ESRF	**Experiment title:** Investigating age-related diminished skeletal mechanosensitivity by high resolution SAXS and WAXS analysis of osteocyte perilacunar tissue	**Experiment number**: **SC-3308**
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Shifts: 12	**Local contact(s)**: Cedric Montero Diego Pontoni	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Julius Wolff Institute, Charité – Universitaetsmedizin:

Marta Aido *

Bettina Willie*

Georg Duda

Max Plank Institute of Colloids and Interfaces:

Michael Kerschnitzki*

Rebecca Hoerth*

Wolfgang Wagermaier*

Report:

Summary

The pathogenesis of bone mineral loss with osteoporosis and aging has been attributed at least in part to a reduced mechanosensitivity of bone(1). The mechanisms leading to decreased mechanoresponsiveness as the skeleton matures remain unclear. It is accepted that osteocytes, organized in a lacuno-canalicular network, sense mechanical stimuli and orchestrate bone (re)modeling(2). Age-related changes in osteocyte perilacunar properties might contribute to bone's reduced responsiveness.
By means of small angle x-ray scattering (SAXS), we studied the hydroxylapatite mineral particles in bone sections of mice of different ages.The left tibiae of the animals were loaded ciclically, while the right tibia served as an internal control.We hypothesized that the bone mineral particle size and orientation of mature perilacunar tissue in adolescent, adult, and elderly mice long bones is altered with aging, whereas newly formed perilacunar tissue from mechanical loading will have similar mineral particle size and orientation independent of age.

Scientific Background

Bone has the ability to adapt to mechanical stimulus to attain an optimized structure in terms of size, shape and matrix properties. However, with aging there seems to be a decrease in the sensitivity of the mechanosensor. This leads to a reduced response of the skeleton to adapt to mechanical loading and thus contributes to the progression of osteoporosis. Bone´s mechanosensor is largely believed to be osteocytes (2). These reside within lacunae and are connected through long dendritic processes located inside the canaliculi. The total lacunar and canalicular surface area in adults is two orders of magnitude greater than the surface

directly remodeled by osteoblasts and osteoclasts, which suggests that osteocytes might be able to remove and add mineral directly from the tissue that surrounds its lacunae (perilacunar tissue). This ability would have important implications in the local lacunar strain field influencing their responsiveness to mechanical stimulation. A recent study has shown that the perilacunar tissue in aged rats is stiffer (3) and thus may attenuate the strain signal that the osteocyte can sense, thereby contributing to osteoporosis. Mechanical stimulation might enhance the perilacunar tissue properties and improve the osteocyte ability to sense and respond to matrix strain in aged individuals.

Materials and Methods

We have used high-resolution small angle x-ray scattering (SAXS) to obtain structural information from hydroxylapatite mineral particles in tibial bone sections.
Cyclic compressive loading was applied on the left tibia of female 10, 26 and 78 wks old C57Bl/6J mice for 2 weeks (n=2/age). The right tibia served as an internal control. All mice were administered calcein 12 and 3 days before euthanasia, allowing identification of newly formed bone with fluorescent microscopy.
The tibiae were collected after the *in vivo* loading experiment, embedded in polymethylmethacrylate (PMMA) and sectioned to a final thickness of 10 μm in water-free conditions. The mineral crystals size, alignment and orientation at the tibial midshaft were measured by SAXS using a monochromatic X-ray beam (13 Kev).The X-ray beam had a diameter of 1 μm at the position of the specimen and the q-range to be covered was approximately 0.1 to 3 nm^{-1}.

Results

Preliminary results suggest that bone mean mineral size (T parameter) and orientation (Rho parameter) is dependent on animal and tissue age. We were able to distinguish bone mineral size and orientation between mature and newly formed tissue, which is identified by the green calcein fluorochrome labels.

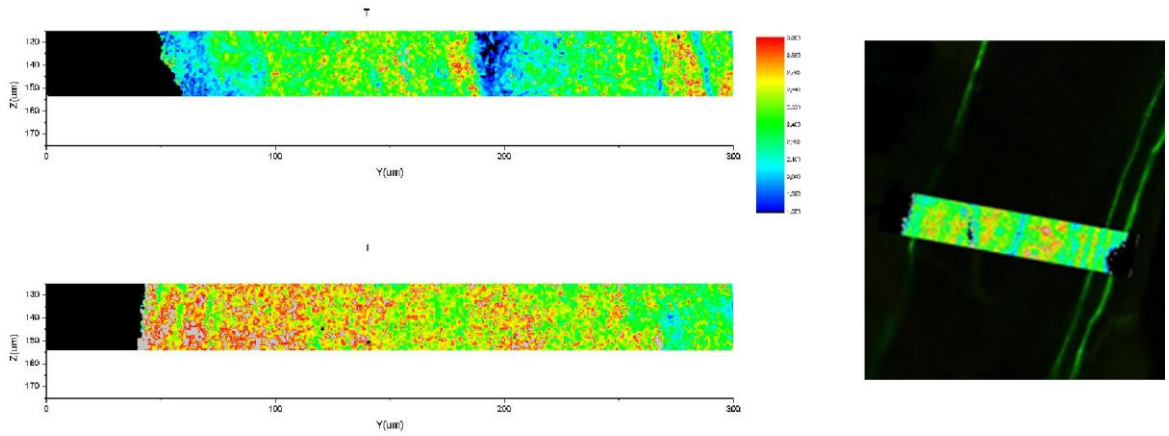

Figure 1: Left) T parameter of an adult : 26 wks old (above) and an elderly: 78 wks old mouse (below); Right) T parameter of the loaded tibia of an adult mice; newly formed bone between the green fluorochrome labels

Understanding how bone mineral properties change with aging, including the properties of the tissue adjacent to osteocytes, may help us better understand mechanisms of bone adaptation. This knowledge would allow us to develop more effective biophysical therapies aimed at inhibiting age-related bone loss and osteoporosis.

References

1. Akkus, O., F. Adar, and M.B. Schaffler, Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. Bone, 2004. 34(3): p. 443-53
2. Tatsumi, S., et al., Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metab, 2007. 5(6): p. 464-75.
3. Nicolella DP, Yao W, Lane N. Estrogen deficiency alters the localized material properties of the peri-lacunar bone matrix in old rats. *30 th Annual Meeting of the American Society for Bone and Mineral Research*. Montréal, Canada 2008.