INTRODUCTION

Rickets is a debilitating disease caused by deficient mineralization at the growth plate during development due to dietary lack of vitamin D [1]. Vitamin D deficiency induces structural disorganization in the growth plate [2] that results in greatly diminished bone growth rates [3] and produces bone with inferior mechanical properties [4]. Studies have documented the tensile and compressive properties of the healthy growth plate [5] and the importance of shear deformation in growth plate mechanical failure [6]. We recently characterized heterogeneous in the shear properties of healthy growth plate [7]. However, it is unknown whether vitamin D deficiency alters growth plate mechanical properties, particularly at the microscale. The goal of this study was to determine how differences in growth plate microstructure induced by vitamin D deficiency are manifested in the tissue microscale mechanical properties.

METHODS

Sprague-Dawley rats were raised for 10 weeks on vitamin D deficient (n = 8) or control (D-replete) (n = 9) diets, as described previously [8]. The proximal tibiae were removed immediately after euthanasia and frozen at -18°C. Tibiae were sectioned by 2 sagittal cuts slightly medial to the center of each bone. The cut section was trimmed to obtain a parallelepiped 4 mm anterior/posterior, 5 mm longitudinal and 1.5 mm medio/lateral, with bone retained on both the epiphyseal and metaphyseal sides (Fig. 1A). Samples were treated with 7 µg/ml 5-DTAF for 2 hours to stain the extracellular matrix. Samples were then mechanically tested in a tissue deformation imaging stage mounted onto a confocal microscope. Samples were imaged at 10x magnification while cyclic shear strain (γω) was applied in the anteroposterior direction at a frequency of 100 mHz and an amplitude of 64 µm.

Two-dimensional maps of shear strain were calculated using digital image correlation analysis on videos of the deforming samples. Previous work using this technique [7] indicated shear strain induces slip between neighboring chondrons, resulting in a distinct spatial pattern in the induced shear strain in the direction of growth (γω) (Fig. 2). To measure the extent of this spatial periodicity, fast Fourier transform (FFT) analysis was performed on the 2D shear strain maps (Fig. 3). The characteristic spatial period of γω in the growth plate was calculated by dividing the size of the region of interest by the mode of the 2D FFT of the strain map.

RESULTS

In both control and vitamin D deficient growth plates, applied shear strain in the anteroposterior direction (γω) induced significant shear strain in the direction of growth (γω) concentrated in bands located in the interterritorial matrix between chondrons (Fig. 4). Growth plates from the control group exhibited highly periodic chondron structure and mechanical properties in the proliferative and hypertrophic zones (Fig. 3A), supporting previous findings [7]. In contrast, the mechanical properties of the proliferative zone of the vitamin D deficient growth plates were less periodic and more variable (Fig. 3B). The average spacing of peak strains in vitamin D deficient growth plates was twice as large as controls, and the standard deviation was 6 times larger. Peak strains in vitamin D deficient growth plates were twice those of controls.

DISCUSSION

This study demonstrates that the growth plate mechanical response to shear loading at the microscale is disrupted by dietary vitamin D deficiency in a manner consistent with the known structural changes caused by the disease. Greater variation in chondron size, orientation, and alignment caused by vitamin D deficiency are manifest as a disruption of the spatially periodic mechanical response of the growth plate to shear loading. However, in vitamin D deficient animals, chondrons were still found to be surrounded by a highly compliant interterritorial matrix (Fig 4B), allowing for substantial intercolumnar slipping. This effect, combined with known changes in columnar growth rates induced by vitamin D deficiency [9] may be an important mechanism contributing to growth plate disorganization in this disease.

SIGNIFICANCE

This study reports that vitamin D deficiency induces changes in the microscale shear properties of the growth plate consistent with known changes in tissue microstructure, thus enhancing understanding of the mechanism of the disease.

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