Accelerated Growth Plate Mineralization and Foreshortened Proximal Limb Bones in Fetuin-A Knockout Mice

Abstract

The plasma protein fetuin-A/alpha2-HS-glycoprotein (genetic symbol Ahsg) is a systemic inhibitor of extraskeletal mineralization, which is best underscored by the excessive mineral deposition found in various tissues of fetuin-A deficient mice on the calcification-prone genetic background DBA/2. Fetuin-A is known to accumulate in the bone matrix thus an effect of fetuin-A on skeletal mineralization is expected. We examined the bones of fetuin-A deficient mice maintained on a C57BL/6 genetic background to avoid bone disease secondary to renal calcification. Here, we show that fetuin-A deficient mice display normal trabecular bone mass in the spine, but increased cortical thickness in the femur. Bone material properties, as well as mineral and collagen characteristics of cortical bone were unaffected by the absence of fetuin-A. In contrast, the long bones especially proximal limb bones were severely stunted in fetuin-A deficient mice compared to wildtype littermates, resulting in increased biomechanical stability of fetuin-A deficient femora in three-point-bending tests. Elevated backscattered electron signal intensities reflected an increased mineral content in the growth plates of fetuin-A deficient long bones, corroborating its physiological role as an inhibitor of excessive mineralization in the growth plate cartilage matrix - a site of vigorous physiological mineralization. We show that in the case of fetuin-A deficiency, active mineralization inhibition is a necessity for proper long bone growth.

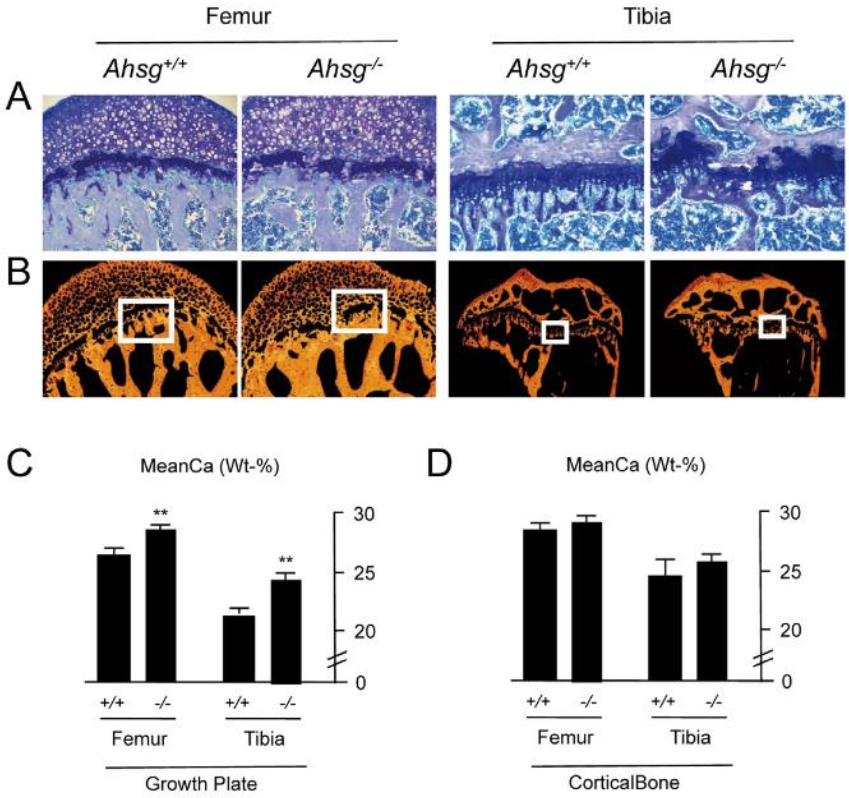

Figure 4. Growth plate morphology and mineralization in *Ahsg+/+* and *Ahsg−/−* mice. (A) Wildtype mice had dark blue stained cartilage cores within metaphyseal trabeculae of the growth plates, whereas *Ahsg−/−* had completed the cartilage-to-bone transition without the remains of cartilage cores within the trabeculae. Moreover, *Ahsg −/−* mice showed pronounced discontinuities in the chondrocyte column organization in comparison to the wildtype mice (Toluidine blue staining). (B) *Ahsg −/−* mice frequently showed thickened calcified bridge formations across their growth plates, which was confirmed by backscattered electron microscopy. The orange and black areas correspond to mineralized and non-mineralized tissue, respectively. (C) The mineral content in *Ahsg−/−* mice was significantly increased in both tibial and femoral growth plates in comparison to wildtype mice as judged by quantitative backscattered electron imaging. (D) In contrast, the mineralization (mean Ca Wt%) of the femoral and tibial cortices was similar in *Ahsg−/−* and *Ahsg+/+* mice.