Exploring the ARNTL and RORc Genes' Impact on Lipid Accumulation in the Liver via the Circadian Rhythm

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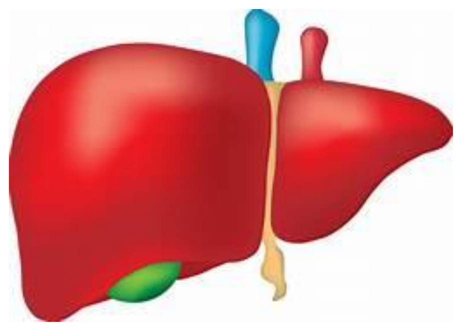
Background and Introduction

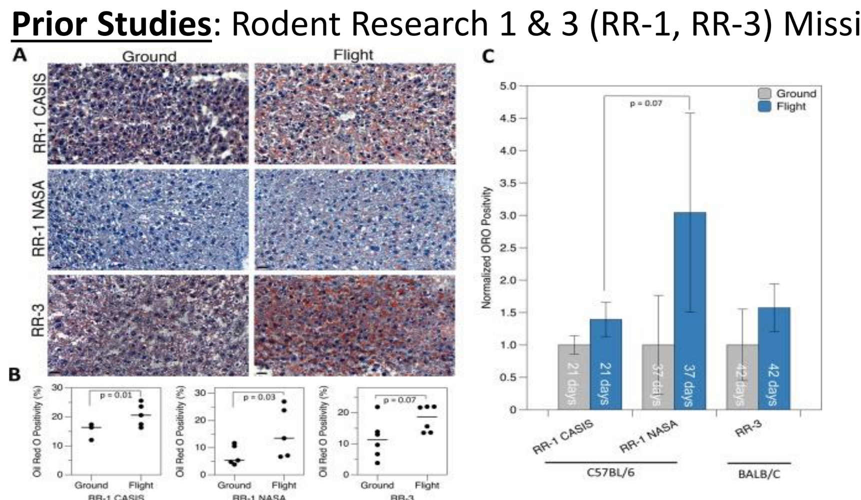
While there are structural differences between mice and human livers, the genetic mechanisms are very similar. Therefore, mice livers and their biological processes can be studied as an accurate representation of the corresponding processes and consequences humans would experience. Understanding the mechanisms that lead to lipid (fatty compounds) accumulation in the liver during spaceflight is important because lipid accumulation very commonly occurs during spaceflight, and this accumulation is linked to obesity and a multitude of other liver disorders in humans. Finding ways to counteract this will help improve the safety of spaceflight and for humans living in space.



Similarities Between Mice and Human Livers:
- Bile and cholesterol production
- Glucose conversion to glycogen
- Blood filtration
- Regulation of circadian rhythm

Manages drug processing & energy regulation from glucose, fats, and proteins.

Prior Studies: Rodent Research 1 & 3 (RR-1, RR-3) Missions



Key Findings:
- A multi-omics analysis of mice, after different space durations, showed lipid accumulation through Oil Red O staining (physical process that marks lipids/fat cells)
- Spaceflight causes lipid dysregulation in the liver. [1]

Other recent research shows: The connection between the circadian clock and lipid metabolism
1. 15-20% of metabolites, including fatty acids, exhibited rhythmic patterns in both mice and humans [2]
2. Albumin, a protein that carries lipids in the bloodstream, shows diurnal oscillations when the circadian rhythm was disrupted. Reducing the regulator of albumin, ARNTL, also led to increased lipid accumulation in mice. [3]

Source Data: OSD-245 titled Transcriptional Analysis of Liver from mice flown on the RR-6 mission [4].

Group	n	Description
FLT LAR	10	Mice flown to ISS (FLT), live animals returned (LAR) after ~29 days.
GC LAR	9	Ground control group with conditions mimicking flight to/from ISS.
LAR Baseline	10	Baseline control group.
FLT ISS Terminal	10	Mice flown to ISS, euthanized on ISS after ~50 days.
GC ISS Terminal	10	Ground control group with conditions mimicking flight to ISS
ISS Term. Baseline	10	Baseline control group.

Description: Original RR-6 study done to evaluate muscle atrophy in mice during spaceflight, and the livers from mice with placebo for the drug being tested were sent to NASA GeneLab for further study.

Data Collection:
- RNA was extracted from the livers and was made available through the GeneLab data repository.

Research Goal: Find the connection between the specific expression of genes and the effects of spaceflight (microgravity & disruption of circadian rhythm), using source data made available by OSD-245, specifically those in the FLT and GC ISS Terminal groups.

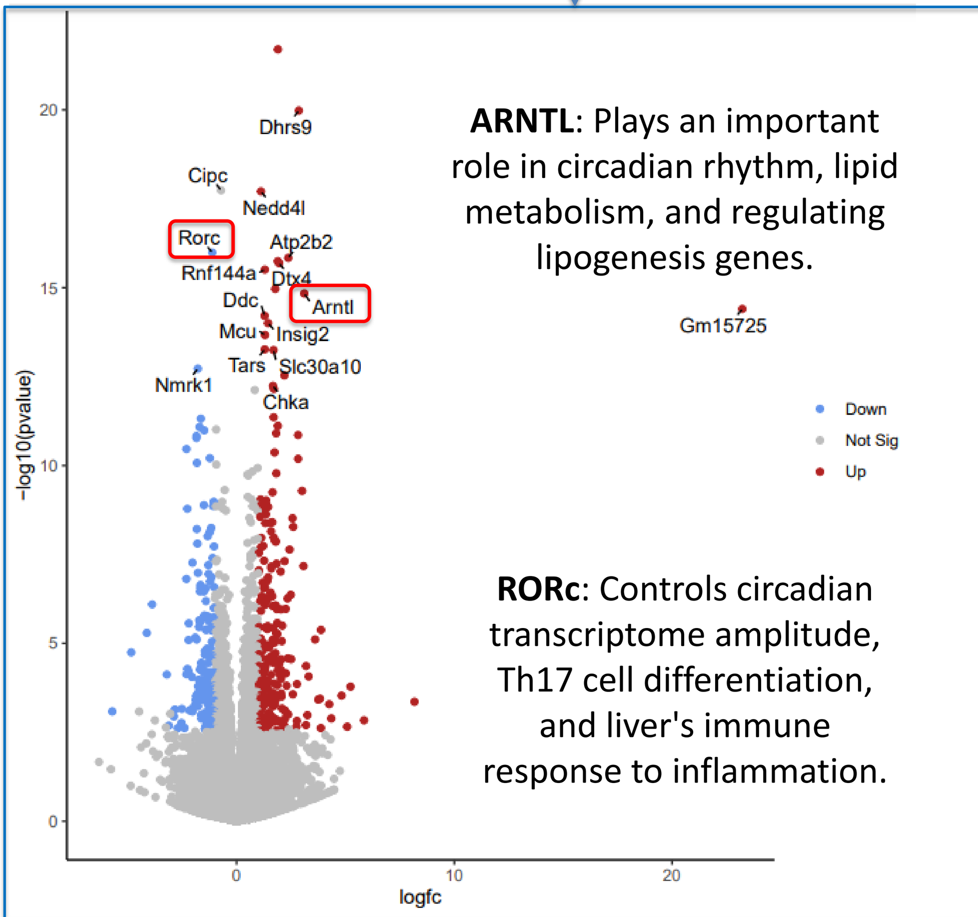
Data Analysis Tools & Methods:

The genetic analysis done in this work used the publicly-available platform of the NASA GeneLab Galaxy platform (UseGalaxy.org)

1. **DESeq2**: A method for differential analysis of count data when doing high-throughput RNA sequencing assays, allowing for more quantitative analysis focused on the strength rather than the mere presence of differential expression. Differential expression analysis enables a comparative study showing the functional relevance of a specific gene with respect to the study's independent variable. [5]
2. **GoSeq**: Gene Ontology (GO) analysis which highlights over or under-represented biological processes based on the input gene list coming from the DESeq2 analysis. This tool provides a qualitative assessment as to the significant biological processes linked to the differentially expressed genes from the DESeq2 tool. [6]
3. **FGSEA**: Fast Gene Set Enrichment Analysis enables the detection of functional enrichment from microarray data. It compares two groups using the signal to noise ratio so that each probe in the microarray is assigned a score to quantify the enrichment. The gene sets are defined on prior biological and biochemical pathways as enumerated in the GoSeq tool. [7]

Data Analysis

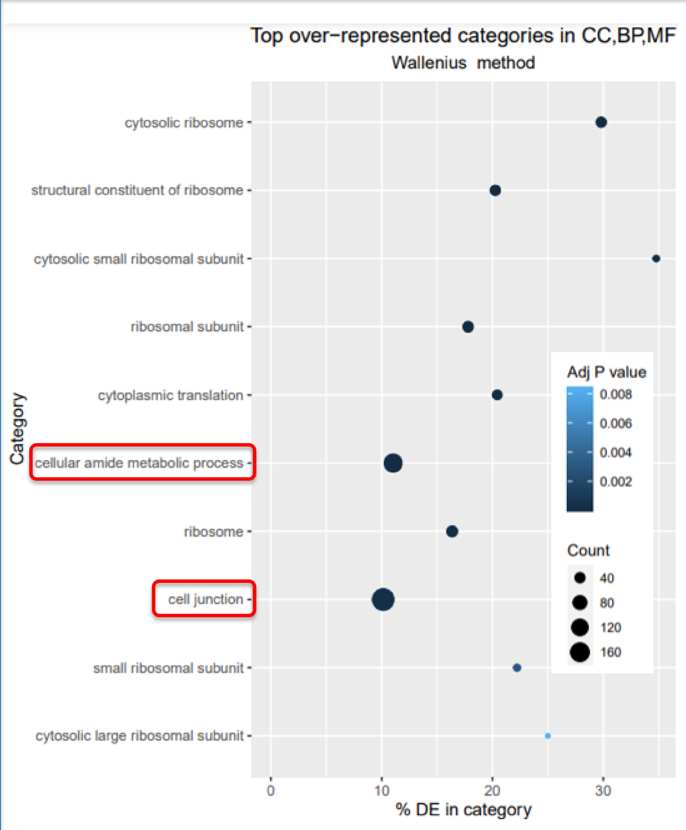
GeneLab Database → DESeq2 Tool → GoSeq Tool → FGSEA Tool



ARNTL: Plays an important role in circadian rhythm, lipid metabolism, and regulating lipogenesis genes.

RORc: Controls circadian transcriptome amplitude, Th17 cell differentiation, and liver's immune response to inflammation.

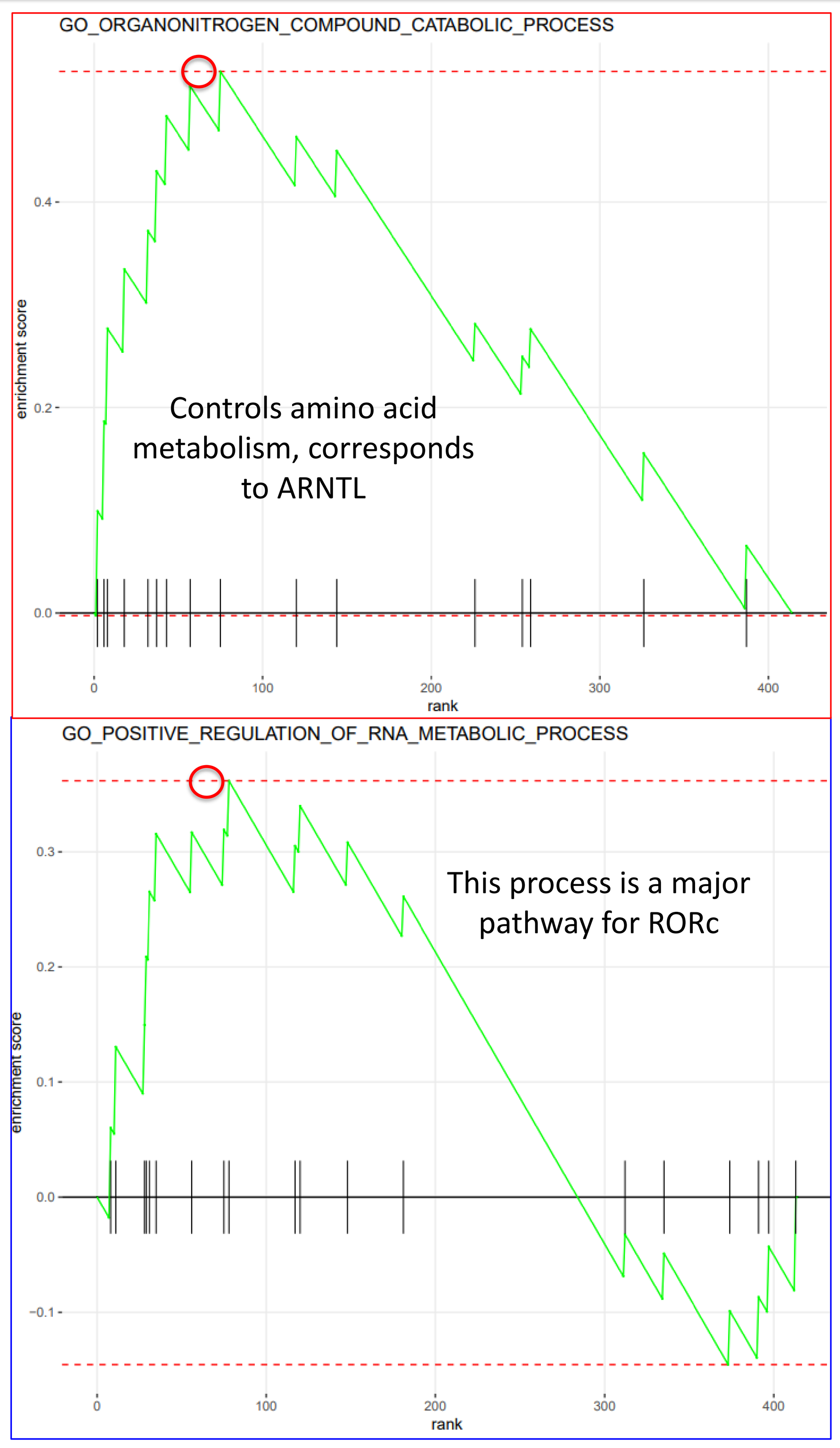
Generate volcano plot
Show the top 20 most significant genes within our comparison groups.



Cellular Amide Metabolic Process: Affects amino acid metabolism, impacting insulin sensitivity, inflammation, and oxidative sensitivity, influencing lipid metabolism & accumulation.

Cell Junction: Dysfunctionality can cause bacterial product leakage, inducing inflammation and disrupting lipid metabolism.

Pathway Analysis
Shows most significant changes in biological processes



Detailed Pathway Analysis
Quantifies significance of specific pathway, by magnitude of the first peak.

Both ARNTL and RORc genes play a big role in the circadian rhythm of the liver and take part in liver metabolic processes.

Proposed Experiment

Hypothesis: Spaceflight conditions disrupt the circadian rhythm due to the upregulation of ARNTL and the downregulation of RORc, thereby impacting liver metabolism and leading to the accumulation of lipids in hepatocytes.

Goals: The first goal for our experiment was to understand the impacts of upregulated ARNTL in microgravity on lipid accumulation in the liver. The second goal for our experiment was to understand the impacts of downregulated RORc in microgravity on lipid accumulation in the liver.

Experimental Setup: We propose sending knockout (KO) mice (*Mus musculus*) models into space, while also having a ground factor. Each group has n=4, and group count is 8, where wildtype has both genes:

Group (n=4)	Wildtype	ARNTL KO	RORC KO
Ground Control	X		
Ground Exp. 1		X	
Ground Exp. 2			X
Ground Exp. 3		X	X
Space Control	X		
Space Exp. 1		X	
Space Exp. 2			X
Space Exp. 3		X	X

Controlled Variables for all mice:
- Female and ~32 weeks of age at start
- Fed 5 grams of Nutrient Upgraded Rodent Food Bar (NuRFB) daily
- Housed in standard Rodent Habitat subject to standard air composition
- "Race-tracking" chosen as a reliable technique for monitoring circadian rhythms
- Experimental duration of 50 days
- Will be euthanized with Euthasol injection, followed by cervical dislocation

Data Collection & Analysis:
- The mice masses to be measured, following by dissection and measurement of the liver
- RNA sequencing to be conducted to measure genetic expression of ARNTL and RORc in the hepatocytes
- Oil Red O (ORO) staining to be used to quantify lipid accumulation in the livers

Dependent Variables: body mass, liver mass, genetic expression of ARNTL and RORc, lipid accumulation

	Expected Outcomes	
	Ground	Space
Exp. 1 ARNTL KO	Significant increase in lipid accumulation, due to non-regulation of albumin, a protein carrier that distributes fat via the bloodstream.	Greater increase in lipid accumulation since the disruption of circadian rhythm will also disrupt expression of RORc.
Exp. 2 RORc KO	Similar increase in lipid accumulation, due to RORc's role in maintaining lipid metabolism.	Moderate lipid increase since disruption of circadian rhythm upregulates ARNTL.
Exp. 3 ARNTL & RORc KO	Greatest increase in lipid accumulation, with dysregulated metabolism and transport.	Similar levels of lipid accumulation as Ground Exp. 3 group.

Expected Conclusion: Our proposed experiment intends to show the direct link between the ARNTL and RORc genes and lipid accumulation in the liver, coupled with counter-balancing effects of spaceflight's microgravity and disruption of circadian rhythm. This will provide a better understanding of the effects of spaceflight on liver health, as well as help advance scientific knowledge to promote the discovery of treatments for liver problems and diseases and find ways to keep astronauts safe during their travels.

References

[1] Beheshti, et al. "Multi-omics analysis of multiple missions to space reveal a theme of lipid dysregulation in mouse liver." Sci Rep 9, 19195 (2019). https://doi.org/10.1038/s41598-019-55869-2
[2] Sinturel, Flore et al. "Circadian rhythm of lipid metabolism." Biochemical Society transactions vol. 50,3 (2022): 1191-1204. https://pubmed.ncbi.nlm.nih.gov/35604112/
[3] Petrenko, Volodymyr, et al. "Lipid Metabolism around the Body Clocks." Progress in Lipid Research, 13 May 2023, https://www.sciencedirect.com/science/article/pii/S0163782723000255
[4] Galazka JM, et al. "Transcriptional analysis of liver from mice flown on the RR-6 mission", NASA Open Science Data Repository, Version 14, http://doi.org/10.26030/sm73-vt20
[5] Love MI, et al. "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." Genome Biol. 2014;15(12):550. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4302049/
[6] Young MD, et al. "Gene ontology analysis for RNA-seq: accounting for selection bias." Genome Biology, 11, R14. 2010. https://bioconductor.org/packages/release/bioc/html/goseq.html
[7] Korotkevich G, et al. "Fast gene set enrichment analysis." bioRxiv. 2019. doi:10.1101/060012, http://biorxiv.org/content/early/2016/06/20/060012.

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