EFFECT OF VITAMIN D RECEPTOR ON BONE GROWTH AND STRENGTH DURING GESTATION

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INTRODUCTION

Increased demands are placed on calcium regulating channels during certain stages of the female reproductive cycle. Pregnancy and lactation in particular substantially alter mineral metabolism to meet the demands of the mineralizing fetus and to supply calcium for the production of milk. This dynamic alteration during pregnancy has not been shown to result in noteworthy bone loss or net effect on mechanical properties, although a positive calcium balance exists [1]. However, the role of vitamin D receptors in calcium metabolism during pregnancy and lactation is not fully understood. Vitamin D is an important regulator of calcium metabolism. Lack of vitamin D results in Rickets due to abnormal mineralization of bone. Mice without vitamin D receptor genes (VDR -/-) normally perish in infanthood. A hypercalcemic diet is needed to provide normal skeletal growth [2].

The objective of this study was to determine the role of vitamin D in skeletal maintenance during pregnancy. Specifically, the objectives were to 1) measure the femoral cortical bone area and moments of inertia 2) determine the maximum bending strength, and 3) compare these parameters for wild type and vitamin D receptor knockout mice at two stages of pregnancy.

METHODS

This study was performed on 10 wild type (+/+) and 10 VDR knockout (-/-) mice. The mice were taken from a larger study, and all protocols were approved by an institutional review board. Mice were fed a diet containing 2% Ca, 1.25% phosphorous, and 20% lactose with 2.2 IU vitamin D_3/g. Four groups of five mice were sacrificed at 16 or 9 days of pregnancy, followed by storage at -80C. Before testing, each animal's right femur was dissected and all soft tissue was removed. Bones were then wrapped in gauze, saturated in PBS and stored in closed plastic tubes at –40C prior to testing.

All femora were scanned using a micro-computed tomography scanner (μ-CT 80, Scanco Medical, Zurich, Switzerland). The bones were placed with the distal end downwards in a small tube allowing reproducible vertical alignment of the bones within the scanner. Thirty images, evenly spaced along the entire length of the femur, were scanned at a resolution of 10 microns in plane. Image data was analyzed using Matlab v. 6.0 imaging processing toolbox (The Mathworks, Natick, MA). A Gaussian filter was used to suppress image noise. To separate cortical bone from marrow and the surrounding saline solution, a thresholding process was used. The centroid, major and minor moments of inertia, and the total bone area were determined for the femoral diaphysis.

Femora were kept hydrated in PBS and thawed to room temperature before biomechanical testing began. Three-point-bending tests were performed by placing the femora on rounded supports with a constant span length of 5 mm. The femora were positioned with the condyles facing down [2]. Loading was performed perpendicular to the longitudinal axis of the diaphysis using a materials testing system (MTS, Eden Prarie, MN) at a rate of 10 mm/min [2]. Displacement and load were recorded using standard software. Maximum strength and maximum deformation were determined directly from the measurement. The bone area, moments of inertia, and strength were compared between the four groups by analysis of variance using Statview software (SAS Institute, Cary, NC). A Fisher LSD post-hoc test was used to detect differences between groups with a significance level of 0.05.

RESULTS

The cortical area was greater in the wild type mice than in the knockout mice at 16 days of pregnancy (Fig. 1). The minimum bone area of the 9 day wild type mice was 0.829 ± 0.059 mm^2 compared to 0.842 ± 0.08 mm^2 in 9 day pregnancy VDR knockout mice. The area of the 16 day wild type mice was 0.979 ± 0.045 mm^2 which was 14% greater than that of the

16 day knockout area of 0.8588 ± 0.048 mm^2 ($p<0.05$) and was also greater than both of the 9 day pregnancy groups ($p<0.05$).
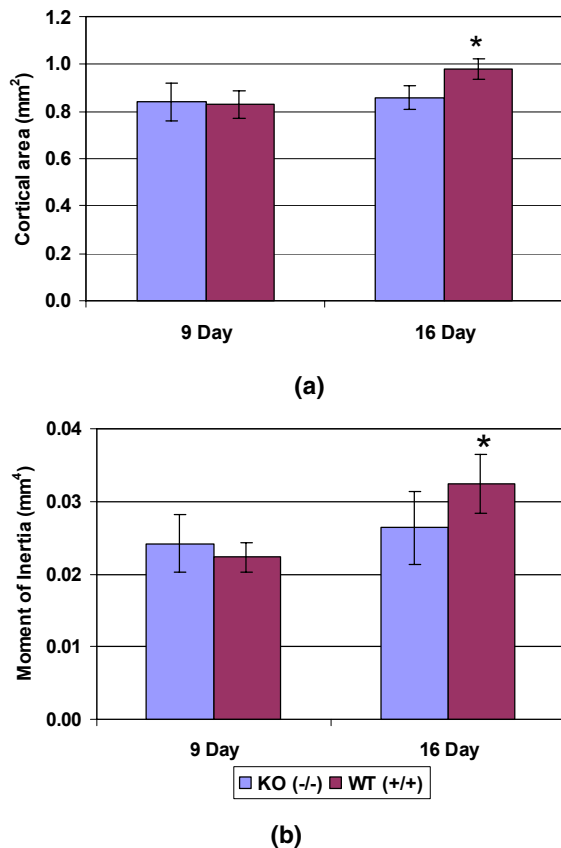

(a)

(b)

Figure 1: a) Cortical area and b) major moment of inertia at the smallest cross-section of the bone (* indicates significant differences over all other groups)

The major area moment of inertia also increased in the wild-type mice at 16 days of pregnancy, and was higher than that in the knockout mice (Fig. 1b). The major moment of inertia for the 9 day wild type mice was 0.02232 ± 0.002 mm^4 and for the VDR knockout mice, 0.02422 ± 0.004 mm^4. For the 16 day wild type mice, the major moment of inertia was 0.03242 ± 0.004 mm^4 which was a 22.99% increase over the VDR knockout 16 day pregnancy value of 0.02636 ± 0.005 mm^4 ($p<0.05$) and was also an increase over the 9 day pregnancy groups ($p<0.05$).

The maximum bending force was higher in the wild type mice than in the knockout mice at 16 days pregnancy, but the difference was not significant ($p=0.1$, Fig. 2). The maximum bending force for the 9 day pregnancy wild type group was 5.980 ± 0.683 lbf, compared to the VDR knockout 9 day bending force of 5.914 ± 0.812 lbf. In the 16 day pregnancy wild types, the maximum bending force was 6.776 ± 0.621 lbf and for the VDR knockout 16 day, the force was 5.664 ± 0.738 lbf.

DISCUSSION

The absence of the vitamin D receptor affects the growth and adaptation of long bones during gestation. There are significant increases in bone cross-sectional area and in major moments of inertia during the gestation period for wild type mice that do not occur in vitamin D receptor knockout mice. Previous studies have indicated that periosteal bone formation rates increase at the end of pregnancy in rats [3], which may have led to the greater cross-sectional area observed in the wild type 16 day pregnancy mice. However, the VDR knockout mice did not have a corresponding increase in cortical cross-sectional area. Differences in the maximum bending load were also observed, but were not statistically significant. This suggests that bone growth and development during pregnancy is regulated by the vitamin D receptor.

One limiting factor in this study was the additional calcium supplementation in the mouse diet. This hypercalcemic diet is required for the knockout mice to reach maturity [2], but may not be necessary for normal adult calcium homeostasis. The lack of age matched non-pregnant controls was also a limitation. However, previous studies indicate no net changes in bone mechanical properties due to pregnancy [1].

Overall, this study indicates that vitamin D is an important regulator of bone growth during pregnancy. Future studies will address skeletal maintenance during adolescence and lactation.
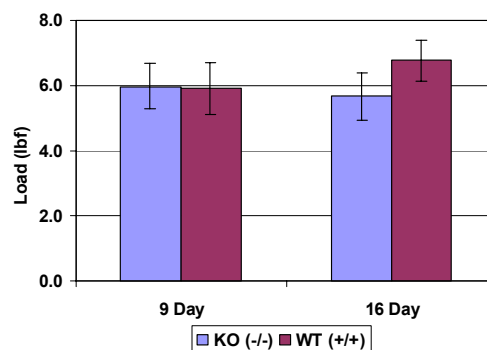

Figure 2: Maximum bending force

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