Examining the Influence of Different Microbiota Compositions on Male Mouse Bone Density and Architecture using Microcomputed Tomography

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Abstract

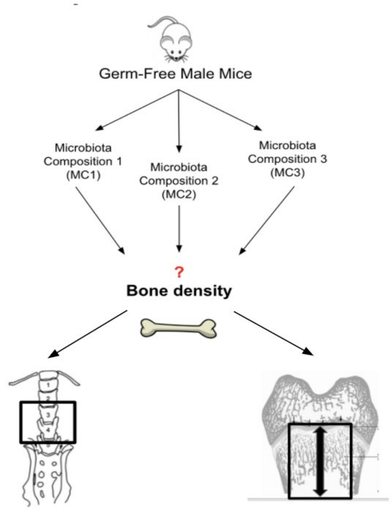
Osteoporosis, a disease that results in bone loss, affects more than 10 million Americans.[1] Bones provide structure, allow for locomotion, and are a storage site for minerals and marrow as an essential organ in the human body. Bones consist of two components: cortical (compact outside shell) and trabecular (cancellous inner region). Cortical bone provides strength and stability, while trabecular bone is more metabolically active because of its architecture which increases its surface area and makes it a site where osteoporosis can readily be seen. My research project tested the effect of different microbiota compositions on bone density architecture. Germ-free adult littermate male mice received one of 3 different microbiota and were examined 4 weeks later. Examination of the trabecular bone parameters of the femur of each mouse revealed no significant differences in trabecular bone volume fraction, bone density or structural parameters (trabecular number, thickness, spacing) between the three different mouse groups, while analysis of the vertebrae showed that microbiota composition may affect trabecular bone volume fraction. The lack of a difference in the femur was surprising, given the important role of the microbiota composition in regulating bone volume. Interestingly, analysis of femoral cortical bone parameters suggests that one of the microbiota compositions may have a negative effect on cortical bone area and overall shape. While more mice are needed to make conclusions, my studies suggest that different microbiota compositions may affect bone components differently. In the future I will determine links between the microbiota components and the bone changes.

Background

- The microbiota is the aggregate of microbes living in and on the human body, and the microbiome is the aggregate of genetic material of the microbiota.
- Bone volume fraction (BVF) is a measure of how much of a bone sample is truly mineralized bone. A low BVF can result in an increased risk for bone diseases such as osteoporosis.
- Prior studies have shown that microbiome composition affects bone health.
- The gut microbiome regulates bone health.

Methods

- Germ-free adult littermate male mice received one of 3 different microbiota and were examined 4 weeks later
- After 4 weeks, the germ-free mice were weighed and harvested for vertebrae, tibia, and gut organs
- Femurs and vertebrae were scanned in a micro-CT machine. Bone length was measured and then 3D images were generated using GEHC Microview for trabecular and cortical analysis.
- All measurements are presented as the mean ± SEM. Significant outliers were removed using the ROUT test. Student's t-test and 1-way ANOVA with Tukey's post-test were performed using GraphPad Prism software version 8 (GraphPad, San Diego, CA, USA).



Results

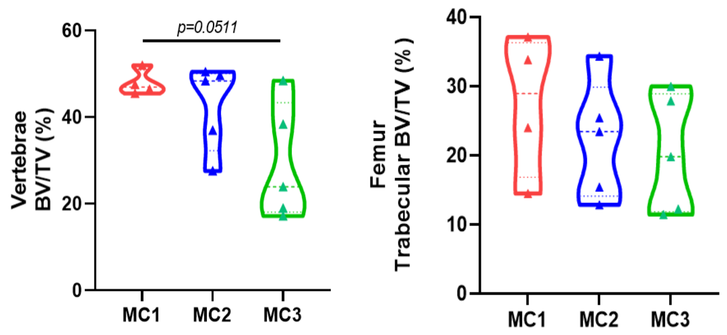


Figure 1: Different microbiota compositions may alter vertebral and femoral trabecular bone density. Bone volume fraction percentage per gram of body weight among the 3 groups. Representative distal femur images show bone trabecular network. Values are averages ±SEM and were analyzed using one-way ANOVA. N_{MC1} =4, N_{MC2} =5, N_{MC3} =5. P-values are as written.

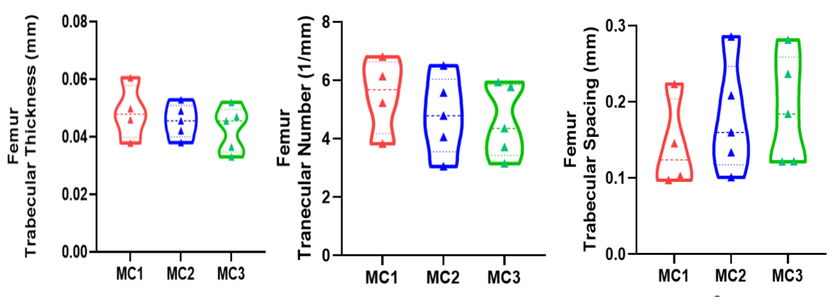


Figure 2: Bone femur microarchitecture. Analysis of trabecular spacing, number and thickness of the femur among the 3 groups. Values are averages ±SEM and were analyzed using one-way ANOVA. N_{MC1} =4, N_{MC2} =5, N_{MC3} =5. P-values are as written. P-values are as written.

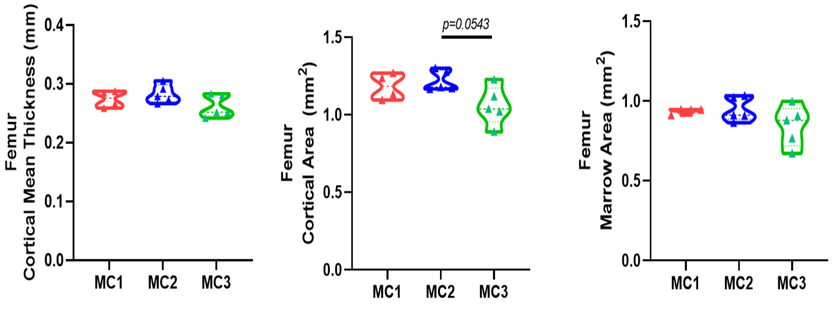
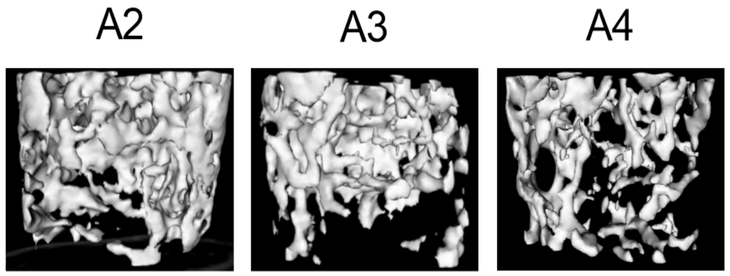


Figure 3: Different microbiota compositions may alter the parameters of cortical bone in femur. Analysis of cortical parameters (mean thickness, area, and marrow area) among the 3 groups. Values are averages ±SEM and were analyzed using one-way ANOVA. N_{MC1} =4, N_{MC2} =5, N_{MC3} =5. P-values are as written. P-values are as written.

Femur Isosurfaces



Vertebral Isosurfaces

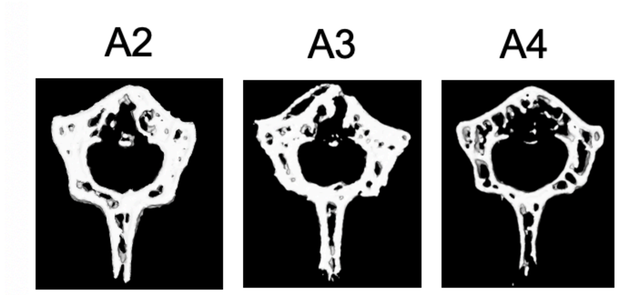


Figure 4: Vertebral and femoral isosurface images. Representative distal femur and lumbar vertebrae images show bone trabecular network.

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Conclusions

- Different microbiota can affect bone health positively or negatively, depending on the composition of the microbiota.
- Different microbiota compositions may affect bone components differently.
- Vertebral BVF is affected more significantly by changes in microbiota composition than femoral BVF.
- Femoral cortical area is affected by microbiota composition.

References

1. **What Women Need to Know**. *National Osteoporosis Foundation*, 22 Mar. 2021