Gene Expression and Structural Skeletal Responses to Long-Duration Simulated Microgravity in Rats

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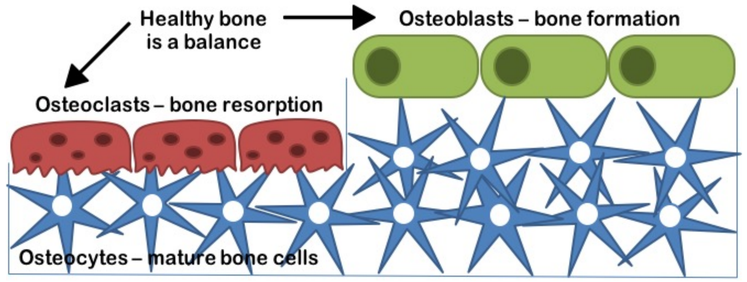
BACKGROUND



- Spaceflight environment (microgravity and radiation) result in decreased bone mass, density, and strength *[Smith et al., 2012]*
- Radiation is thought to increase oxidative damage through the production of excess ROS, which in turn may be a potential mechanism for bone loss
- Bone loss is due to the increased activity of bone-resorbing osteoclast cells and changes in bone-forming osteoblast cells
- Cancellous bone fails to recover years after spaceflight *[Carpenter et al., 2010]*

EXPERIMENTAL AIMS

(1) Understand the signaling pathways and molecular mechanisms causing bone loss due to prolonged simulated weightlessness
(2) Determine effects of prolonged simulated weightlessness on bone integrity, particularly by understanding the relationship between oxidative stress and bone turnover

HYPOTHESIS: *Simulated microgravity leads to the temporal regulation of oxidative defense genes and pro-bone resorption factors, showing progression and eventual plateau during long-term unloading, and that transient changes at early time points in these pathways precede skeletal adaptations to long-duration unloading.*

METHODS

(1) Ground-based model for weightlessness: Simulated weightlessness using hindlimb unloading (HU) model in Long-Evans rats (male, 3-months old)
(2) Experimental Design: Animals were unloaded for up to 90 days. Samples were collected from short term (7 days) and long-term time points (90 days HU with or without an additional 90 days of full weightbearing as recovery period)
(3) RNA extraction: RNA was extracted from cortical bone tissue of femoral shaft. Bones were crushed over liquid nitrogen using tissue crusher and then homogenized. RNA was extracted using TRIzol method, and purified using Qiagen spin-column purification
(4) RNA quality and qPCR: RNA quality was tested using Nano-Drop. For qPCR, subset of samples were selected based on animals' body mass.

RESULTS: RNA Quality Assessment & Quantitative PCR Analysis

Pilot: RNA concentration and quality by Nano-Drop and BioAnalyzer

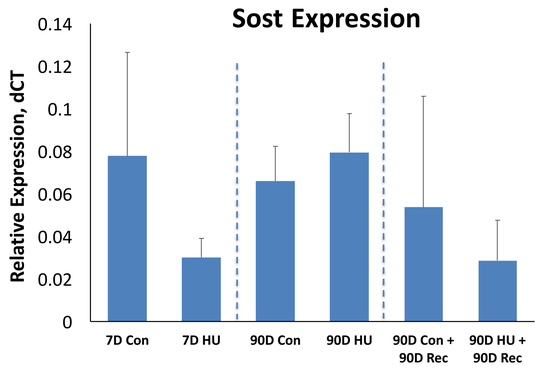
RNA Extraction Results (individual values)			
Treatment	**Nucleic Acid Concentration (ng/μL)**	**260/280**	**RIN**
HU 7 Days	340.6	2.1	9.8
	341.0	2.1	9.9
	203.0	2.1	8.5
Control 7 Days	197.4	2.1	6.5
	232.1	2.1	9.5
	149.4	2.1	9.5
HU 90 Days	98.7	2.0	<4.0*
	294.0	2.1	9.6
	81.1	2.0	9.2
Control 90 Days	270.9	2.1	7.2
	215.9	2.1	9.5
	122.8	2.0	9.6
HU 90 Days + Recovery 90 Days	294.0	2.1	9.2
	81.9	2.0	10.0
	72.6	2.0	9.3
Control 90 Days + Recovery 90 Days	119.0	2.2	9.7
	145.2	2.0	9.7
	177.2	2.1	9.7

All samples from the femoral shaft of male, 3-month old rats.
*Technical artifact, RNA quality was acceptable.

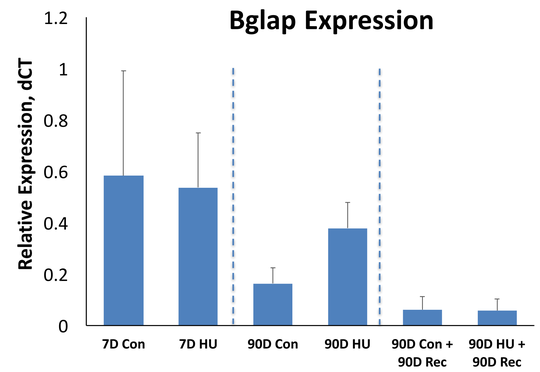
Conclusion: Majority of samples evaluated to date (a subset of the total) were of high quality (RIN>7) and acceptable concentration for use in qPCR and RNA-seq applications. Concentrations were sufficient for further assays.

- RNA-seq will be used to identify oxidative stress and bone resorption-related changes for acute and long-term time points.

Pilot: Expression Levels of Genes Related to Bone Formation



Sclerostin **(Sost)** is an inhibitor of bone formation



Bone gamma-carboxyglutamic acid-containing protein **(BGLAP or Osteocalcin**) is secreted by osteoblasts and osteocytes. It promotes bone mineralization and calcium ion homeostasis

LEGEND
7D Con = Control 7 Days. 7D HU = HU 7 Days. 90D Con = Control 90 Days. 90D HU = HU 90 Days. 90D Con +90D Rec = HU 90 Days + Recovery 90 Days. 90D HU + 90D Rec = Control 90 Days + Recovery 90 Days.

Error bars indicate SD. N=3/group. Expression levels of genes of interest were normalized to that of RPL19.

Results presented are early preliminary data. T-test was used to compare treatment group with corresponding control group; gene expression between treatment and corresponding controls was not significantly different but trends were observed, specifically in BGLAP expression of 90D HU versus 90D controls. More data points will be added to increase statistical power.

SUMMARY & FUTURE WORK

Preliminary qPCR results show trends towards differential gene expression in select HU versus corresponding control groups, indicating transient and temporal changes occur during simulated microgravity. More extensive and detailed analyses of changes in gene expression levels are in progress using RNA-seq. Further, bone structure and microarchitecture, as well as strength and material properties, will be quantified using μCT and mechanical testing to determine the relationship between changes in gene expression and bone structure and material properties.
Findings from this work will shed light on gaps in knowledge about recovery of bone strength, risk factors for poor recovery of bone density and strength, and the temporal changes in oxidative stress-related pathways.

ACKNOWLEDGEMENTS

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