicellular organ that communicates with and affects its host in numerous ways [4]. The composition of the GM is modulated by a number of environmental factors such as diet and antibiotic treatments [5–7]. Molecules produced by the gut bacteria can be both beneficial and harmful and are known to affect endocrine cells in the gut, the enteric nervous system, gut permeability, and the immune system. At homeostasis, the GM provides colonization resistance with epithelial and immune balance, protecting the host from invading bacteria, viruses, and possibly other classes of pathogens. Perturbations in this balance can be caused by pathogens, antibiotic treatment, and diet causing inflammation, tissue destruction, and dysbiosis that may lead to disease development [8]. Perturbed microbial composition has been postulated to be involved in a range of inflammatory conditions, within and outside the gut, including inflammatory bowel diseases, rheumatoid arthritis, multiple sclerosis, diabetes, food allergies, eczema, and asthma, as well as obesity and the metabolic syndrome [9,10]. The inflammation seen

### Glossary

**Bone mineral density (BMD):** is highly correlated with bone strength and is commonly measured and used in the clinic to predict fracture risk. According to the World Health Organization's guidelines, osteoporosis is diagnosed when the BMD is more than or equal to 2.5 standard deviations below that of a young adult reference population.
**Dysbiosis:** refers to an imbalance between putative species of 'protective' versus 'harmful' intestinal bacteria.
**Gnotobiotic:** is an animal that is free of bacteria or contaminants or into which a known microorganism or contaminant has been introduced for research purposes.
**Inulins:** are plant-derived polysaccharides. The inulins belong to a class of dietary fibers known as fructans.
**Microbiota:** refers to the microflora and microfauna in an ecosystem.
**Osteoblasts (OBs):** are cells that make bone by producing a matrix that then becomes mineralized. The skeleton is preserved by a balance between the activity of OBs that form bone and OCLs that break it down.
**Osteoclasts (OCLs):** are cells that break down bone and are responsible for bone resorption. OCLs are large multinucleate cells that differentiate from hematopoietic stem cells in the bone marrow.
**Prebiotics:** are non-digestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and act as a substrate for commensal bacteria. They help promote the growth and activity of advantageous bacteria in the gut.
**Probiotics:** the Food and Agriculture Organization of the United Nations and the WHO (FAO/WHO) definition of probiotics is 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Probiotics such as lactobacilli and bifidobacteria are beneficial to the host because they help improve the intestinal bacterial balance.

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*Keywords:* osteoporosis; osteoimmunology; gut microbiota.

1043-2760/
 http://dx.doi.org/10.1016/j.tem.2014.11.004

in inflammatory bowel diseases such as Crohn's disease and ulcerative colitis is associated with alterations in the diversity and richness of the GM [11–13]. A study in monozygotic twins, discordant or concordant for Crohn's disease, showed an increased ratio of adherent invasive *Escherichia coli* to *Faecalibacterium prausnitzii* in diseased twins [14]. *F. prausnitzii* has anti-inflammatory functions indicating that the ratio of pathogenic to more protective species is of importance [15]. In a large multicenter analysis of fecal microbiota profiles in patients with Crohn's disease, a fecal microbiota profile predicting disease activity was identified. The genus *Bifidobacterium* was significantly decreased during the active phase of Crohn's disease and increased to healthy levels during the remission phase [16]. Species of the *Bifidobacterium* genus are normal inhabitants of a healthy human gut and alterations in intestinal bifidobacteria levels, or species composition, are often present in cases of GM dysbiosis [17]. However, despite the large number of studies linking alterations in the GM to inflammatory diseases, it is unclear whether these alterations are the cause or consequence of these diseases. Gut-associated inflammatory and autoimmune conditions have been associated with low bone mass, suggesting a connection between the gut and bone [18,19]. The GM affects the host's immune status and it is well established that there is a connection between the immune system and bone metabolism, suggesting that the GM might affect bone metabolism via altered immune status. Here we review current knowledge supporting a role for the GM in the regulation of bone metabolism.

## Bone metabolism – the immune connection

The skeleton is remodeled by bone-forming OBs and bone-resorbing OCLs [20]. Parathyroid hormone, vitamin D, retinoids, thyroid hormones, cortisol, inflammatory cytokines, and sex-steroids are all important regulators of skeletal remodeling and thereby the risk of osteoporosis. Besides providing structural support, the skeleton also serves as a niche for mesenchymal and hematopoietic progenitors. OBs are derived from pluripotent mesenchymal stromal cells while OCLs are derived from hematopoietic stem cells that also generate immune cells [21]. OCLs are specifically derived from the myeloid-monocyte lineage of hematopoietic cells and it is the local microenvironment that determines whether the myeloid precursor cell will differentiate into a macrophage, a myeloid dendritic cell, or an OCL. The presence of macrophage colony stimulating factor (M-CSF) leads to increased proliferation and survival as well as upregulated expression of receptor activator of nuclear factor-κB (RANK) in OCL precursor cells. This allows RANK ligand (RANKL) to bind and start the signaling cascade that leads to OCL formation [21].

The association between inflammation and bone loss is well established and in auto-immune diseases such as rheumatoid arthritis, osteoclastic bone resorption is driven by inflammatory cytokines produced by activated T cells [22]. The estrogen deficiency that occurs at menopause results in increased formation and prolonged survival of OCLs. This is suggested to be due to a number of factors including loss of the immunosuppressive effects of estrogen, resulting in increased production of cytokines promoting osteoclastogenesis, and direct effects of estrogen on OCLs [23,24]. Several studies indicate that low-grade inflammation affects physiological bone turnover and plays a role in pathological skeletal conditions such as osteoporosis. Moderately elevated serum levels of high sensitivity C-reactive protein (hsCRP), as an estimate of low-grade systemic inflammation, are reported to be associated with low BMD, elevated bone resorption, bone loss, and increased fracture risk [25–28]. In line with these data, blockade of the inflammatory cytokines tumor necrosis factor alpha (TNFα) and interleukin 1 (IL-1) leads to a decrease in bone resorption markers in early postmenopausal women [29]. In addition, mice depleted of T cells *in vivo* by treatment with anti-CD4 and anti-CD8 antibodies are protected against ovariectomy (ovx)-induced bone loss [30]. The mechanism involves an upregulation of TNF-producing T cells in the bone marrow of ovx mice, further arguing for a role of T cells and T cell-produced cytokines in bone turnover [31]. To summarize, imbalances in bone remodeling may lead to bone loss and osteoporosis.

## The GM as a regulator of bone mass

At birth, we are immediately colonized with bacteria from our mother and the environment. The GM is varied at first but stabilizes towards an adult-like configuration during the 3-year period after birth [32,33]. During this time, the neonatal immune system rapidly matures under the influence of the GM and environmental factors such as diet, intestinal infections, antibiotic treatments, and breast-feeding. The GM composition changes with age and is extremely variable between older individuals (>65 years) [34–36]. The GM presents a vast source of potential antigens for the host's immune system to cope with. At homeostasis, there is a symbiotic relationship between the host and resident microbes that helps in food digestion and protects from invading pathogens. However, under pathological conditions that compromise the host's ability to limit the microbiota's entry from the intestines, species can invade host tissue and cause disease. Altered immune stimulation or release of metabolic products by the GM can also result in disease. Dietary changes, antibiotic treatments, or pathogens can shift the composition of the GM, and thereby disturb the balance in metabolic and immune regulatory networks that normally restrain intestinal inflammation [9,10,37,38]. The use of gnotobiotic animals and the development of effective sequencing technologies have made it possible to characterize the effects and the composition of the GM. Studies have shown that germ-free (GF) animals have immature mucosal immune systems with poorly developed gut-associated lymphoid tissue (GALT). Furthermore, GF mice have a reduced number of $CD4^+$ T cells in the spleen and fewer and smaller germinal centers within the spleen, suggesting that the GM is capable of shaping systemic immunity [39].

It was recently shown that absence of GM in GF mice leads to increased bone mass, compared to conventionally raised (CONV-R) mice [40,41]. It was found that both the spongy trabecular bone and the compact cortical bone was affected with the trabecular bone volume/tissue volume (BV/TV) increased by 39% in the distal femur of GF compared with CONV-R mice [40]. The increased BV/TV was