SIGNIFICANT VARIATIONS IN BONE MASS BETWEEN INBRED STRAINS OF MICE ARE EVIDENT DURING EMBRYONIC DEVELOPMENT

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Introduction: Identifying the factors contributing to variations in peak bone mass are important for reducing fracture risk. The influence of genetic and environmental factors on peak bone mass are not fully understood. When during growth and development do these differences in bone mass arise? Significant variations in external morphology between inbred mice (C57BL/6J, C3H/HeJ, and CBA/J) have been reported at different stages of embryonic development [1]. Others reported variations in peak bone mass of a similar group of inbred mice at four months of age [2]. These studies suggest that the differences in adult peak bone mass are present at birth. Subsequent analyses revealed these differences in bone mass to be variations in cross sectional geometric parameters such as area and moment of inertia [5]. It is important to understand how these variations arise because these two traits play important roles in whole bone mechanical function. In this study, we tested the hypothesis that variations in bone geometry (area and distribution) between inbred strains will be apparent during embryogenesis. We tested for differences in morphology of the femurs, vertebrae, and calvaria from A/J, C57BL/6J, and C3H/HeJ inbred strains. These three strains were chosen because of known differences in adult bone cross sectional geometry [5]. We propose to examine the femur, calvaria, and vertebrae since these bones are derived from different cell populations (lateral plate mesenchyme for the femur and ectomesenchyme for the calvaria and vertebrae). In this study, we present the femur data.

Methods: Embryos at specific gestation dates were generated via timed pregnancies of inbred strains of A/J, C57BL/6J, and C3H/HeJ mice. The presence of a vaginal plug determined gestation day 0.5 (E0.5). Pregnant females were sacrificed at E14.5, E16.5, E18.5, and P1 (Postnatal Day 1). At least 2 sets of embryos were generated per strain per gestation date. All mice were raised under identical conditions and handled according to the institute's guidelines. After euthanizing, one set of embryos were prepared for analyses of external dimensions using alcian blue and alizarin red [4]. For assessment of cross sectional morphology, the femurs, spine, and calvaria from a second set of embryos at each gestation date were fixed in 10% neutral buffered formalin, dehydrated, cleared, and embedded in poly-methylmethacrylate. Samples were ground, polished, stained with toluidine blue, and imaged. The cross-sectional area, the periosteal and endosteal diameters, and the polar moment of inertia were quantified. The polar moment of inertia is a measure of the distribution of mass in space and is independent of orientation. This procedure was conducted every 200-300 microns through the length of the femur. The embryonic cortex consisted of a trabeculated structure. The geometric parameters were calculated over the entire region of the trabeculated tissue such that the area and the moment of inertia would be apparent values and not exclusively of the bone itself. The trabeculae in the narrow region was not included in the analysis since this will be resorbed. Morphological properties were compared using 1-way ANOVA with n=5 per inbred strain at E18.5 and at P1.

Results: The results from the alcian blue and alizarin red staining (data not shown) revealed qualitative differences in the sizes of the thoracic cavity and relative amounts of hyaline cartilage to bone in the thorax between the three inbred strains. C3H/HeJ consistently exhibited the widest thoracic cavity at E18.5 and P1. An examination of the femurs revealed that periosteal diameters of the C3H/HeJ and the C57BL/6J were similar but that A/J exhibited a smaller periosteal diameter. These results revealed gross differences in external skeletal morphology between the three inbred strains. The cross-sectional morphology has been assessed for E18.5 and P1 to date (Fig. 1). Significant differences in femoral cross-sectional area and polar moments of inertia were observed between the three inbred strains E18.5 and P1 (p<0.05, ANOVA). C57BL/6J exhibited significantly greater area and polar moments compared to the other two strains (Fig. 2). No differences in area or polar moment of inertia were observed between A/J and C3H/HeJ. These results revealed significant variations in geometric parameters between strains that were not evident from examination of external skeletal morphology.

Discussion: Significant variations in femoral cross-sectional morphology were observed between three inbred mice known to have variations in adult bone morphology [5]. These results suggest that variations in peak bone mass originate during embryonic development. Furthermore, these results suggest that differences in peak bone mass may arise from differential gene expression (spatial-temporal and levels) during embryogenesis [1]. These relative variations in bone morphology exist during embryonic development but do not fully account for the differences observed at peak bone mass. The cross sectional morphology at peak bone mass is a result of variations in geometry at birth, as shown here, plus variations in morphology imposed during growth and development. Adult A/J mice have femurs with relatively small cross-sectional area and moment of inertia, consistent with observations at E18.5 and P1. Adult C57BL/6J mice have femurs with a small cross-sectional area (same as A/J) but large moment of inertia (like C3H/HeJ). However, at E18.5 and P1, C57BL/6J exhibited relatively large area and moment of inertia. Morphologically, at E18.5 and P1, C3H/HeJ and A/J are comparable, but based on the results at peak bone mass, C3H/HeJ may exhibit greater cortical growth rates than A/J during growth and development. It is likely that these initial differences in bone cross sectional geometry may have an effect on growth and development.

These differences in bone morphology may originate as early as mesenchymal condensation, chondroblast differentiation [4], or osteoblast differentiation [1] during embryonic development. It is unclear whether bone/cartilage interactions in early endochondral ossification during embryonic development play a role [3] in determining long bone cross sectional geometry. During growth and development, these differences may be a result of variations in the rates of modelling and remodelling that occur in cortical bone.

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