The Impact of Aging on Osteocyte Lacunar Canalicular Turnover Depends on Strain Environment

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INTRODUCTION: The expansive osteocyte lacunar-canalicular system (LCS) has an active role in mineral homeostasis[1]. Osteocytes can form and remove bone along their LCS in a process called LCS turnover[2,3]. Because the LCS surface area is immense[4], genetic mouse models that impede LCS turnover decrease bone fracture toughness[5], and these genetic models produce an aging-like phenotype to LCS geometry[5], a compelling hypothesis is that LCS turnover has an essential role in maintaining bone quality but that this process diminishes with aging. In support of this idea, recent work[6] demonstrates that LCS turnover impacts bone quality immediately around individual osteocytes. However, the impact of LCS turnover on whole bone tissue is not known because the scale and frequency of this process have not been determined. Osteocytes are abundant within both cortical and cancellous bone, which have markedly different metabolic activities[7], but it is unknown if LCS turnover depends on the skeletal site. Furthermore, osteocytes are mechanosensitive cells, and their signaling activity depends on tissue strain[8,9]. However, it is not known if osteocytes located in distinct strain environments differently engage in LCS turnover. LCS turnover can be monitored in undecalcified bone through the presence of perilacunar fluorochrome labels, which indicates osteocyte bone mineralization. As we show, serial fluorochrome labels allow measuring the dynamics of LCS bone turnover. This study aims to test the hypotheses that (1) the prevalence and persistence of LCS bone mineralization decrease with aging and that (2) LCS bone mineralization depends on tissue strain and osteocyte location.

METHODS: Skeletally-mature young adult (5-mo) and early old age (22-mo) female C57BL/6Nia mice received two injections of fluorochrome labels, at two specific dates. These dates included 16d, 8d, 4d, or 2d before the euthanasia. The injections were administered in a manner where each mouse received one injection of alizarin and one injection of calcein, but the specific timing and sequence of the injections varied for each group (n=8-10/group). To ensure that label identity was not confounded with the specific timepoints, some mice received the calcein injection first and the alizarin injection second. Other mice received injections in the opposite order. No confounding effects of label identity were observed. Proximal and distal right femurs were embedded, sectioned in transverse (for cortical midshaft) or sagittal (for metaphysis) directions, and polished to a mirror finish. Confocal laser scanning microscopy, along with accompanying surface images, was used to visualize bone-mineralizing (labeled) and non-bone-mineralizing (not labeled) osteocyte lacunae for both age groups. The percentage of bone-mineralizing osteocytes was measured for lamellar, non-lamellar, and total (lamellar + non-lamellar) cortical bone within A/P/M/L regions of interest (ROI) at femoral midshaft (Figure 1A&C) and for cancellous bone (Figure 1B) at the metaphysis, using custom MATLAB analysis. In cortical samples, we assessed the impact of intracortical strain on bone-mineralizing osteocytes by dividing each ROI into three distance sections: the first 30%, middle 30-70%, and last 70-100% of cortical thickness (Figure 1D). We conducted immunohistochemistry on paraffin-embedded left femurs to evaluate the percentage of MMP14 positive lacunae. Mixed model ANOVA was used to test if the percentage of bone-mineralizing osteocytes or the percentage of MMP14+ lacunae depend on ROI, intracortical strain, label date, or aging. All animal procedures were approved by the university IACUC.

RESULTS: For the young mice, osteocyte participation in mineralizing their surroundings was highly abundant in both cortical and cancellous bone (>80% 2d-labeled lacunae in both tissues). Aging reduced the population of bone-mineralizing osteocytes ($p < 0.001$) in cortical bone (-48%, Figure 1E), for both lamellar (-49%) and non-lamellar (-46%) compartments of cortical bone, and cancellous bone (-49%, Figure 1F). Label presence decreased with further injection dates, likely indicating LCS bone resorption. In cortical bone, labels administered 16 days before euthanasia were 44% ($p < 0.001$) less abundant in young mice and 61% ($p < 0.001$) less abundant in old mice compared to 2d labels. Cancellous bone had 28% and 81% ($p < 0.001$) fewer 16d labels compared to 2d labels in young and old mice, respectively. There were fewer MMP14+ lacunae in old bones compared to young bones (-10%, $p < 0.001$).

LCS turnover depends on the bone strain environment but differently for young and old mice (interaction $p < 0.001$, Figure 1 H & G). In young mice, ROI had no significant impact on bone-mineralizing osteocytes but within each ROI, the osteocytes closest to the periosteal surface (70-100% of cortical thickness-highest intracortical strain) had the lowest participation in mineralizing the tissue (70-100% vs. 0-30%: -8%, $p = 0.017$). In old mice, more osteocytes in the medial ROI (closest to femur neutral axis) participated in mineralizing their surroundings compared to those in the other three ROIs (e.g., M vs A: +44% $p<0.001$). Notably, the decay of 16-day labels was slower in the medial ROI in comparison to the other ROIs. The middle intracortical section (30-70% of cortical thickness) had the smallest population of bone-mineralizing osteocytes (30-70% vs. 70-100%: -25%, $p = 0.009$), and regions near to endocortical and periosteal surfaces had similar percentages of bone-mineralizing osteocytes. Strain environment did not impact the MMP14+ lacunae abundance.

DISCUSSION: This study presents new evidence that osteocyte participation in mineralizing their surroundings is highly abundant in both cortical and cancellous bone of young adult mice but decreases with aging. LCS bone resorption also decreased with aging, but the turnover dynamics are similar between osteocytes from young and old mice. These results suggest that activated osteocytes engage in consistent LCS turnover behavior in cortical bone. The large decline in LCS turnover in aging suggests significant implications for bone quality. These data add to other evidence for the same strain, sex, and ages of mice that LCS turnover increases the compliance of bone local to osteocytes[6]. Thus, decreased LCS turnover in aging could affect the material properties of the perilacunar tissue, potentially impacting how osteocytes perceive mechanical cues. Our results also demonstrate that the impacts of aging on LCS turnover depend on both cortical strain environment and the intracortical strain. These data support a potential link between LCS turnover and osteocyte mechanosensation. Together, these datasets demonstrate that LCS turnover likely influences the bone quality of a substantial amount of tissue and may contribute to reduced osteocyte mechanosensation and bone fracture resistance with aging.

SIGNIFICANCE: We demonstrate that the impact of aging on LCS turnover depends on strain environment. LCS turnover has the potential to have an important role in the decline of bone tissue fracture resistance and mechanosensitivity in aging and could represent an overlooked therapeutic target.

REFERENCES: [1]Qing+, JBMR, 2012; [2]Vahidi+, Bone, 2021; [3]Heveran+,Op Int, 2023; [4]Buenzli+, Bone, 2015; [5]Dole+, Cell Rep., 2017; [6]Rux+, Bone, 2022; [7]Hadjidakis+, Ann. NY Ac. Sci., 2006; [8]Lewis+, PNAS, 2017; [9]Holguin+, JBMR, 2016

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IMAGE: Figure 1. Evaluation of osteocyte bone mineralization in cortical and cancellous bone and different strain environments.

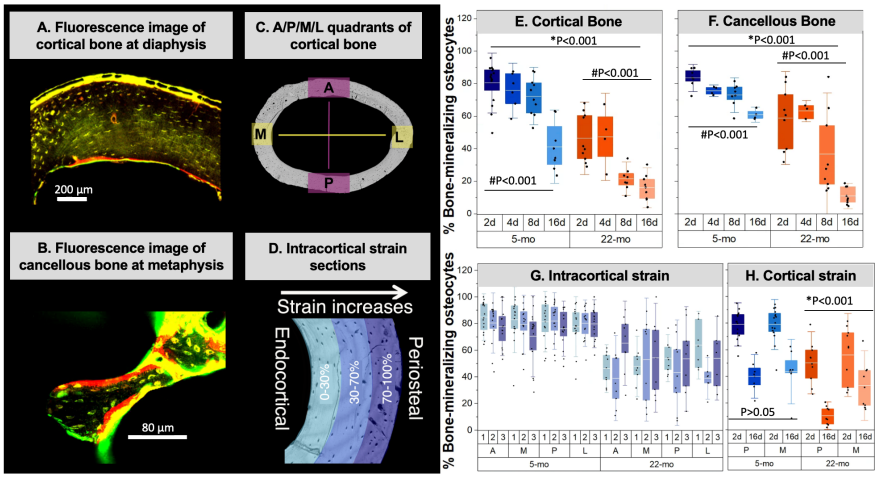

Figure 1. CLSM images of A) cortical bone and B) cancellous bone were used to assess the percentage of bone-mineralizing osteocytes. The impact of tissue strain on LCS turnover was analyzed in C) A/P/M/L ROIs of cortical bone as well as D) three intracortical sections. Aging decreased the prevalence of LCS turnover in E) cortical and F) cancellous bone. The impact of G) intracortical strain and H) distinct cortical ROIs on LCS turnover depended on age.