

Introduction

One of the many missions of NASA is to develop a safer agent of space travel for astronauts. According to NASA, the average astronaut will be exposed to anywhere between 50 and 2,000 mGy of effective radiation. In a more relative manner, this is about 150 to 6,000 chest x-rays [1]. Space radiation is known to induce DNA damage that can lead to a number of health defects [2]. Galactic Cosmic Radiation (GCR) is a primary type of radiation seen during space travel that comes from the milky way. Radiation exposure has a direct relationship with how far away astronauts are from the Earth [1], so as space travel becomes more intense so does radiation exposure for astronauts. The objective of this study is to use micro-indentation to observe the material properties of Long Evans Rats bone that have been treated with GCR with respect to dosage, sex, and indentation location.

Methods

Study Design

Animal work within the study followed IACUC approved protocols of Johns Hopkins University and Brookhaven Laboratory. A cohort of Long Evans rats (n=6, 4 female and 2 male) were irradiated with 50cGy of protracted GCR at the NASA Space Radiation Laboratory at Brookhaven National Labs, along with corresponding sham controls (n=6, 2 female and 4 male). These rats were then euthanized 90 days post irradiation. Hind limbs were collected at The College of New Jersey then stored in 70% ethanol.

Embedding, Cutting and Polishing

Hind limbs were then dried and embedded into epoxy resin. Each puck was cut using a low-speed diamond saw. The bone pucks were then polished using various grit sandpapers, starting with 320grit using water and elevating to cloth with 0.04um silica suspension and water.



Figure 1,2: Photos taken during the process of cutting the bone pucks with the diamond saw.



Indentation testing

Spherical micro-indentation was used to measure the material properties of the bones in the study; this was chosen in order to avoid damage to the specimen. A .3mm diameter ruby tip is used to perform the test and each specimen is indented in four anatomical locations; lateral, anterior, posterior, and medial. The indentation sequence contained a 10um depth at a 45 second hold. Specific locations for indentation was based on the homogeneity of the area and additional indents were performed based on significant deviances of peak force and greatest contact force. Prior to testing bones were hydrated in saline for 24 hours and remained hydrated during testing by creating a meniscus with a dropper and saline.

Using a MATLAB code, the results for each test are fitted into a 3-parameter viscoelastic Maxwell model and the instantaneous and long-time shear modulus were collected in order to examine the stiffness of a specimen.



Figure 3: Photo taken with the dino-lite of micro-indenter tip and rat bone.

Statistical Analysis

Data was analyzed using a statistical linear mixed model approach.

Results

There were no statistically significant differences observed for bone material properties found between sham and irradiated when considering the long-time ($P=0.686$) and instantaneous ($P=0.292$) shear moduli.

There was a statistical difference between the anatomical positions in which the bone was indented when considering instantaneous shear modulus ($P=0.033$). There was no statistical significance when considering long-time modulus, although a small p-value ($P=0.102$) was found. A post hoc tukey test was performed on the individual anatomical positions, this demonstrated that each anatomical position within instantaneous and long-time shear moduli had significance or traces of trends of statistical differences when being compared to the medial region of the bone. Statistical significance was found within instantaneous shear modulus when comparing the medial and posterior region ($P=0.032$). Small p-values were also found within instantaneous shear modulus when comparing the medial and anterior ($P=0.080$) along with medial and lateral ($P=0.214$). When observing long-time modulus, small p-values were discovered when comparing medial and anterior ($P=0.122$), medial and lateral ($P=0.156$), along with medial and posterior ($P=0.272$).

Trends in sex differences were found in both long-time ($P=0.338$) and instantaneous ($P=0.133$) shear moduli. These trends are demonstrated in the box and whisker plots below (Figure 3.).

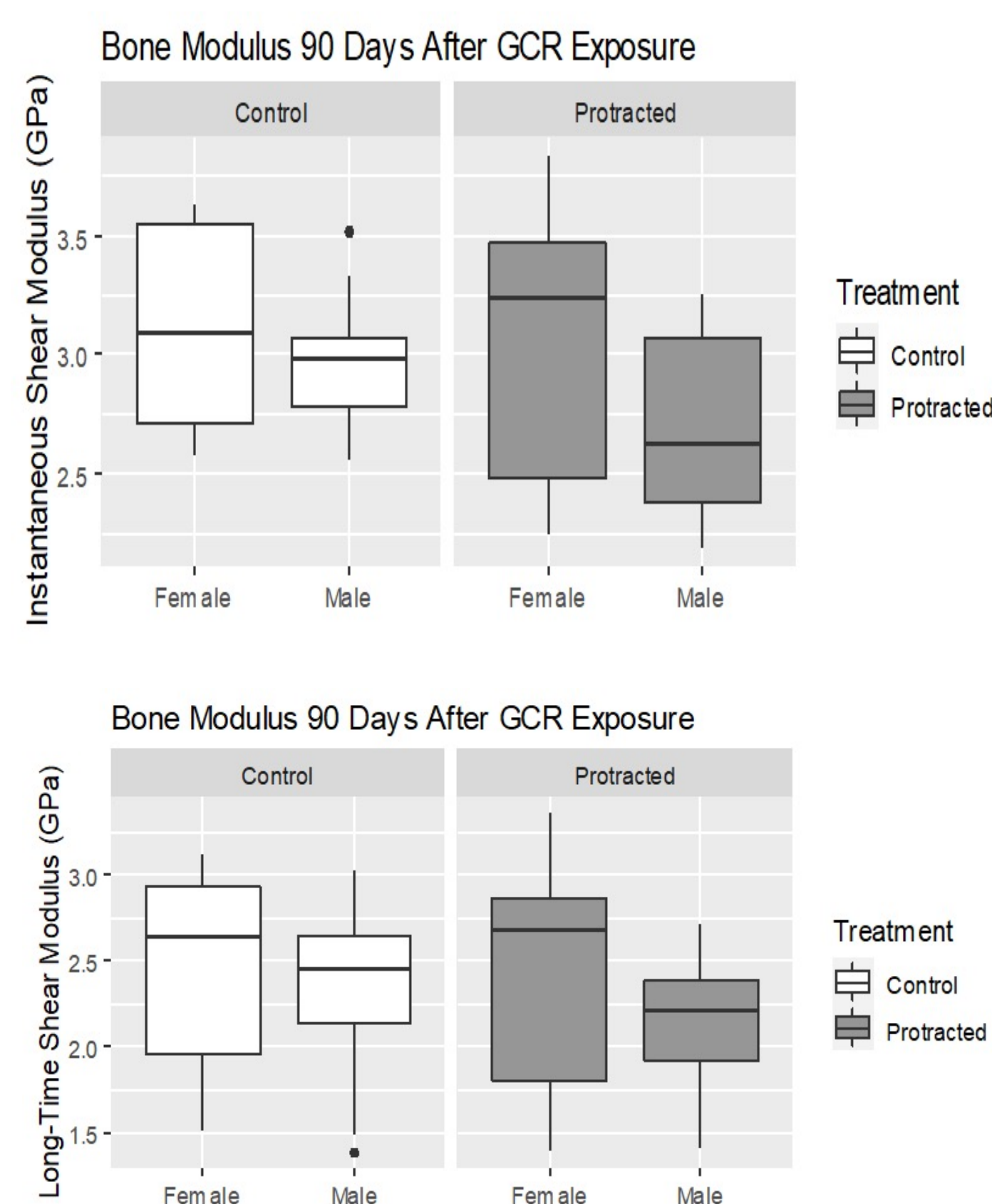


Figure 4: Box and whiskers (mean with 25th and 75th percentiles) plots for instantaneous(top) and long-time(bottom) shear moduli of bone for both female vs male rats.

Discussion

The lack of statistical power is due to the small sample size. The remainder of this animal cohort was delayed due to COVID-19. Due to infinite half-space and homogeneity assumptions, any observable porosity in the cortical bone were avoided. Avoiding these pores could confound the bone material quality, especially if radiation increases porosity.

According to the findings, the medial region of the bone resulted in larger material bone properties when being compared to the other regions of the bone (anterior, posterior, and lateral). Since the medial is one of the thinnest areas of the bone, the assumption of infinite half-space may not have always been met. This can contribute to the differences seen in the medial regions of the bone compared to the other regions.

The study presented that females had higher instantaneous and long-time shear moduli when compared to males, in other words, the females bone material properties were stronger upon testing. The trends in radiation effects between male and female correspond with a previous study that suggests that estrogen-containing specimens develop less severe cataracts in response to ionizing radiation when compared to specimens containing no estrogen [3].



Figure 4: Rat femur under microscope taken while examining porosity of each bone.

Conclusion

As NASA works to man a mission to mars it is important that the effect of radiation on the human body is studied. Using spherical micro-indentation, the material properties of rat femurs exposed to sham and 50cGy of protracted GCR were studied. Results concluded a trend in differences between specific anatomical positions, the medial region of the bone was seen to have a relative difference when compared to other anatomical positions. Trends were also seen when comparing male to female material bone property strength, females having stronger material properties. Future work looks to include a large sample size in order to increase statistical power, as well as dive deeper into studying the relationship between sex and radiation exposure. Studying the effects of protracted and acute radiation is also a field that future work hopes to include.

Acknowledgements

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References

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