INTRODUCTION: Variation in bone traits in the elderly is mainly established in adulthood [1]. However, how adult variation in bone traits is established is still unclear. Studies have shown that the interaction of a specific set of cortical and trabecular traits contributes to load bearing of cortico-cancellous structures for bone mechanical function [2, 3]. Currently, there are two possible explanations to how bone traits interact - either trabecular remodeling with age leads to a coalescence of bone tissue to form the cortical shell [4] or, genotypic variation in cortical bone traits leads to an adaptive response of trabecular bone [3, 5]. To provide better insight into which traits are genetically determined "primary" variants and which are "secondary" adaptive traits, we examined the temporal sequence of bone trait development in the lumbar vertebral body of three inbred mouse strains – each with distinct genetic variation in their bone trait sets [6]. Adult A/J mice are known to have narrow/slender bones with low cortical area and trabecular volume. The C57BL/6J strain is more robust/wide, and has increased trabecular volume compared to A/J. C3H/HeJ mice are also known to have a robust bone phenotype however a high cortical area is paired with low trabecular volume. We assume the traits that mature early influence the traits that mature later during growth. Therefore, we expect that growth patterns among bone traits in these three different strains will provide insight into the underlying biology of how genetic variation in specific sets of bone traits functionally adapt to establish interactions as seen in adulthood.

METHODS: Female (n=6/strain) and male (n=3/strain) A/J (AJ), C57BL/6J (B6) and C3H/HeJ (C3H) mice were purchased from Jackson Laboratory at 6-8 weeks of age and used to establish breeding colonies. The Institutional Animal Care and Use Committee approved handling and treatment of animals. Female and male vertebral were harvested from each mouse strain at 1, 7, 14, 28, and 112 days of age (n=5/age/strain). Vertebrae were fixed with 10% phosphate buffered formalin then prepared for plastic embedding using polymethylmethacrylate resin. L3 vertebrae were sectioned along the mid-transverse plane and L4 vertebrae were sectioned in the mid-coronal plane. Sections were polished to 300-micron thickness in the mouse lumbar vertebral body. At 7-10 days of age, RCA was determined using a two-way ANOVA and Students' t-test. Overall significance was set as p<0.05.

RESULTS: Temporal analyses of postnatal development showed that variation in trabecular traits preceded the variation in cortical bone traits in the mouse lumbar vertebral body. At 7-10days of age, RCA was indistinguishable among the different strains (Figure 1A). However, at 7 days, trabecular BV/TV varied significantly among the strains in a manner consistent with the differences observed by adulthood (112 days) (Figure 1B). Throughout growth, BV/TV was highest in B6 mice, intermediate in AJ mice, and lowest in C3H mice. At 14days of age, values of RCA began to differentiate into characteristic cortical traits as seen at in adulthood. Morphological images of AJ, B6 and C3H lumbar vertebral body along the mid-coronal plane showed trabecular volume at 7 days was similar to that at 112 days (Figure 2). At 7 days, endochondral ossification regions along the lateral sides and superior and inferior ends of the vertebral body allowed for bone expansion and later development of cortical traits as seen in adulthood. Therefore, the data suggested that variation in genotype-specific trabecular traits were established earlier in bone development, which was later followed by adaptive changes in cortical bone.

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