Microgravity-Induced Renal Sodium and Fluid Retention:

transcriptomics reveals upregulation of the Arachidonic Acid metabolism pathway

Ayma Rizwan[1], Priya Tamura[2], Shlok Bharati[3], Isabella Chiu[4], Jennifer Claudio[5], Elizabeth Blaber[6]

Terre Haute South Vigo High School (Terre Haute, Indiana)[1], Palo Alto High School (Palo Alto, California)[2], Panther Creek High School (Frisco, Texas)[3], Lynbrook High School (San Jose, California)[4], Blue Marble Space Institute of Science (WA)[5], Rensselaer Polytechnic Institute (NY)[6]

Introduction

The human body's remarkable ability to adapt to extreme environments is put to the ultimate test during spaceflight. One of the most significant physiological challenges is the cephalad fluid shift, the redistribution of blood volume towards the upper body due to acute weightlessness in space. Microgravity-induced fluid retention contributes to spaceflight associated neuro ocular syndrome and orthostatic intolerance after re-entry. Little is known about the kidney's role in regulating sodium and fluid retention during spaceflight. Our objective is to analyze NASA spaceflight data to characterize the kidney's gene expression responses to long duration spaceflight.

Background

Microgravity induced **cephalad fluid redistribution** causes a reduction in kidney perfusion[1]. Spaceflight experiments demonstrate that kidneys respond to lower perfusion by **increasing sodium reabsorption,** which exacerbates fluid retention[2]. This response appears to be mediated through an **aldosterone independent** mechanism (Figure 1). However, the molecular mechanisms behind sodium reabsorption leading to positive sodium balance are not clear.

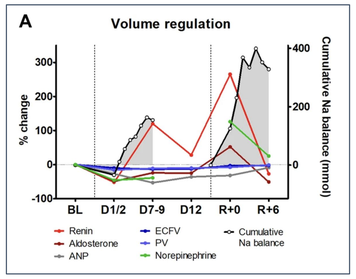


Figure 1: Long duration spaceflight causes an increase in sodium reabsorption, leading to increased cumulative sodium balance.[2]

Metadata Analysis

Spaceflight conditions

NASA Open Science Data Repository OSD-513[3] was collected as part of the RR-23 payload by the SpX-21 mission. Male mice (C57BL/6J wild type), approximately 16-17 weeks in age, were launched into space (n=10) or maintained as ground controls (n=10) under identical conditions. Kidneys at this age are fully mature but not yet affected by aging-related decline. The mission used Rodent Flight Hardware with 12h light/12h dark to mimic a standard circadian cycle, and an Ad libitum food schedule with Nutrient Upgraded Rodent Food Bars.

Figure 2: Rodent Flight Hardware, as used on the OSD-513 spaceflight group and control group.

RNA collection and sequencing

Mice were exposed to spaceflight conditions for 38 days before returning to Earth alive for euthanization by bilateral thoracotomy with sedation and tissue collection. Post-tissue collection, each left kidney was immersed in RNALater for 24 hours at 4˚C. RNA was then extracted and stored at -80 ˚C before the left kidneys were sequenced. Raw FASTQ files were run through quality control, trimmed, and aligned to the reference genome prior to quantification of gene counts.

Differential Gene Expression Analysis

Differences between space-flown and ground control mice in the data set were drawn through Jupyter notebook differential gene analysis, conducted with python's implementation of DESeq2. We used Principal Component Analysis (PCA) and Volcano plots to visualize differential gene expression. We used ShinyGo's KEGG analysis, DAVID, and STRING to evaluate enriched pathways and identify protein function.

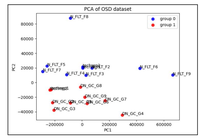


Figure 3: PCA Plot depicting distinctive expression patterns between the spaceflight (in blue) and ground control (in red)

Transcriptomics Analysis & Results

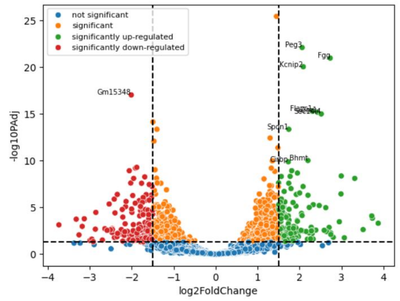


Figure 4: Volcano displaying distribution between the p-values, prioritizing genes for pathway enrichment and biological interpretation. Log10 Adjusted P Value indicates gene significance and log2 Fold Change indicates direction.

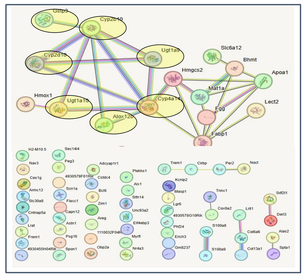


Figure 5: Nodal network of proteins generated by STRING[4] representing interactions between products of differentially expressed genes. Proteins involved in arachidonic acid metabolism are circled with yellow, suggesting that microgravity-induced changes in protein expression may converge on this pathway.

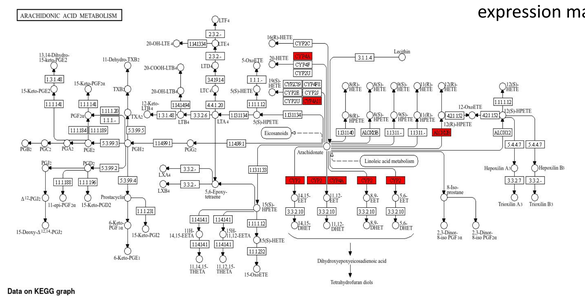


Figure 6: Full Arachidonic Acid Metabolism Pathway, generated by QuickGo's KEGG[5], with our dataset's differentially expressed genes highlighted in red, contextualizing individual gene-level changes within the broader metabolic pathway.

Selected Upregulated Genes, Protein Function, Relevance and Homology[4][6]

Genes and Functions	Relevance	Homology Models
Cytochrome pathway: • Fatty-acid oxidation & detoxification enzymes. • Convert AA → 20-HETE • 20-HETE helps regulate sodium handling, vascular tone, and tubuloglomerular feedback.	Upregulation suggests accelerated production of 20-HETE, reducing its natriuretic effect and promoting sodium retention under microgravity.	Cyp4a14, Cyp2b10, Cyp2d10
UDP-glucuronosyltransferase pathway: • Glucuronidation enzymes → makes molecules water-soluble for excretion. • Clear reactive AA-derived metabolites to prevent buildup.	Increased Ugt1a means faster clearance of 20-HETE, removing its inhibition on sodium reabsorption, potentially driving fluid overload in microgravity.	Ugt1a9, Ugt1a10

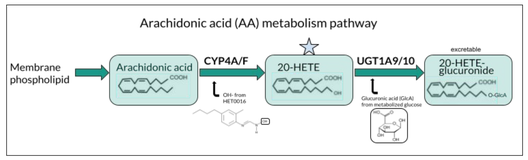


Figure 7: The arachidonic acid metabolism pathway.[7] The enzymes Cyp and Ugt, both differentially expressed in the microgravity-affected mice, facilitate metabolism and excretion of 20-HETE.

References Cited

[1] Norsk, P., Asmar, A., Damgaard, M., & Christensen, N. J. (2015). Fluid shifts, vasodilatation and ambulatory blood pressure reduction during long duration spaceflight.

[2] Olde Engberink, R.H.G., van Oosten, P.J., Weber, T. et al., npj Microgravity 9, 29 (2023). The kidney, volume homeostasis and osmoregulation in space: current perspective and knowledge gaps.

[3] Galazka , J., Zawieja , D., Gebre, S., Costes , S., Boyko , V., Dinh , M., Chen, Y., Houseman , C., Lai Polo, S., Peach , K., Vallota-Eastman, A., Saravia-Butler, A., Oribello , J., Torres , A., Novak , B., Yehia , J., & Han, C. (2025). Transcriptional profiling of kidneys from mice flown on the RR-23 mission. NASA Open Science Data Repository, Version 5. https://osdr.nasa.gov/bio/repo/data/studies/OSD-513

[4] STRING. (2000). STRING: functional protein association networks. String-Db.org. https://string-db.org/

[5] QuickGO. (2001, August). Www.ebi.ac.uk. https://www.ebi.ac.uk/QuickGO/annotations

Hypothesis & Proposed Experiment

We hypothesize that microgravity-induced upregulation of genes in the arachidonic acid metabolism pathway facilitates the elimination of 20-HETE, which in turn increases sodium reabsorption.

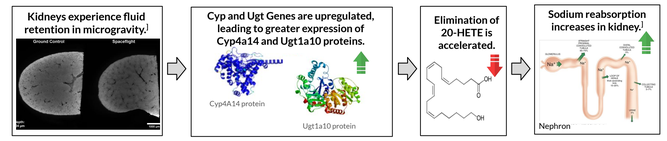
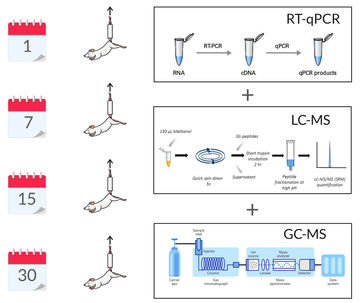


Figure 8: Depiction of our hypothesis' line of reasoning.

Specific Aim 1: Characterize the time course of gene expression of Cyp4A, Ugt1A, 20-HETE production & elimination in simulated microgravity.



Specific Aim 2: Determine the effect of inhibiting 20-HETE metabolism on urine sodium excretion in mice in simulated microgravity.

experimental condition	treatment	outcome
Ground control		
Ground control + Gemfibrozil		
Artificial microgravity		
Artificial microgravity + Gemfibrozil		

Significance & Future Direction

- Our analysis of NASA's OSD-513 dataset reveals upregulation of multiple Cyp and Ugt genes involved in the arachidonic acid metabolism pathway.
- These transcripts encode enzymes which metabolize eicosanoids, lipid-based signalling molecules such as the natriuretic molecule 20-HETE, pointing to accelerated breakdown of 20-HETE as a novel mechanism underlying spaceflight-induced fluid retention.
- Our proposed experiments are among the first to test the hypothesis that 20-HETE is a key regulator of natriuresis during microgravity exposure.
- The results of this experiment have the potential to offer new therapeutic avenues for both spaceflight-induced fluid retention and other syndromes of aldosterone independent sodium retention or increased intracranial pressure.

Acknowledgements

We would like to thank NASA Genelab for High Schools and our mentors for their help and guidance with our project. We are beyond grateful for this opportunity to explore this research at a deeper level. Special thanks to Jennifer Claudio, Elizabeth Blaber, and James Casaletto for their assistance in this immersive research experience we're beyond grateful to have been part of.

References Cited cont.

[6] SMART. (1998, May). Smart.embl.de. https://smart.embl.de/

[7] Tallima, H., & El Ridi, R. (2018). Arachidonic acid: Physiological roles and potential health benefits – A review. Journal of Advanced Research, 11, 33–41. https://doi.org/10.1016/j.jare.2017.11.004

Microgravity Induced Disruptions in Renal Sodium and Fluid Retention:
transcriptomics reveals upregulation of the Arachidonic Acid metabolism pathway

Priya Tamura[1], Ayma Rizwan[2], Shlok Bharati[3], Isabella Chiu[4], Jennifer Claudio[5], Elizabeth Blaber[6]

Palo Alto High School (Palo Alto, California) [1], Terre Haute South Vigo High School (Terre Haute, Indiana)[2], Panther Creek High School (Frisco, Texas)[3], Lynbrook High School (San Jose, California)[4], Blue Marble Space Institute of Science (WA)[5], Rensselaer Polytechnic Institute (NY)[6]

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Microgravity induced **cephalad redistribution c**auses a reduction in kidney perfusion. Spaceflight experiments demonstrate that kidneys respond to lower perfusion by **increasing sodium reabsorption,** which exacerbates fluid retention. This response appears to be mediated through an **aldosterone independent** mechanism (Figure 1). However, the molecular mechanisms behind sodium reabsorption leading to positive sodium balance are not clear.

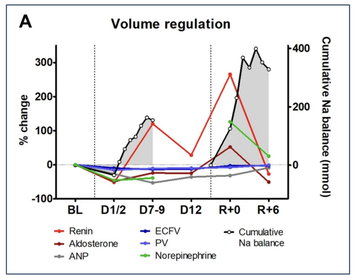

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Fig. 6: Rodent Flight Hardware used for mice in spaceflight.

RNA collection and sequencing

Mice were exposed to spaceflight conditions for 38 days before returning to Earth alive for euthanization by bilateral thoracotomy with sedation and tissue collection. Post-tissue collection, each left kidney was immersed in RNALater for 24 hours at 4˚C. RNA was then extracted and stored at -80 ˚C before the left kidneys were sequenced. Raw FASTQ files were run through quality control, trimmed, and aligned to the reference genome prior to quantification of gene counts.

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Transcriptomics Analysis & Results

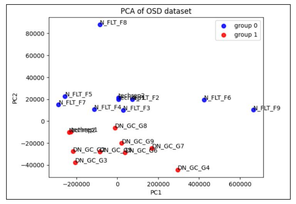
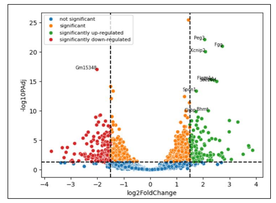


Figure 4: PCA Plot depicting distinctive expression patterns between the spaceflight (in blue) and ground control (in red), proving microgravity effect on mice gene expression is statistically strong enough to separate samples by condition.



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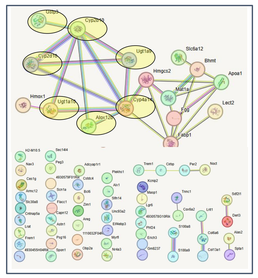

Figure 4: Nodal network of proteins generated by STRING representing interactions between products of differentially expressed genes of the dataset. Unlinked proteins at the bottom depict isolation of each differentially abundant proteins. Proteins involved in arachidonic acid metabolism are circled with yellow, suggesting that microgravity-induced changes in protein expression may converge on this pathway.

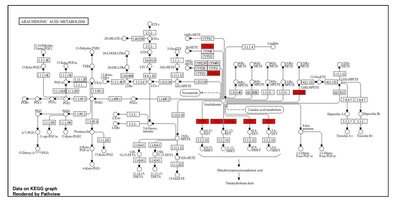

Figure 5: Full Arachidonic Acid Metabolism Pathway, generated by QuickGo's KEGG, with our dataset's differentially expressed genes highlighted in red, contextualizing individual gene-level changes within the broader metabolic pathway.

Research on Up/Downregulated Coded Proteins (STRING [Ref])

KEY: Upregulated, Significant. Downregulated, Significant. Upregulated. Downregulated.

Genes	Functions	Relevance	Homology
Cytochrome pathway (Cyp): Cyp4a14, Cyp2b10, Cyp2d10	• Fatty-acid oxidation & detoxification enzymes. • Support liver and kidney metabolic homeostasis. • Convert AA → 20-HETE (major AA metabolite in kidney). • 20-HETE helps regulate sodium handling, vascular tone, and tubuloglomerular feedback.	Upregulation suggests accelerated metabolism of 20-HETE, reducing its natriuretic effect and promoting sodium retention under microgravity.	Cyp4a14, Cyp2b10, Cyp2d10
UDP-glucuron-osyltransferase pathway (Ugt): Ugt1a9, Ugt1a10	• Glucuronidation enzymes → make molecules water-soluble for excretion. • Clear reactive AA-derived metabolites to prevent buildup.	Increased Ugt1a means faster clearance of 20-HETE, removing its inhibition on sodium reabsorption, potentially driving fluid overload in microgravity.	Ugt1a9, Ugt1a10
Gstp3 (Glutathione S-transferase pi 3)	• Glutathione conjugation → protects cells from reactive compounds. • Neutralizes oxidative byproducts from AA metabolism.	Upregulation indicates increased AA metabolic flux, supporting the idea that microgravity ramps up 20-HETE pathway activity and downstream elimination.	
Alox12b (Arachidonate 12-lipoxygenase, 12R-type)	• Lipoxygenase → converts AA to 12-HpETE / 12-HETE. • Generates AA-derived signaling lipids **with roles in inflammation and differentiation.**	Upregulation may reduce AA availability for 20-HETE, contributing to lower 20-HETE levels and enhanced sodium reabsorption.	

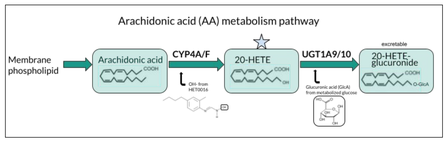

Figure 6: The specific chemical changes taking place in the arachidonic acid metabolism pathway with two of the main enzymes (Cyp and Ugt), both differentially expressed in the microgravity-affected mice.

Hypothesis

We hypothesize that microgravity-induced upregulation of genes in the arachidonic acid metabolism pathway facilitates the elimination of 20-HETE, which in turn increases sodium reabsorption.

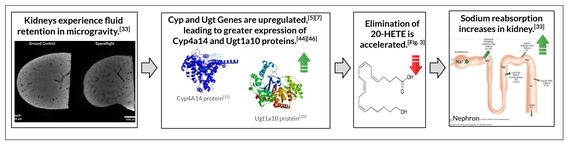

Figure 7: Depiction of our hypothesis' line of reasoning.

Proposed Experiment

Specific Aim 1: Characterize the time course of gene expression of Cyp4A, Ugt1A, 20-HETE production & elimination in simulated microgravity.

We'll measure gene expression and 20-HETE metabolites in microgravity-simulated mice to see whether Cyp4A and Ugt1A upregulation precedes increased 20-HETE elimination.

Specific Aim 2: Determine the effect of inhibiting 20-HETE metabolism on urine sodium excretion in mice in simulated microgravity.
This will be done using
Using a hindlimb suspension model, we'll measure hormones and sodium excretion, testing whether blocking 20-HETE metabolism reverses microgravity-induced sodium retention.

Significance and Future Direction

- Our analysis of NASA's OSD-513 dataset reveals an upregulation of Cyp4a and Ugt1a, genes involved in the AA pathway, pointing to accelerated breakdown of the natriuretic molecule 20-HETE as a novel mechanism underlying spaceflight-induced fluid retention.
- Our proposed experiments are among the first to test the hypothesis that 20-HETE is a key regulator of natriuresis.
- The results of this experiment have the potential to offer new therapeutic avenues for both spaceflight-induced fluid retention and other syndromes of aldosterone independent sodium retention or increased intracranial pressure.