Bone Morphology Regulates Mineralization in Mice during Growth and Development

Faillace, ME; Ramcharan, M; Jepsen, KJ

INTRODUCTION: Mechanical functionality of the skeleton is a complex trait that is determined by the composition and morphology of the tissue. Our group has previously shown a link between bone robustness and tissue mineralization, whereby increased robustness across A/J, C3H, and B6 mice was linked to a increase in tissue mineral density and ash content [1]. We have further shown that this interaction is established during post-natal growth [2]. Although we know that morphology is linked to composition across genotypes we do not know how or when osteoblasts are producing different matrices at specific bone sites, and across genotypes. Examination of mineral is important in understanding mechanical functionality; an increase in mineralization does lead to increased stiffness, however increased functionality comes at the expense of an increase in brittleness.

The goal of this study is to understand how osteoblast mineralization is regulated by morphology during growth and development. By using the natural variation in bone diameter at the proximal (narrow) and distal (wide) sites of the femoral shaft, we can determine how morphology and composition co-vari independent of genetic background. Despite differences in the local mechanics and growth patterns along the femoral shaft, we hypothesize that there will be a common biological paradigm among inbred mouse strains, whereby external body size regulates mineralization.

METHODS: 8-week-old A/J, B6, and C3H male and female mice were purchased from The Jackson Laboratory (Bar Harbour, ME). 3 breeding pairs/strain were used to generate female pups at 10, 14, 28, 31, or 56 days of age. The Institutional Animal Care and Use Committee approved the handling and treatment of the animals. Sample Prep: Left femora (n=3/strain/timepoint) were harvested at sacrifice. The femoral head and condyles were removed to expose the proximal and distal surfaces. Bones were cleaned of marrow, dehydrated in successive 70, 80, 90, and 100% ethanol for 48 hours, and embedded in epoxy (Epo-thin: Buehler, Lake Bluff, IL). The proximal and distal ends of the embedded blocks were polished with a series of fine abrasive compounds, until the bone surface was exposed, flat and reflective. Fourier Transform Infrared Imaging (FTIRI): Bone blocks were analyzed using FTIRI to determine mineral and protein content. IR-images of the cross-sections of the proximal and distal femurs were collected in reflection mode using a Bruker Hyperion 3000 FT-IR Microscope at the National Synchrotron Light Source (Brookhaven National Laboratory, Upton, NY). Reflection data was transformed to absorption using a custom Matlab Kramer’s Kronig routine [3]. Mineralization (phosphate:protein) was determined from IR images.

RESULTS: FTIRI analysis revealed that mineralization in all three strains increased over time for the proximal and distal sites (Figure 1). Mineralization was higher in the proximal femur compared to the distal femur for all three strains, and highest in A/J and C3H mice compared to B6 for both bone sites. Further, the mineralization profile for the proximal femur from A/J and C3H mice was much steeper when compared to B6 mice.

DISCUSSION: As hypothesized, our results are consistent with previous studies that showed bone cells precisely regulate mineralization [1, 4-5] to compensate for variation in external bone size during growth and development. The results confirmed compositional differences across the three strains [4], as well as between bone sites within each animal. We further saw that there was a strong correlation between bone morphology and tissue composition that developed after 14 days. Proximal-distal differences within each strain can be partially explained by differences in average tissue-age, as well as differences in the growth process. It appears mineral deposition via endosteal expansion and cortical drift are similarly regulated regarding mineralization. Further, comparison of the proximal and distal femur within one strain can be used as an internal control to determine how morphology regulates composition, independent of genetic background.

The correlation between morphology and composition was expected, and suggest that osteoblasts increase mineral content to compensate for the smaller cross-sections. Mineral content has been correlated with mechanical properties [5], such as elastic modulus. We have further shown that external bone size also plays a role in this relationship.

In conclusion, to understand what drives osteoblasts to form different matrices across the three mouse strains, we examined the relationship between bone composition and morphology. We have shown a general biological paradigm, independent of genetic background or location that regulates mineralization according to external bone size.

SIGNIFICANCE: Understanding how composition is linked to morphology has a significant impact on understanding how bone formation is physiologically regulated so that functional bones are formed across a genetically diverse population.


ACKNOWLEDGEMENTS: NIH AR056639. NSLS supported by the United States Department of Energy contract DE-AC02-98CH10886.