The Effects of Pregnancy on the Mechanical and Material Properties of Bone

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Introduction

Pregnancy affect's a woman's body due to hormonal changes, anatomical changes, and so on. One of the organs impacted is bone. It is known that pregnancy affects bone mineral density [1], which can increase bone fracture risk and osteoporosis.

Changes in hormone levels such as progesterone and estrogen have been shown to cause changes in ligament laxity of collagen, which can potentially affect bone [2,3,4]. Parathyroid hormone (PTH) is also known to fluctuate throughout pregnancy, affecting bone metabolism [5]. Due to the limited number of studies investigating pregnancy, and that none have investigated bone mechanical and material properties, this study investigates these properties.

Methods

Study Design

All animal work was performed under IACUC approved protocols of The College of New Jersey. In this study, Pregnant CGS rats (n=24) on Day 18 of pregnancy were euthanized for another study. Control CGS rats (n=15) were age matched. Both groups had both hind limbs were removed and frozen prior to testing.

Biomechanical Testing

After thawing, soft tissue was removed from the femur, and then soaked in 0.1% PBS overnight prior to testing. An Instron Universal Testing System (Model 5967, Instron, Norwood, MA) was used for three-point bend testing until failure. A constant span of 15 mm was used, and the femur was loaded at a rate of 0.02 mm/s in the middle of the anterior shaft (Figure 1). Force and deflection data were taken during the experiment and used to calculate the linear stiffness, max load, failure load, and energy to failure using MATLAB (Mathworks, Natick, MA).

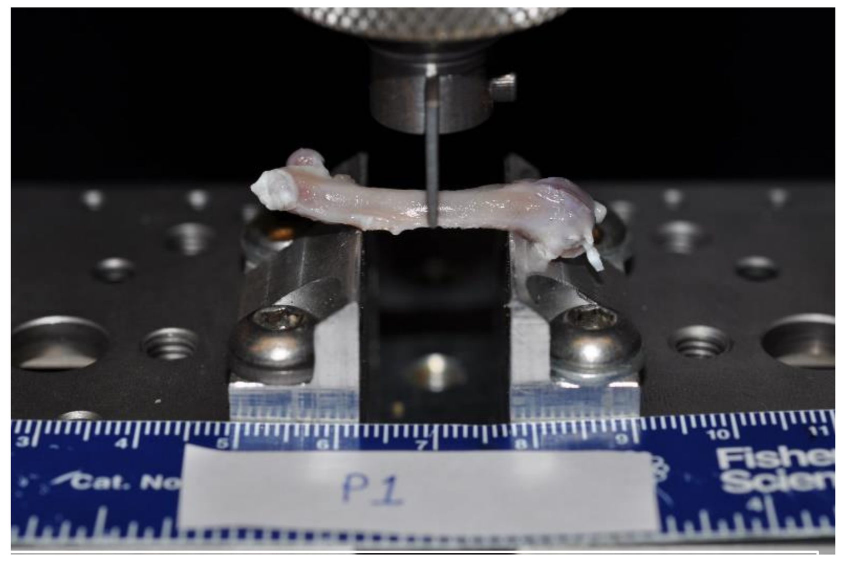

Figure 1: Three-Point Bend Testing Set-up.

Embedding and Polishing

The samples were dried and then embedded in epoxy resin. The resulting disks were cut with a diamond saw and polished with a series of increasingly fine grit papers, followed by a polishing cloth with 0.04 µm silica suspension (Figure 1).

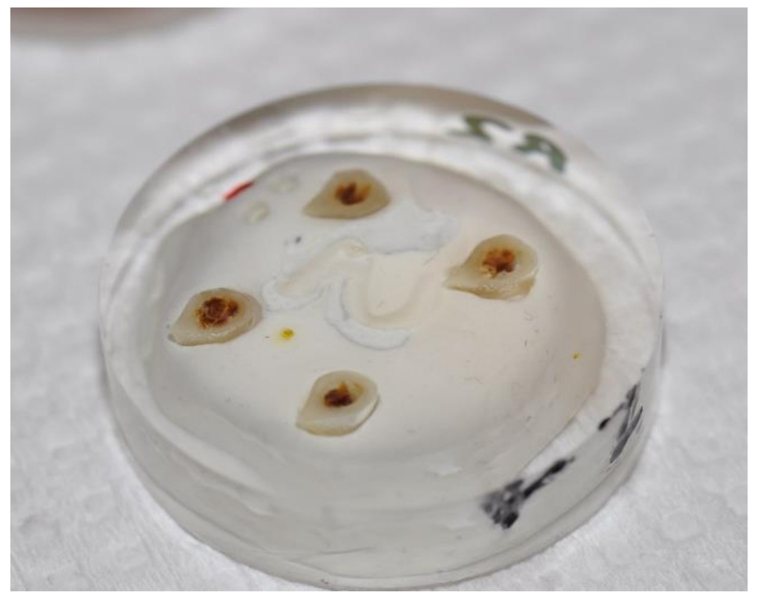

Figure 2: The polished and embedded samples in an epoxy disk.

Indentation Testing

A spherical micro-indenter outfitted with a DinoLite microscope was used. At each anatomical location (Figure 3), where areas to be indented were picked based on the local porosity. A 150 µm radius ruby tip was used. The target depth was 15 µm with a 30 second hold. Force-displacement data was measured with a 10 N load cell (0.1 N resolution) at a sampling rate of 200 Hz. Samples were rehydrated prior to testing, which would aim between the pores. The resulting force-displacement data was analyzed by fitting the force relaxation response to a 3-parameter viscoelastic Maxwell solid to obtain the instantaneous and relaxed shear moduli [6,7].

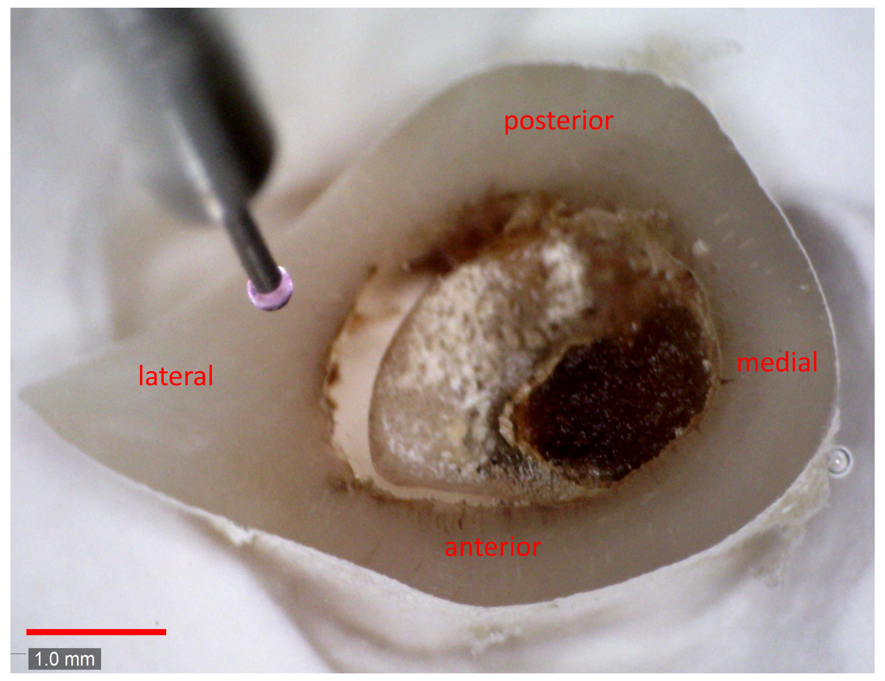

Figure 3: A micro-indentation test on the lateral area of the specimen

Statistical Analysis

The resulting properties were statistically analyzed using a standard least squares model. For the mechanical properties, the limb and the treatment were considered fixed effects, whereas the rat was considered a random effect. For the material testing, the limb, the location, and the treatment were fixed effects, whereas the rat was a random effect. Effect tests and post-hoc Tukey tests were performed for each interaction.

Results

Biomechanical Testing

The treatment was significant for the linear stiffness, maximum load, failure load, and energy to load (p=0.004, <0.001, 0.002 and 0.009 respectively). The limb did not influence any of the four properties. For each of these properties, the bone strength of the pregnant samples was higher than the controls (Figure 4).

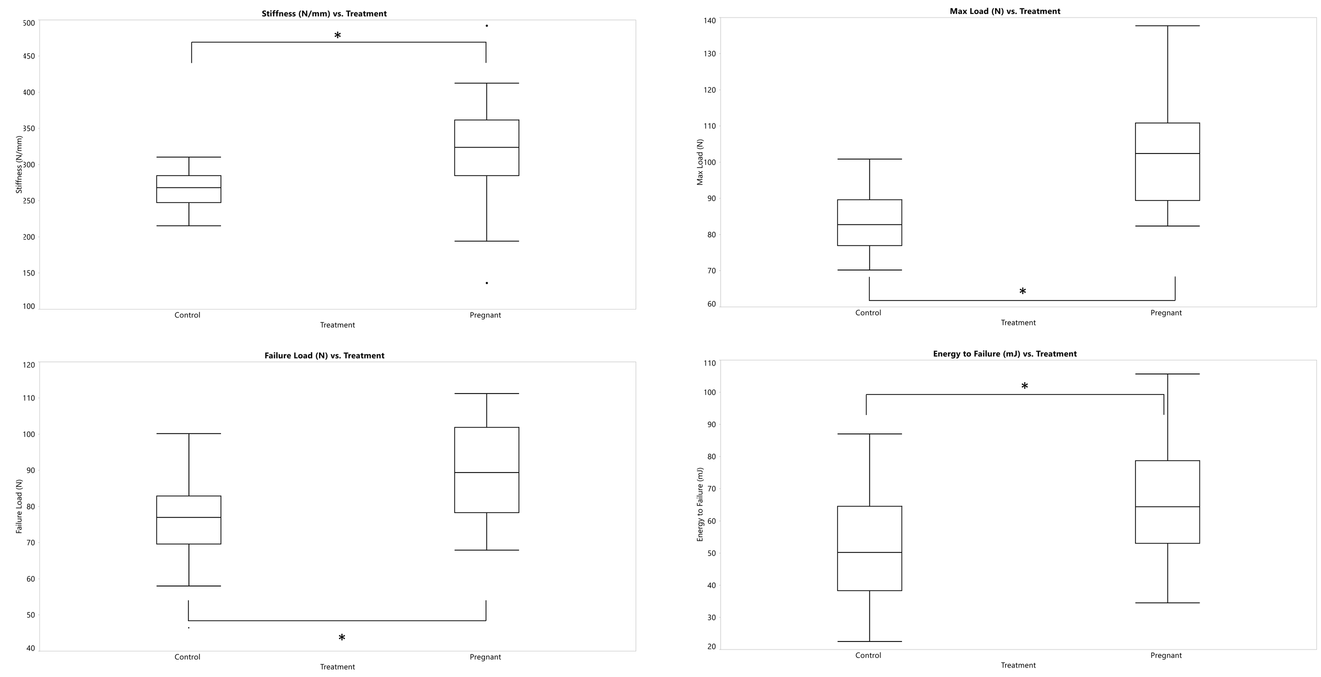

Figure 4: Top Left: Stiffness vs. Treatment. Top Right: Max Load vs. Treatment. Bottom Left: Failure Load vs. Treatment. Bottom Right: Energy to Failure vs. Treatment.

Material Testing

The relaxed shear modulus and the modulus ratio had treatment as a significant effect (p=0.022 and <0.001 respectively) (Figure 5). Treatment was not significant for the instantaneous shear modulus (p=0.179). Location and limb were significant for all three properties.

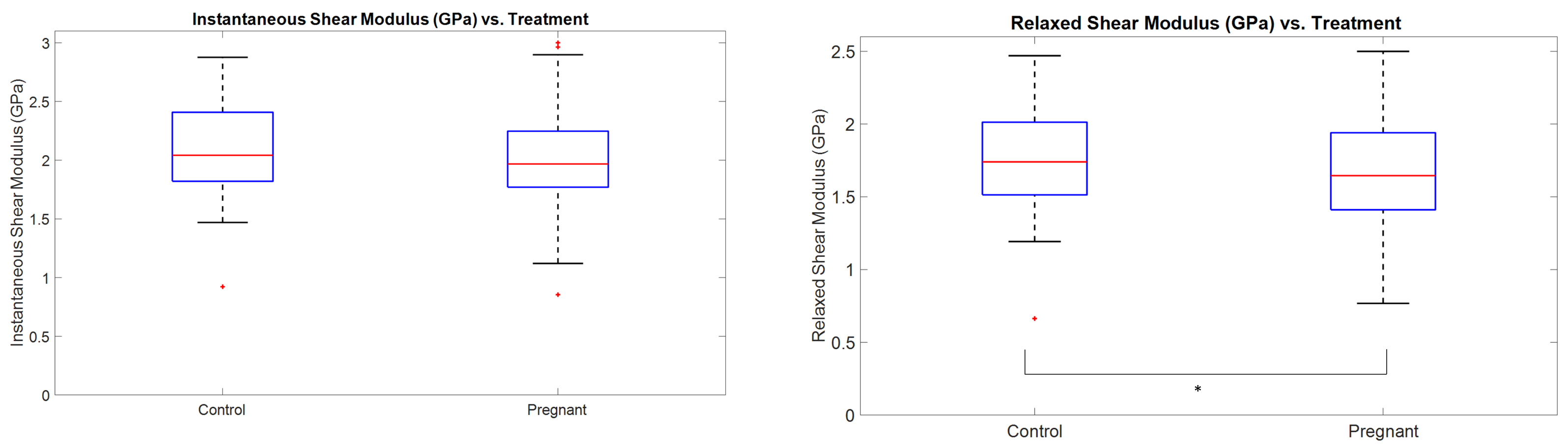

*Figure 5: Left: Instantaneous Shear Modulus (GPa) vs. Treatment. Right: Relaxed Shear Modulus (GPa) vs. Treatment. * signifies significance.*

Discussion

The three-point bend testing investigates both tensile and compressive elements at the structural level, whereas the spherical micro-indentation testing only investigates the compressive elements at the material level. Spherical micro-indentation is not affected by factors such as animal weight or changes in the overall bone anatomy, both of which are possible factors that could affect three-point bend testing.

Spherical micro-indentation primarily investigates the inorganic portion of the bone. Due to the relaxed modulus, and therefore ratio, being the only properties affected, it is likely only the organic matrix affected. The instantaneous modulus would likely show the inorganic crystalline structure being affected by factors such as hormonal changes due to pregnancy but was not affected. It is suspected that the organic portion of the matrix is responsible for the lower relaxed shear modulus with increased laxity due to higher levels of progesterone and estrogen [2,3,4]. PTH levels also change during pregnancy, affecting bone turnover and metabolism, increasing standard deviation within bone properties.

One limitation of this study is within the three-point bend testing. Due to the nature of how the pregnant samples were obtained, it was not possible to obtain the animal weights. With pregnancy, the animals put on weight, which by Wolff's Law, will increase bone structural strength. In future studies, finite element analyses, histology, and bone ashing should be done to investigate any potential changes in bone porosity or changes in the ratio of inorganic to organic matrices within the bone. These studies would provide additional information regarding bone microarchitecture. Additional material testing, such as microhardness testing, should be completed at a smaller length scale in order to minimize the effects of porosity, which are present in spherical-micro-indentation. Additional investigations into the changes of bone over the course of pregnancy would provide data about possible mechanisms as well.

Conclusions

Pregnancy does have an impact on bone at both the structural and material levels. Bone properties from three-point bend testing show an increase in strength and is likely due to changes in the organic matrix portion of the bone. Changes in the inorganic matrix were not as apparent, as spherical micro-indentation showed that the instantaneous shear modulus was not shown to significantly change, whereas the relaxed shear modulus and the modulus ratio were shown to change. It is hypothesized that the organic matrix is the portion of bone primarily affected by pregnancy, however, the inorganic matrix is also mildly affected, as seen with a wide standard deviation among samples.

Acknowledgements

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References

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