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BULK PROPERTIES OF THE MURINE SPINE ARE MAINTAINED DURING 30-DAYS OF MICROGRAVITY ON THE INTERNATIONAL SPACE STATION

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INTRODUCTION

As NASA prepares to send astronauts on longer duration spaceflight missions, understanding the detrimental effects of microgravity on human health is important. It has been well established that spaceflight negatively affects bone, with bone loss up to 1-2% for every month in space [1, 2]. Furthermore, astronauts are 4.3X more likely to experience lumbar disc herniation after returning to Earth [3]. While measures have been established to counteract bone loss, there are outstanding questions regarding the impact of spaceflight on spine mechanobiology.

Previous reports from short duration missions (≤15 days) showed changes in rodent disc biochemical content and mechanical properties after spaceflight [4-7]. However, the impact of longer duration microgravity on disc biomechanics is not well understood and may provide insight into elevated risk of herniation after spaceflight. Thus, the primary aim of this study was to examine murine disc biochemical content and lumbar vertebral body microstructure after 30-days on the International Space Station (ISS).

METHODS

Rodent Research-10. Lumbar spines from the Rodent Research-10 (RR-10) mission were collected as part of a collaboration with the RR-10 Investigator Team. Mice (B6129SF2/J) were 16 weeks at launch (day 0) and included four groups: a baseline control (BL, euthanized on day 0, n=10), a ground control (GC, Rodent Research housing on Earth, n=10), a vivarium control (VIV, normal mouse housing on Earth, n=12), and a spaceflight experimental group (SF, n=10). After 30 days, mice were euthanized in orbit and carcasses were flash frozen and returned to Earth for dissection. Lumbar spines were collected and stored at -80C until processing for biochemistry and µCT imaging.

Biochemical Analysis. The L1-L2 discs were removed and refrozen at -80C. Frozen discs (n=8-10/group) were lyophilized overnight before digestion in papain. Digests were evaluated for DNA content with the PicoGreen Assay, sulfated glycosaminoglycan (GAG) content with the Dimethylmethylene blue (DMMB) assay, and collagen content with an oxidized hydroxyproline content (OHP) assay. GAG content and OHP content were normalized to DNA content, as the weights of the small discs could not be reliably measured.

µCT. The L3-L5 lumbar columns were wrapped in saline-polyethylene glycol-soaked gauze to maintain hydration and scanned at 4.0 µm voxel size (60 kV, 166 µA, 0.25 mm aluminum filter) using a Skyscan 1272 (Bruker). The L4 vertebra was reconstructed and segmented in nRecon to isolate the middle 50% of the vertebral body. Trabecular bone was manually segmented from cortical bone, and automated analyses for bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and trabecular number (Tb.N) were conducted by CT Analyser.

Statistics. To provide robust statistical analysis against normality assumptions, a one-way ANOVA and permutation test combined approach was used. Briefly, the F-statistic generated by the one-way ANOVA was compared to a distribution of F-statistics generated from 10^3 permutations of the experimental data. Post-hoc pairwise comparisons were conducted similarly, using a combined t-test and permutation approach. A Bonferroni post-hoc adjustment was used to account for multiple comparisons. Significance was determined at $p \leq 0.05$ and data is presented as mean±standard deviation.

RESULTS

Biochemical Analysis. Spaceflight did not affect intervertebral disc DNA, GAG, or OHP contents (**Figure 1**). Discs from the spaceflight group had an average of 1771.8±294.2 ng of DNA per disc, which did not differ from discs in the baseline control, ground control, or vivarium control groups ($p>0.6$). Similarly, loading did not affect GAG or OHP contents, with spaceflight discs containing an average of 21.94 µg GAG/µg DNA and 15.59 µg OHP/µg DNA ($p>0.4$).

µCT Analysis. Similarly, spaceflight did not affect lumbar vertebral body bone microstructure (**Figure 2**), with one-way ANOVA indicating no statistically significant effects of loading on BV/TV

(p=0.17), Tb.Th (p=0.056), Tb.Sp. (p=0.44), and Tb.N (p = 0.37; **Figure 3**).

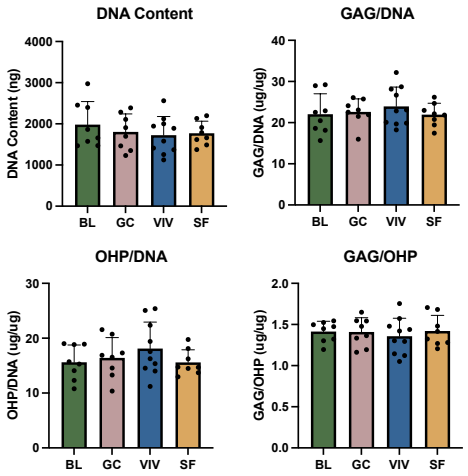

Figure 1. Disc biochemical content is not significantly affected by spaceflight (p > 0.4). BL=baseline control, GC=ground control, VIV=vivarium control, SF=spaceflight.

DISCUSSION

Results presented here differ from previously reported data. In one of the first studies on spaceflight and the intervertebral disc, Pedrini-Mille et al. reported decreases in the proteoglycan/OHP ratio in the annulus fibrosus of lumbar discs from rats flown on COSMOS 2044 (14-day flight) [4]. This was supported by a later publication from STS-48, which reported decreases in proteoglycan/OHP ratio in lumbar and thoracic discs of neonatal rats after 5 days of spaceflight [5]. While data presented here investigates GAG content, sulfated GAGs compose the major proteoglycans of the disc, and GAG content is directly proportional to proteoglycan content. Furthermore, numerous studies utilizing simulated weightlessness demonstrated decreases in disc GAG content after unloading [8, 9]. Thus, the consistency in disc biochemical content between spaceflight and control groups in this study was unexpected.

Similarly, the lack of changes in trabecular bone of the vertebral body were also surprising. While data presented here show no significant spaceflight effect on BV/TV, Gerbaix et al. report a 36% decrease in L3 BV/TV after 30-days of spaceflight on Bion-M1 [10]. Berg-Johansen et al. report similar results from the same flight in the caudal vertebrae [11]. On the contrary, a recent study from Rodent-Research 4 demonstrated less bone loss, with changes in L4 BV/TV marked as significant by two-way ANOVA, but not after pairwise post-hoc comparisons [12]. Notably, preliminary results from other weight bearing sites such as the femoral head (unpublished), show significant losses in BV/TV, suggesting that bone loss may be dependent on anatomical location and loading modality rather than spaceflight itself.

There are several factors that may contribute to differences between previous reports and observations presented here, including flight duration and animal housing. While Bion-M1 was the same duration as RR-10, Bion-M1 housing utilized smooth walled cylinders that limited ambulation [13], whereas the Rodent Research hardware has wire mesh. After 14 days in the wire mesh habitat, mice have been observed to develop a unique compensatory mechanism of "race tracking", which involves highly repetitive, fast running in circles around the habitat. Another behavior is backflipping, whereby mice somersault repeatedly [15]. Though not quantified, these behaviors were observed in the mice used for this study. Lastly, the Animal Enclosure Module (AEM), used in the space shuttle era, also utilized wire mesh, but experiments did not last longer than 15 days [14]. Given that axial spinal loading of quadrupeds is primarily due to muscle contraction, the lack of differences seen here may be explained by the compensatory mechanisms developed by mice in longer duration spaceflight (> two weeks).

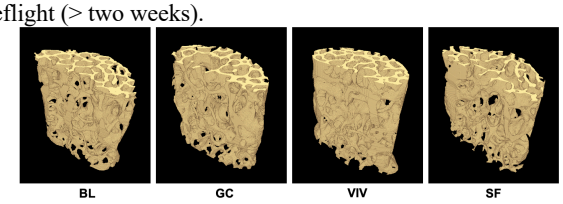

Figure 2. Representative reconstructions of lumbar vertebral body.

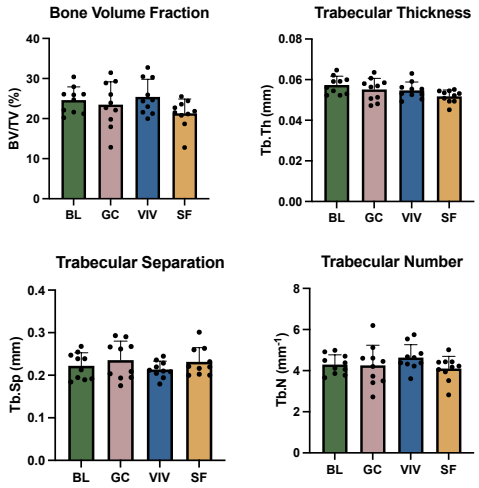

Figure 3. Vertebral body microstructure (p > 0.05).

However, as with all spaceflight studies, there are challenges that limit these findings, including a small sample size limiting statistical power. Furthermore, it is possible that other properties, such as mechanical behavior, bone remodeling rates, and other skeletal sites in the same mice experience more drastic differences with longer spaceflight.

Rodent spaceflight studies are extremely helpful in understanding the effects of microgravity on the human body, but the difference in loading modalities between quadrupeds and bipeds is critical for lumbar spine loading. Data presented here suggests that adaptation to the Rodent Research hardware after 30-days of spaceflight may reload the quadruped spine, compensating for microgravity. Further research into this topic may help clarify appropriate rodent models for understanding the impact of long duration spaceflight on spine health in humans.

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