Senescence-accelerated mouse prone 8 exhibits osteoporosis with increased osteoid

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INTRODUCTION: Osteosarcopenia is a condition where both muscle mass and bone density decrease, increasing the risk of falls and fractures, thus shortening healthy lifespan. Senescence-Accelerated Mouse (SAM) prone 8 (SAMP8) mice exhibits a short life span due to promoted oxidative stress status[1]. SAMP8 mice are widely used to study the aging-associated diseases such as cognitive decline[2] or sarcopenia[3]. Mitochondrial DNA deletion increases in SAMP8 mice[4], which results in mitochondrial dysfunction and acceleration of aging[5]. Some reports suggest that SAMP8 mice are an ideal animal model of osteosarcopenia, however, the bone phenotype is controversial. The bone mineral density (BMD) of the lumbar spine of SAMP8 mice was comparable to that of control SAM resistant 1 (SAMR1) strain[6], while other study showed the bone volume of the femoral metaphysis were significantly decreased[7]. Another study demonstrated that the blood examination of markers of the bone formation and the resorption were decreased in aged SAMP8 mice, suggesting the low bone turnover status[8]. However, bone histomorphometric analysis has not been reported. In this study, we conducted bone histomorphology in SAMP8 mice to examine whether indeed there is a decrease in bone mass, the balance between bone formation and resorption in such cases, and the underlying mechanisms.

METHODS: This study was approved by the Institutional Animal Care and Use Committee of Kagoshima University. Although the bone mass of SAMP8 mice starts to decrease at 2 months of age[7], they develop sarcopenia starting from 8 months of age[8]. Therefore, to investigate the osteosarcopenia phenotypes, we utilized 8-month-old SAMP8 and SAMR1 mice (n = 6). The mice were euthanized with excess isoflurane and subjected to sampling of bone (femora and tibiae). Bone μCT and histomorphometry analyses were performed by Isozo Inc. Total RNA was purified from femora using TRIzol reagent, and subjected to RT-qPCR analysis. For evaluation of mitochondria dysfunction of tibial bone marrow cells, 4-month-old SAMP8 and SAMR1 mice were used. Statistical significance was evaluated by unpaired Student's t test.

RESULTS SECTION: We confirmed the features of sarcopenia and osteoporosis in SAMP8 mice. μCT analysis of femora showed that the trabecular bone volume was significantly decreased in SAMP8 mice (Fig. 1). Bone histomorphometry analysis of tibiae showed that bone resorption in SAMP8 mice was comparable to SAMR1, whereas bone formation (calcein apposition) was enhanced, contradictory to the bone loss. However, osteoid was increased in SAMP8 bone (Fig. 2), resembling osteomalacia. We examined the levels of calcium, phosphorus, active vitamin D3, and FGF23 in the blood, but none of them showed any reasonable changes. However, the mitochondrial membrane potential was decreased in SAMP8 bone marrow cells (Fig. 3), while the lactate content of supernatant was increased, suggesting the mitochondria dysfunction in bone cells.

DISCUSSION: The abnormal osteoid accumulation seemed to be the major mechanism of the osteopenia of SAMP8 bone, and this was accompanied by the mitochondrial dysfunction of bone cells. Because collagen cross-links abnormalities by oxidative stress reduces degree of bone mineralization[9], whereas mitochondrial Sirt3 contributes to the bone loss caused by aging[10], the mitochondrial dysfunction, resulting in factors like ATP depletion, which serves as a precursor for calcium phosphate, may be a potential cause of osteoporosis in SAMP8 mice.

SIGNIFICANCE/CLINICAL RELEVANCE: We have demonstrated that SAMP8 mice indeed exhibit the phenotype of osteosarcopenia and indicated their utility as valuable tools in the development of treatments for age-related musculoskeletal instability. Additionally, our results suggested the potential effectiveness of mitochondrial mediator drugs.

REFERENCES:

1. Chiba, Y. *et al. Neurochem Res* **34**, 679-687 (2009).
2. Akiguchi, I. *et al. Neuropathology* **37**, 293-305 (2017).
3. Guo, A. Y. *et al. Exp Anim* **64**, 425-433 (2015).
4. Fujibayashi, Y. Yamamoto, S. Waki, A. Konishi, J. Yonekura, Y. *Neurosci Lett* **254**, 109-112 (1998).
5. Nakahara, H. *et al. Free Radic Biol Med* **24**, 85-92 (1998).
6. Zhang, N. Chow, S. K. H. Leung, K. S. Lee, H. H. Cheung, W. H. *Exp Gerontol* **97**, 1-8 (2017).
7. Sanada, Y. *et al. Arthritis Res Ther* **24**, 235 (2022).
8. Guo A.Y.. *et al. Exp Anim* **64**, 425-422 (2015).
9. Saito, M. Fujii, K. Marumo, K. *Calcif Tissue Int* **79**, 160-168 (2006).
10. Ling W., *et al*., *JCI Insight* **6**, e146728 (2021)

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