Bone loss with aging is independent of age-related gut microbiome dysbiosis in mice

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ABSTRACT

Emerging evidence suggests an important role of the microbiome in bone health. The microbiome sustains a dynamic status that constantly adapts to the host physiology and fitness. During the aging process, the microbiome integrates and responds to aging signals from the host, which in turn impacts host health. However, it is unclear whether microbial dysbiosis with aging contributes to age-related bone loss.

Here, age-specific microbial signatures were characterized and their roles in age-related bone loss were investigated in a murine model. 16s rRNA gene sequencing of fecal community samples from 3- and 24-month old CB6F1 males indicated an age-dependent shift towards a Firmicutes-dominant community with an alteration in energy and nutrient metabolism potential. Muscipirillum, a pathobiont associated with host inflammation, was increased in the aged microbiome. An integrative analysis of 16s predicted metagenome function and LC-MS fecal metabolome revealed enrichment of amino acid biosynthesis pathways in aged mice. Microbial methionine and S-adenosyl methionine metabolism were increased in the aged mice, which have previously been associated with the host aging process. Collectively, aging caused microbial taxonomic and functional dysbiosis in mice. We tested whether the young vs old microbiome differentially impacts bone turnover by colonizing 4 or 8-week old germ-free (GF) mice by fecal transplant from 3- or 24-month old specific-pathogen-free (SPF) mice. We assessed trabecular and cortical bone by micro-computed tomography 1 and 8 months after colonization. The effect of microbial colonization on bone phenotypes was independent of the microbiome donor age. To assess if the gut microbiome was required for age-related bone loss, we compared the trabecular and cortical bone structure of 24-month old CB6F1 GF female mice compared to that of littermates colonized at 8-weeks of age. The 24-month old bone phenotype was indistinguishable between colonized and GF mice. We next examined bone loss from 3 to 24 months in CB6F1 GF compared to SPF mice. Neither female nor male GF mice were protected from age-related bone loss. In conclusion, our study indicates age-related bone loss occurs independent of age-related gut microbial dysbiosis.

INTRODUCTION

Age-related osteoporosis represents a major health burden in the elderly. Due to the rapid growth of the aged population, the number of osteoporotic fractures is increasing, placing a heavy burden on economics and health care systems. The annual direct cost from osteoporosis is estimated to be $25.3 billion by 2025 in US. While guidelines for osteoporosis treatment are well established, they are mostly limited to anti-resorptives. The long-term benefit of these therapies is complicated by increasing recognition of emerging side-effects. Therefore, the development of new preventative or therapeutic strategies for age-related osteoporosis is urgently needed.

Emerging evidence suggests an important role of the microbiome in bone health, representing a potential therapeutic target for osteoporosis. In the present study, we identified age-specific microbial taxa and metabolic features by 16s rRNA gene sequencing and LC-MS in a murine model. We investigated whether host bone phenotype is associated with gut microbiota and fecal metabolites during the aging process.

RESULTS

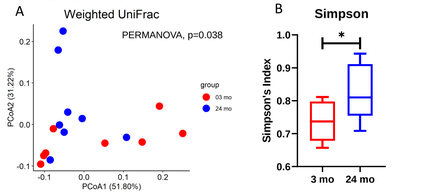


Figure 1 Distinct microbial diversities between young and old mice. (A) Microbial β-diversity was visualized by principal coordinate analysis (PCoA) plot based on weighted UniFrac distance. PERMANOVA with 999 permutations were used to test significant differences between young and old mice, suggesting age-dependent microbial structure differences. (B) Simpson index was used to measure α-diversity, suggesting an increase of community α-diversity in the old mice

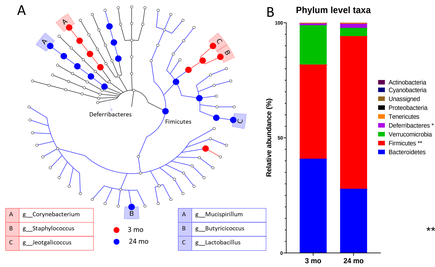


Figure 2 Differential fecal microbial compositions between young and old mice. (A) Linear Discriminant Effect Size (LEfSe) analysis was performed and visualized by Cladoplot (dots highlighted with red or blue indicate p<0.05 and log (LDA)>2). (B) Relative abundance of taxa was calculated at the phylum level (* for p<0.05, **, p<0.01). Both analyses showed the old microbiome shifted towards a Firmicutes-dominant community.

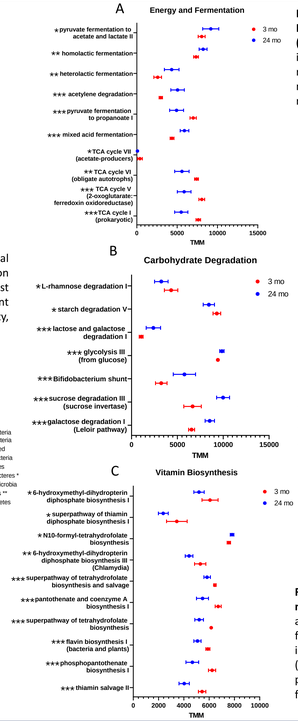


Figure 3 (left) Microbial metagenome function prediction by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) based on 16s rRNA gene. (A) Fermentation related pathways were increased while 4 TCA cycle related pathways were decreased in the old microbiome. (B) Microbial saccharolytic potential was decreased in the old microbiome. (C) B vitamin biosynthesis potential was decreased in the old microbiome. * for p<0.05, **, p<0.01 *** for p<0.001

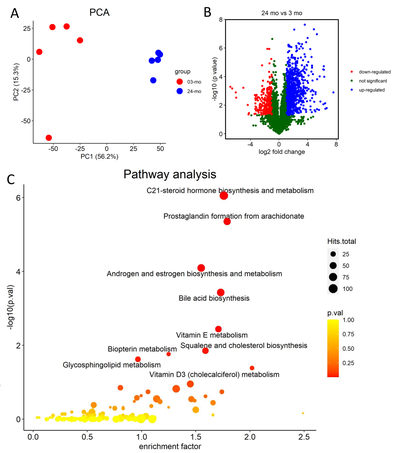


Figure 4 Distinct fecal LC-MS metabolome profiles between young and old mice. (A) PCA plot showed two clearly separate clusters according to the mice age, indicating distinct metabolome profiles between the young and old mice fecal samples. (B) Volcano plot showed 1549 metabolic features were increased while 638 were decreased in the old mice (p<0.05 and log2FC>1). (C) Significant differentially expressed metabolic features were enriched in 9 pathways (p<0.05). For (C), size of the bubbles represents the total metabolic features hitting within the pathway. Color of the bubbles represents p value.

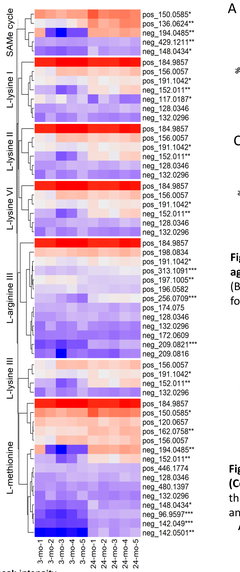


Figure 5 Integrative analysis of 16s predicted metagenome and LC-MS metabolome. An integrative analysis of 16s predicted metagenome and LC-MS metabolome was performed by a customized gene set enrichment analysis (GSEA). Metabolites involved in amino acid biosynthesis were significantly enriched in the old mice (p<0.05).

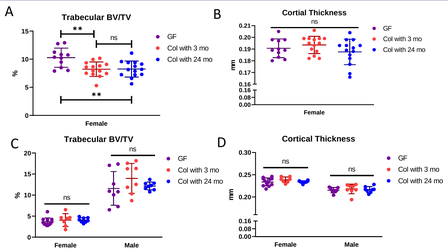


Figure 6 Effect of colonization on bone phenotype of gnotobiotic mice is independent of age of donor microbiome. Trabecular bone volume fraction (A,C) and cortical thickness (B,D) were similar in gnotobiotic mice colonized with either 3-mo or 24-mo fecal microbiome for either short-term (1-month; A,B) or long-term (8-month; C,D). **, p<0.01

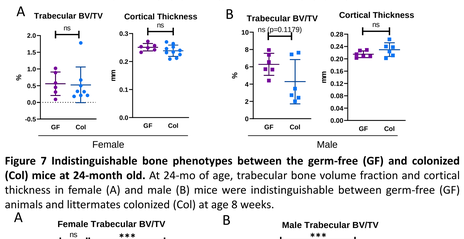


Figure 7 Indistinguishable bone phenotypes between the germ-free (GF) and colonized (Col) mice at 24-month old. At 24-mo of age, trabecular bone volume fraction and cortical thickness in female (A) and male (B) mice were indistinguishable between germ-free (GF) animals and littermates colonized (Col) at age 8 weeks.

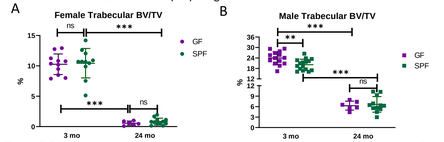
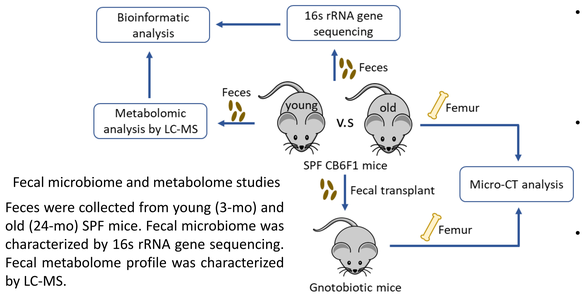


Figure 8 Germ-free mice are not protected from age-related trabecular bone loss. An age-dependent decrease was observed in (A) female and (B) male trabecular bone volume fraction (BV/TV) but no significant difference was observed between germ-free (GF) and conventional (SPF) mice. **, p<0.01 *** for p<0.001

STUDY DESIGN



Fecal microbiome and metabolome studies

Feces were collected from young (3-mo) and old (24-mo) SPF mice. Fecal microbiome was characterized by 16s rRNA gene sequencing. Fecal metabolome profile was characterized by LC-MS.

Gnotobiotic mice studies

- Feces from young (3-mo) and old (24-mo) SPF mice were fecal transplanted to gnotobiotic mice (2-mo). Femur samples were collected for micro-CT analysis after 1-mo and 8-mo colonization.
- Feces from young (3-mo) SPF were fecal transplanted to gnotobiotic mice (2-mo). Femur samples were collected for micro-CT analysis after 22-mo colonization and compared to their GF littermates.
- Femur samples were collected from SPF mice and GF mice at 3-mo and 24-mo old for micro-CT analysis.

mo, month, SPF: specific pathogen free, GF, germ-free

Mouse image was downloaded from pinclipart.com

CONCLUSIONS

- Distinct profiles of fecal microbial community and metabolome profiles were observed between the young and old mice.
- The old microbiome was featured by a Firmicutes-dominant community with an alteration of energy and nutrient metabolism potential.
- An integrative analysis of 16s predicted metagenome function and LC-MS fecal metabolome revealed amino acid biosynthesis pathways were enriched in the old microbiome.
- Age-related bone loss occurred independent of age-related gut microbial dysbiosis
- Future studies may focus on therapeutic effect of microbial modulation by prebiotics/probiotics on age-related bone loss.

ACKNOWLEDGEMENTS

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