

The Gut Microbiome Regulates Osteocyte Transcription in a Sexually Dimorphic Manner

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INTRODUCTION: Age-related decline in bone quality is a major contributor to skeletal fragility. The gut microbiome consists of a diverse community of microorganisms, such as bacteria, fungi, and viruses, which play a key role in human health and aging. One way in which the gut microbiome influences host tissues is through the production of microbiota-derived metabolites, which are released from the gut and distributed to distant organs through the systemic circulation. Recently, microbiota-derived metabolites have been implicated in age-related decline of host tissue functions, such as in Alzheimer's disease¹. However, the role of the gut microbiome in regulating skeletal aging remains unknown. Our previous work has shown that alteration of the gut microbiome using antibiotics impaired bone tissue strength in mice², but the cellular and molecular mechanisms underpinning this process remain unclear. Osteocytes comprise over 90% of the cellular composition of bone and play a key role in bone aging³. Osteocyte regulation of bone quality is also sexually dimorphic⁴. The goals of this study are to: (i) evaluate the effect of chronic alteration of gut microbiome on osteocyte signaling in aged male and female mice, and (ii) identify circulating microbiota-dependent metabolites that may be mediating this effect.

METHODS: Animal procedures received prior approval from the local Institutional Animal Care and Use Committee. Alteration of the gut microbiota was performed using antibiotics (1g/L ampicillin, 0.5 g/L neomycin). Male and female C57BL/6 mice were divided into two groups: (i) unaltered from birth to 22 months of age and (ii) continuous dosing with ampicillin and neomycin (Amp+Neo) from 1 to 22 months of age. Femoral section modulus was measured using micro-computed tomography as previously described⁵. RNA isolated from osteocyte-enriched humeri (lacking periosteum, soft tissues, and epiphyses) was sequenced using an Illumina NovaSeqS4 and 150-bp paired-end reads (n=5/group/sex). Differential gene expression analysis was performed using the DESeq2 package⁵ in R. Differentially expressed genes (DEGs) with FDR<0.1 were considered significant. KEGG over-representation analysis was performed using the identified DEGs for male and female mice separately. Significantly enriched KEGG pathways with FDR<0.05 were subjected to unbiased hierarchical clustering using the treeplot function within the clusterProfiler package^{6,7} in R. Microbiota-dependent metabolites (n=3/group/sex) were identified from serum using an unbiased mass spectrometry pipeline developed in a recent study⁸ and validated for the detection of microbial metabolites. MS-DIAL 4.60 was used for peak picking, annotation, and alignment. Statistical analysis was performed using MetaboAnalyst. Metabolites with FC>3 and p<0.05 were considered significant.

RESULTS: Chronic alteration of the gut microbiome resulted in a significant reduction in section modulus in male mice, but had minimal effect in female mice (Fig. 1A). Differential expression analysis comparing osteocyte-enriched cortical bone from mice with altered gut microbiota (Amp+Neo) to unaltered controls yielded a total of 2,205 differentially expressed genes in males, but only 12 genes in females (Fig. 1B). Only three genes were found to be common between the two sets. Subsequent pathway analysis of differentially expressed genes revealed 20 significant KEGG pathways in males and one significant pathway in females (Fig. 1C). Male-specific pathways that were influenced by alteration of the gut microbiota broadly included cell metabolism, biosynthesis, and cell cycle regulatory signaling. Analysis of microbiota-derived circulating metabolites identified 62 differentially abundant metabolites, of which 17 were differentially abundant by sex, 14 were differentially abundant in response to changes in the gut microbiome in both sexes, and 31 showed differential abundance by microbiome depended on sex (Fig. 1D).

DISCUSSION: Our data establishes an effect of the gut microbiome on cortical bone signaling. We observed sex-specific responses to alteration of the gut microbiome at the systemic, tissue, and cellular level (i.e., microbiota-derived metabolites, femoral section modulus, and osteocyte gene expression, respectively). The observation that alteration of the gut microbiota led to cortical bone gene expression changes involved in cell cycle regulation, senescence, and metabolism suggests that osteocytes may be acting as potential mediators linking the gut microbiome to skeletal aging. The identification of circulating microbiota-derived metabolites that are associated with sex-specific perturbations to the microbiome provides a novel axis of interrogation by which we may further evaluate the molecular basis of microbiota-dependent changes in bone health and aging. Our future work will focus on identifying individual metabolite-gene expression relationships that may be involved in the regulation of bone tissue strength.

SIGNIFICANCE: This study uncovers a sexually dimorphic role of the gut microbiome in regulating bone health. These key insights implicating the microbiome as a potential driver of skeletal aging could pave the way for novel interventions that harness microbiome-based therapeutics to prevent and treat aging-associated skeletal disorders.

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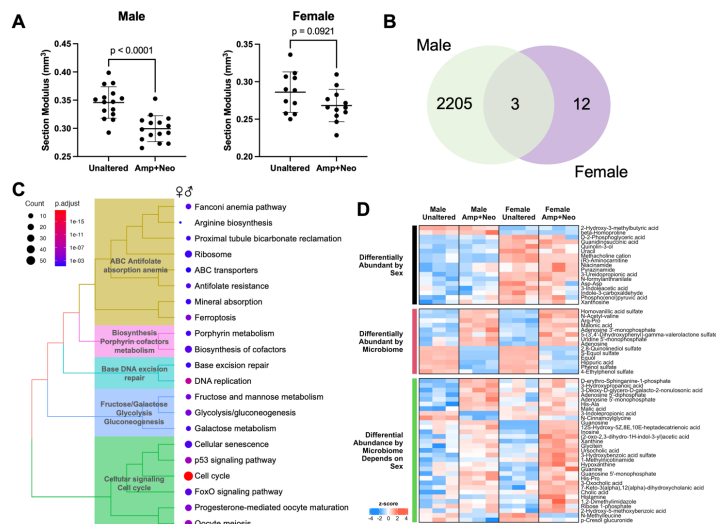


Figure 1. (A) Section modulus of mouse femurs from antibiotic-treated (Amp+Neo) male and female mice compared to unaltered controls. (B) Venn diagram of total and overlapping DEGs in osteocytes from male and female mice. (C) Top KEGG pathways based on male and female DEGs. (D) Top differentially abundant microbiota-dependent metabolites from serum.