In this issue of the JCI, Li et al. show that germ-free mice, when chemically castrated, do not lose bone — a finding that unequivocally establishes a role of gut microbiota in mediating hypogonadal bone loss. Additionally and not unexpectedly, probiotics reversed hypogonadal osteopenia in sex steroid–deficient mice by preventing the disruption of gut barrier function and dampening cytokine-induced inflammation. The authors propose that TNFα is a key mediator; however, it is very likely that other molecules — including IL-1, IL-6, IL-17, RANKL, OPG, and CCL2 — modulate probiotic action. The results of this study highlight the potential for repurposing probiotics for the therapy of osteoporosis. Future placebo-controlled clinical trials will be required to establish safety and efficacy of probiotics in reducing fracture risk in people.
In this issue of the JCI, Li et al. show that germ-free mice, when chemically castrated, do not lose bone—a finding that unequivocally establishes a role of gut microbiota in mediating hypogonadal bone loss. Additionally and not unexpectedly, probiotics reversed hypogonadal osteopenia in sex steroid–deficient mice by preventing the disruption of gut barrier function and dampening cytokine-induced inflammation. The authors propose that TNFα is a key mediator; however, it is very likely that other molecules—including IL-1, IL-6, IL-17, RANKL, OPG, and CCL2—modulate probiotic action. The results of this study highlight the potential for repurposing probiotics for the therapy of osteoporosis. Future placebo-controlled clinical trials will be required to establish safety and efficacy of probiotics in reducing fracture risk in people.

A new player in sex steroid deficiency–related bone loss

Osteoporosis is a leading cause of morbidity in the increasing population of aging adults. In postmenopausal women, fracture incidence far exceeds the combined incidence of breast cancer, stroke, and myocardial infarction. Bone loss arises from accelerated resorption by osteoclasts, which outpaces the accompanying increase in bone formation by osteoblasts (1). Postmenopausal osteoporosis has traditionally been solely attributed to declining estrogen levels. However, the rapid loss of bone during the late perimenopausal transition, particularly when estrogen levels are relatively normal, is proposed to be mediated, at least in part, by rising follicle-stimulating hormone levels (FSH levels) (2, 3). Nonetheless, these hormonal changes do not fully explain the increased bone formation, high BM T cell counts, or macrophage activation that have been noted across the menopausal transition (4). Alterations in immune cell function have largely been attributed to increased production of TNFα, which further enhances osteoclast formation and function (5, 6). Consistent with this, ablation of the Tnfα gene in mice prevents gonadectomy-induced bone loss, osteoclast and osteoblast activation, and accompanying immune cell aberrations (5). However, the mechanisms that drive TNFα production during hypogonadal states remain relatively unclear. In this issue, Li et al. (7) show that gut microbiota play a fundamental role in the enhanced TNFα production that occurs upon the induction of estrogen deficiency in mice.

It has previously been shown that lowering the inflammatory burden does suppress the effects of estrogen deficiency. For example, TNFα or IL-1 blockade in early postmenopausal women decreases bone resorption markers (8). Likewise, mice in which Tnfα or Il6 is deleted are relatively resistant to ovariectomy-induced bone loss (9). Li et al. evaluated how gut microbiota contribute to the inflammation seen with estrogen deficiency by using a strategy in which mice raised in an environment devoid of gut bacterial colonization (germ-free mice) were chemically castrated with leuprolide (7). Impressively, these hypogonadal, germ-free mice did not experience the marked bone loss that occurred in hypogonadal mice with unperturbed gut flora. Likewise, osteoclast numbers were not increased in germ-free mice. Even more impressive was the observation that the reintroduction of flora into germ-free mice reversed the osteoprotection exerted by an absence of microbiota; this, in essence, proved a fundamental role for gut microbiota in regulating skeletal integrity. Mice raised in a germ-free environment are indeed known to have increased bone mass and fewer osteoclasts (10). The findings of Li et al. now suggest that the same pathways are responsible for the bone loss accompanying sex steroid deficiency.

Germ-free mice maintain barrier function

What is the mechanism through which gut microbiota mediate bone loss during sex steroid deficiency? It is widely accepted that inflammation—a phenomenon characterized by widespread immune cell dysregulation and hypercytokinemia, which in turn causes increased bone resorption and decreased bone formation (12, 13). It is also known that the loss of gastrointestinal barrier function can itself cause

**Conflict of Interest:** M. Zaidi is/has been a consultant for Merck, Novartis, Roche, Gerson Lehmann Group, and Guidepoint. He will be entitled to proceeds from any licensing agreement between Icahn School of Medicine at Mount Sinai and any commercial entity for a US patent relating to the use of follicle-stimulating hormone (FSH) blockers in preventing bone loss.

inflammation and T cell activation. Consistent with this, Li et al. show that estrogen deficiency causes the loss of barrier function, leading to endotoxemia (Figure 1, ref. 7). In contrast, germ-free mice maintained adequate barrier function, thus preventing the microbiota from inducing inflammation. Therefore, inflammation induced by sex steroid deficiency in a setting of normal microbiota should lead to T cell activation, which in turn would be expected to increase the production of proinflammatory cytokines, including TNFα (14). That TNFα can itself cause bone loss by increasing osteoclast bone resorption has been proven unequivocally in Tnfa-expressing transgenic mice. Now, Li et al. have established a permissive connection between sex steroid deficiency, gut microbiota, inflammation, and TNFα production by showing that hypogonadal germ-free mice are unable to increase TNFα-expressing CD4+ T cells.

The benefits of probiotics
The identification of a critical role for gut microbiota in mediating the bone loss of sex steroid deficiency begs the question as to whether this pathway can be utilized to
provide a therapeutic advantage for osteoporosis patients. Exposure of weaning mice to antibiotics increases bone mass (15), suggesting that alteration of the normal microbiota may, in fact, recapitulate the beneficial skeletal actions of a germ-free environment. An alternative strategy in lieu of antibiotics would be to use probiotics—live microorganisms that provide health benefits to the host (16). Probiotics have been long known to increase bone strength in chickens (17) and to improve eggshell hardness (16). Several other notable observations underscore the premise that altering the microbiota using probiotics can alter bone remodeling and/or bone mass in hypogonadal states. First, while estrogen deficiency impairs calcium absorption, probiotics reverse this effect and suppress bone resorption (18). Second, the probiotic bifidobacterium blunts the decreases in bone mass and increases in osteoclast numbers in hypogonadal rats (19). Li et al. have further solidified these observations by showing that two different probiotic classes are able to prevent the bone loss that is secondary to estrogen deficiency (7). However, probiotics also independently caused an increase in bone mass in sham controls without hypogonadal hyperresorption, suggesting a possible enhancement of osteoblastic bone formation.

These beneficial effects of probiotics on bone formation lead to a further question: can probiotics reverse hypogonadal bone loss by decreasing proinflammatory cytokines, including TNFα and IL-1β, and/or by increasing antiinflammatory cytokines such as IL-10 (20)? Several lines of evidence attest to this being the case, at least in part. Probiotics do decrease TNFα and IL-1β levels and increase production of the antioestoclastogenic cytokine osteoprotegerin (OPG) in hypogonadal mice (21). They also inhibit Tnfα and Il6 expression and upregulate Il10 in a collagen-induced arthritis model (22)—effects that are likely exerted via NFkB dephosphorylation (23). Importantly, media from the probiotic Lactobacillus is able to decrease both macrophage Tnfα expression and osteoclastogenesis (24, 25). Consistent with these findings, Li et al. note that probiotic-fed hypogonadal mice display significant decrements in Tnfα and Rankl in both the intestine and BM (7). Furthermore, probiotics decreased gut permeability by activating the same MAP kinases that are induced nongenomically by estrogen. This action likely results from the production of small estrogen-like molecules that may behave as selective estrogen receptor modulators with differential actions on bone. It is therefore not surprising that probiotics display sex-specific actions on bone mass in mice (26).

Conclusions
If TNFα inhibition is indeed central to the action of probiotics in preventing hypogonadal bone loss, sequestration of TNFα by the monoclonal antibody infliximab in people—for example, those suffering from inflammatory bowel disease—would be expected to reduce bone loss. Surprisingly, such an effect was not found in a cohort of patients with Crohn’s disease (27), leaving the question open as to whether TNFα is the sole mediator of probiotic action on bone. In fact, multiple studies underscore the complexity of TNFα action in terms of the multiplicity of target genes that it modulates, as well as the temporal oscillations in gene expression that it elicits (28, 29). More directly, transcriptional profiling of the peripheral blood from people fed Lactobacillus has not only been shown to reduce expression of multiple NFκB-responsive genes, but also to inhibit the osteoclastogenic chemokine CCL2 (30). This makes CCL2 and its receptor CCR2 potential mediators of probiotic action, particularly as the genetic deletion of either leads to reduced osteoclastogenesis (31). Additionally, IL-17, which has been recently identified as a modulator of hypogonadal bone loss, is reduced by probiotic administration. In contrast, probiotics increase TGFβ1 to enhance Tregs in murine models of inflammatory disease (32) and therefore prevent ovariectomy-induced Treg downregulation (21). As Tregs inhibit osteoclast differentiation, this action is likely to be osteoprotective (33). It is therefore possible that secreted molecules other than TNFα may play critical roles in mediating the effects of probiotics on bone.

Acknowledgments
M. Zaidi acknowledges the NIH for support (AG40132, AG23176, AR65932, and AR67066).

Address correspondence to: Mone Zaidi, Endocrinology, PO 1055, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, New York 10029, USA. Phone: 212.241.8797; E-mail: Mone.zaidi@mssm.edu.

17. Mutus R, Kocabagli N, Alp M, Acar N, Eren M, Gezen SS. The effect of dietary probiotic...
supplementation on tibial bone characteristics and strength in broilers. Poult Sci. 2006;85(9):1621-1625.


