

# **Biospecimen Retrieval from NASA's Rodent Research-1: Maximizing Science Return from Flight Missions**

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# Background

- Spaceflight experimentation poses various challenges:
  - Limited number of samples
  - Limited number of flights
  - Limited capability to process tissues on orbit
- Biospecimen Sharing Program (BSP) was developed over 50 years ago by the NASA Ames Research Center to maximize science return

# Background (Cont'd)

- Ground-based tests were performed to maximize science return from the Rodent Research-1 Validation flight

# Rodent Research-1

- Launched on SpX4: September 21, 2016
- 20 female adult mice:
  - 10 for NASA's validation mission
  - 10 for the National Lab's science experiment
- Mission duration on the ISS:
  - 33 days (NASA's Validation)
  - 16-17 days (Novartis)
- Animals euthanized and stored at  $<-80^{\circ}\text{C}$  on the ISS until return to Earth on SpX5 (Feb 2015)
- **BSP dissection**
  - First thaw: April 2015
  - Second thaw: March 2016

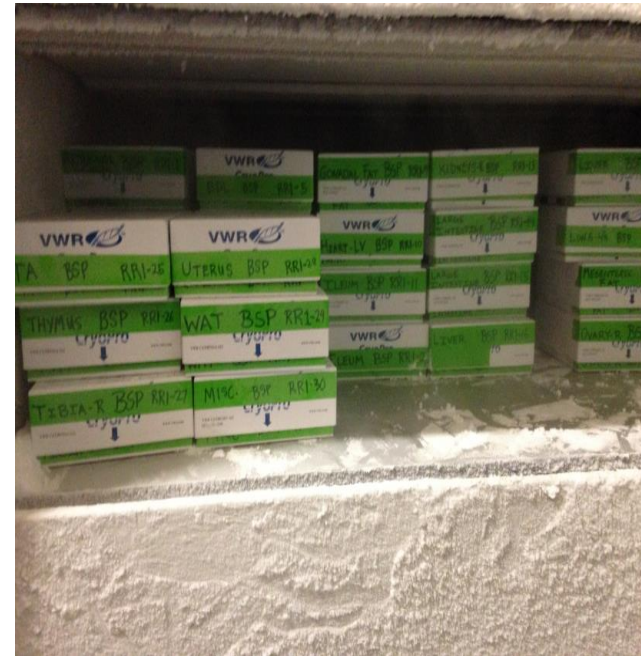


# Rodent Research-1 BSP

- Ground-based tests were performed to maximize science return from the Rodent Research-1 Validation flight:
  - Tissue Preservation Test: assess the quality of tissues collected from frozen carcasses
    - Results presented at 2014 ASGSR
    - Manuscript recently accepted (PLOS One, 2016)
  - Additional tissue quality test to optimize science return from the RR-1 BSP dissections
    - Carcasses were frozen using the on-orbit timelines for RR-1 (NASA's Validation flight)

# RR-1 First Thaw BSP Dissection

- Carcasses were euthanized and stored at  $-80^{\circ}\text{C}$  for over 3 months on ISS and for  $\sim 2$  months at Ames upon return to Earth (total  $> 5$  months at  $-80^{\circ}\text{C}$ )
- 32 different types tissues were retrieved from 40 mice including 10 mice each from flight, ground controls, baseline and vivarium controls, yielding total of 3280 vials of tissues



Ames Life Science Data Archive

# RR-1 First Thaw BSP Dissection (cont'd)

- BSP tissues have been distributed to the scientific community through the Ames Life Science Data Archive (LSDA)
  - To locate available biospecimens, visit the LSDA at:  
<http://lsda.jsc.nasa.gov>
- Select samples were provided to Russian research colleagues at the Institute for Biomedical Problems (IBMP)
- NASA GeneLab project: Liver samples were provided for “omics” analyses (transcriptomics, epigenetics and proteomics)
  - Some of the data are currently publically available:  
[https://genelab-data.ndc.nasa.gov/genelab/search\\_studies/?q=RR1](https://genelab-data.ndc.nasa.gov/genelab/search_studies/?q=RR1)

# RR1 BSP Dissection workflow

## Dissector 1

Thaw frozen carcass

## Dissector 2

Dorsal Skin  
Eyes  
Brain

### Left or Right hindlimb:

Gastrocnemius  
Soleus  
Tibialis anterior (TA)  
Extensor digitorum longus (EDL)  
Quadriceps

Femur  
Tibia

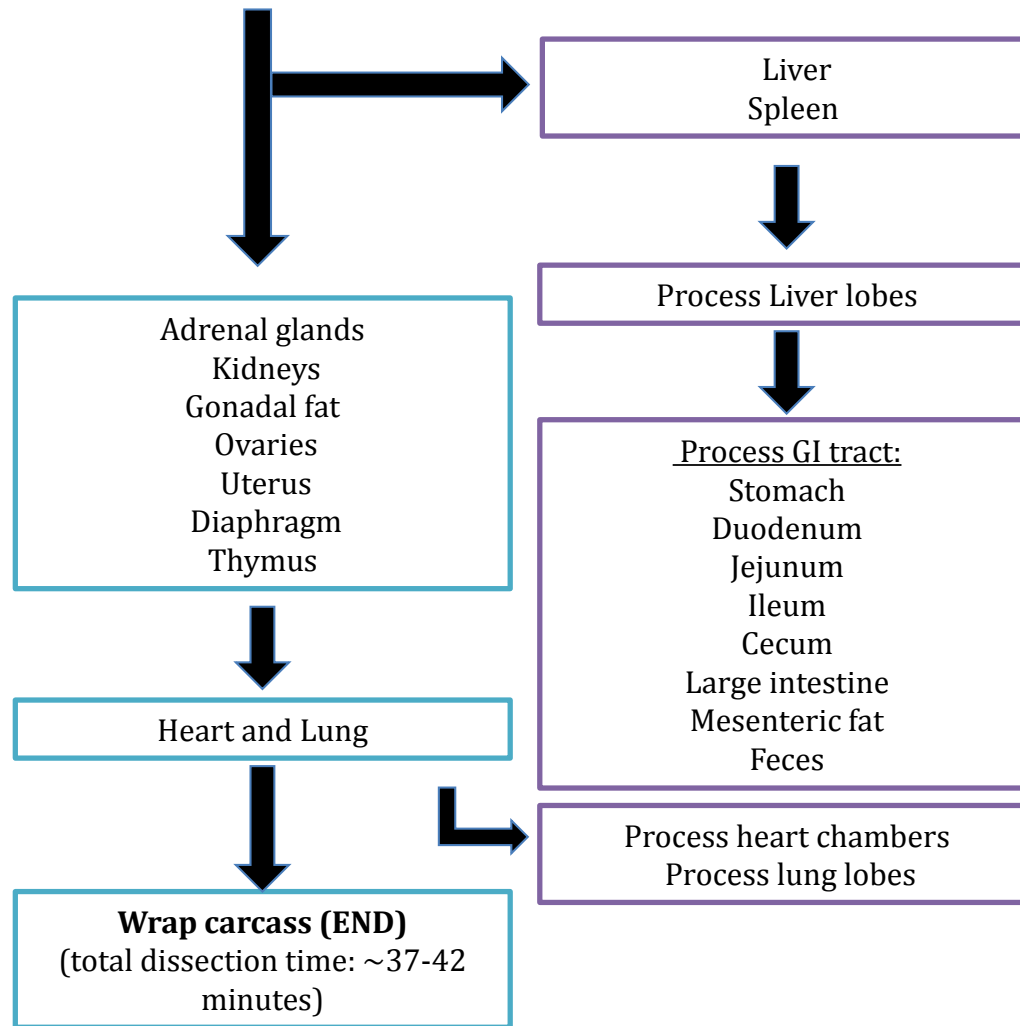
### Other hindlimb:

Gastrocnemius  
Soleus  
Tibialis anterior (TA)  
Extensor digitorum longus (EDL)  
Quadriceps

Femur  
Tibia



# BSP Dissection workflow (cont.)

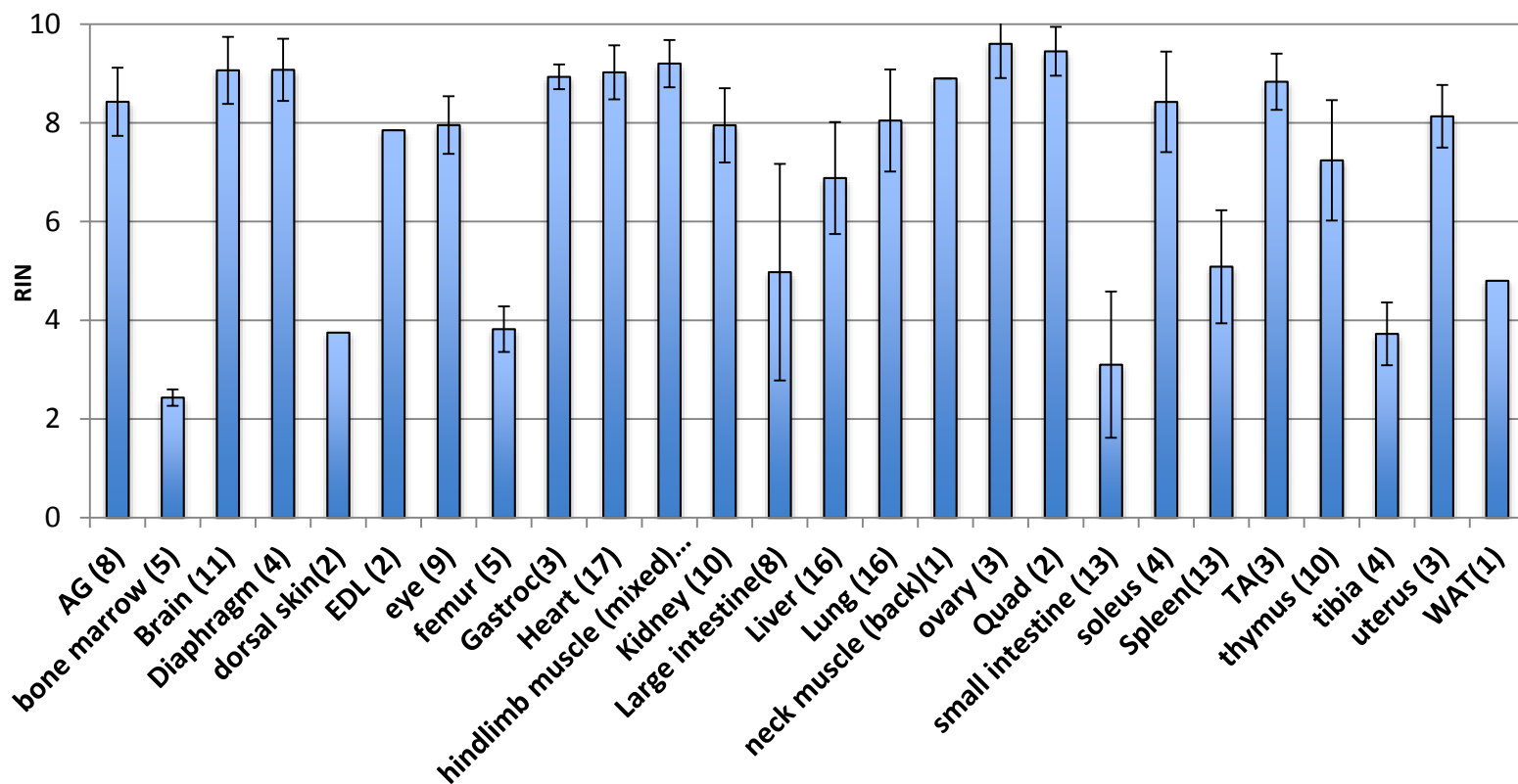


# Tissue Quality Testing: assess RNA quality of various tissues collected from frozen carcasses (1<sup>st</sup> thaw dissection)

High Quality



Low Quality



# RR1 Second Thaw BSP Dissection

- 40 carcasses previously dissected during the first thaw dissection (10 per group from flight, ground controls, vivarium and baseline) and stored for about 11 months at Ames Biospecimen Sharing Facility freezer (-80°C) were dissected.
- Additional 7 different types of tissues were retrieved from each of the 40 remaining carcasses.

# Second Thaw Tissues

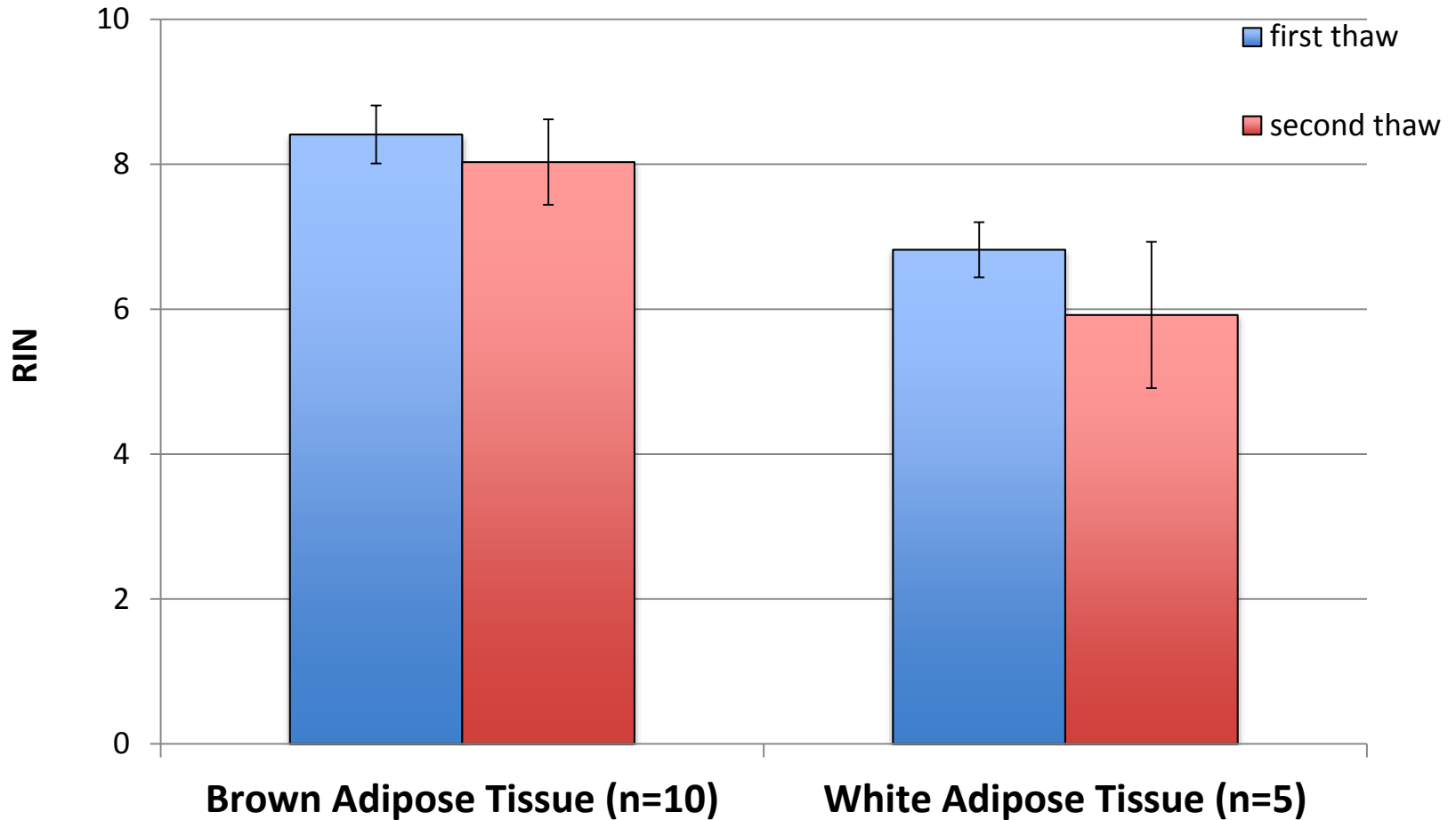
Tissues
Pelvis
Spine
Aorta
Forearms and Tail
Vagina
Brown Adipose Tissue
Neck muscles



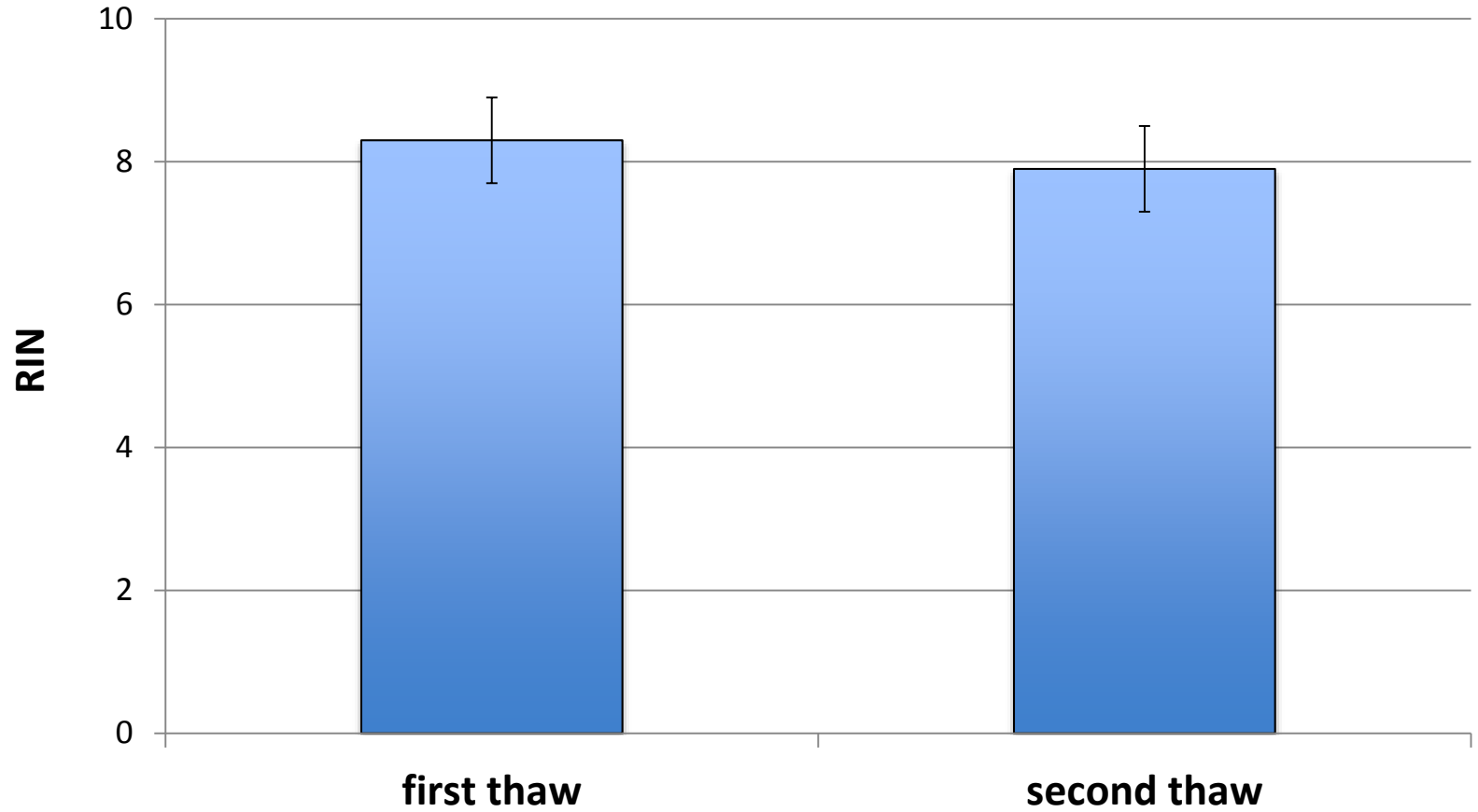
# Tissue Quality Test for the RR-1 second thaw dissection

- Objective:
  - To assess if the tissues collected from the second thaw dissection are viable
- Methods:
  - Tissues were collected from frozen carcasses that were subjected to 2 cycles of freezing and thawing
  - Timelines simulated the on-orbit RR-1 procedures and the first thaw dissection to assess the quality of the second thaw tissues.
  - RIN (RNA integrity number) values of select tissues, including kidney, brain, diaphragm, white adipose tissue (WAT) and brown adipose tissue (BAT) were determined as a measure of tissue quality

# Adipose Tissues

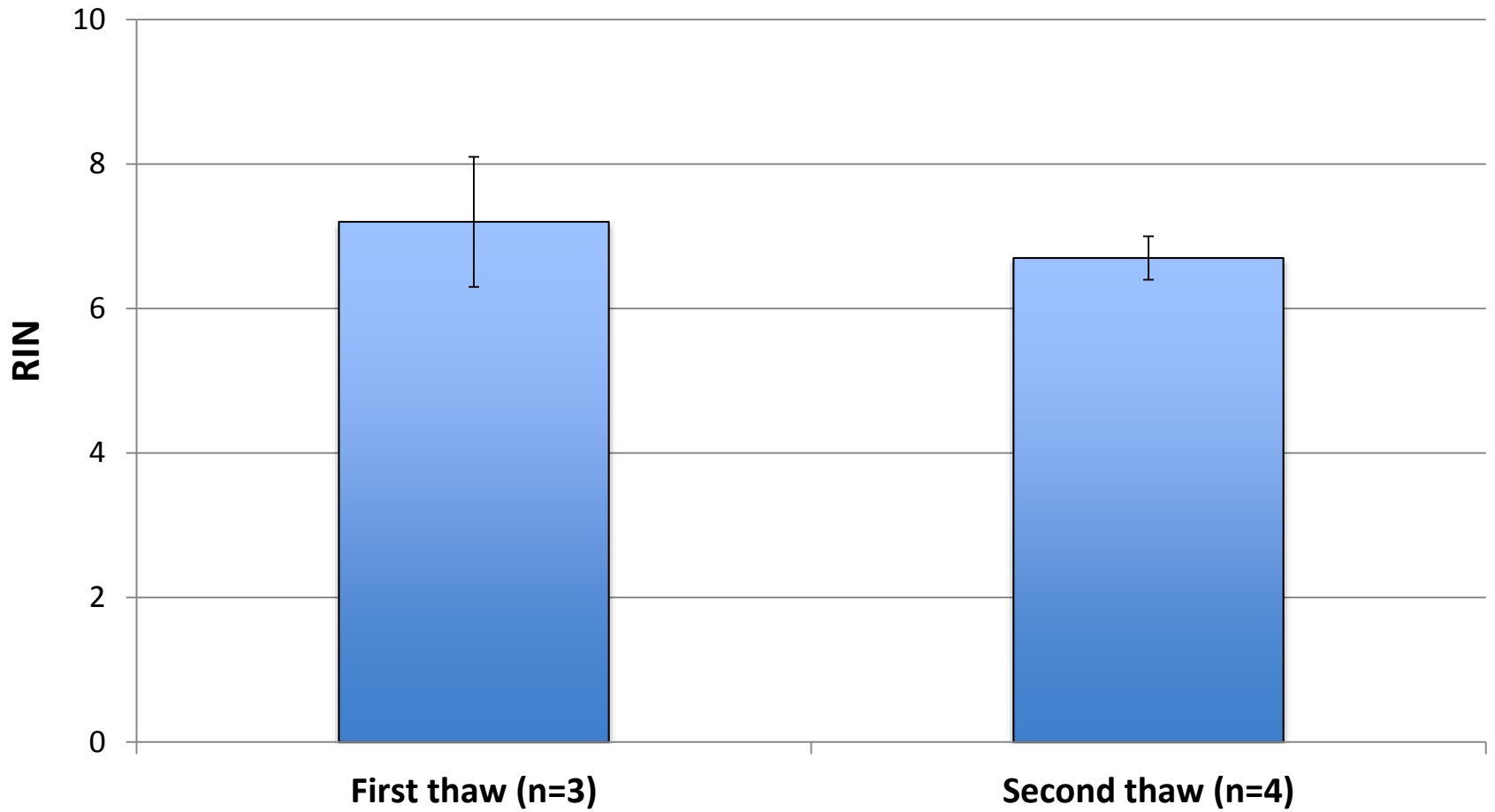


# Brain



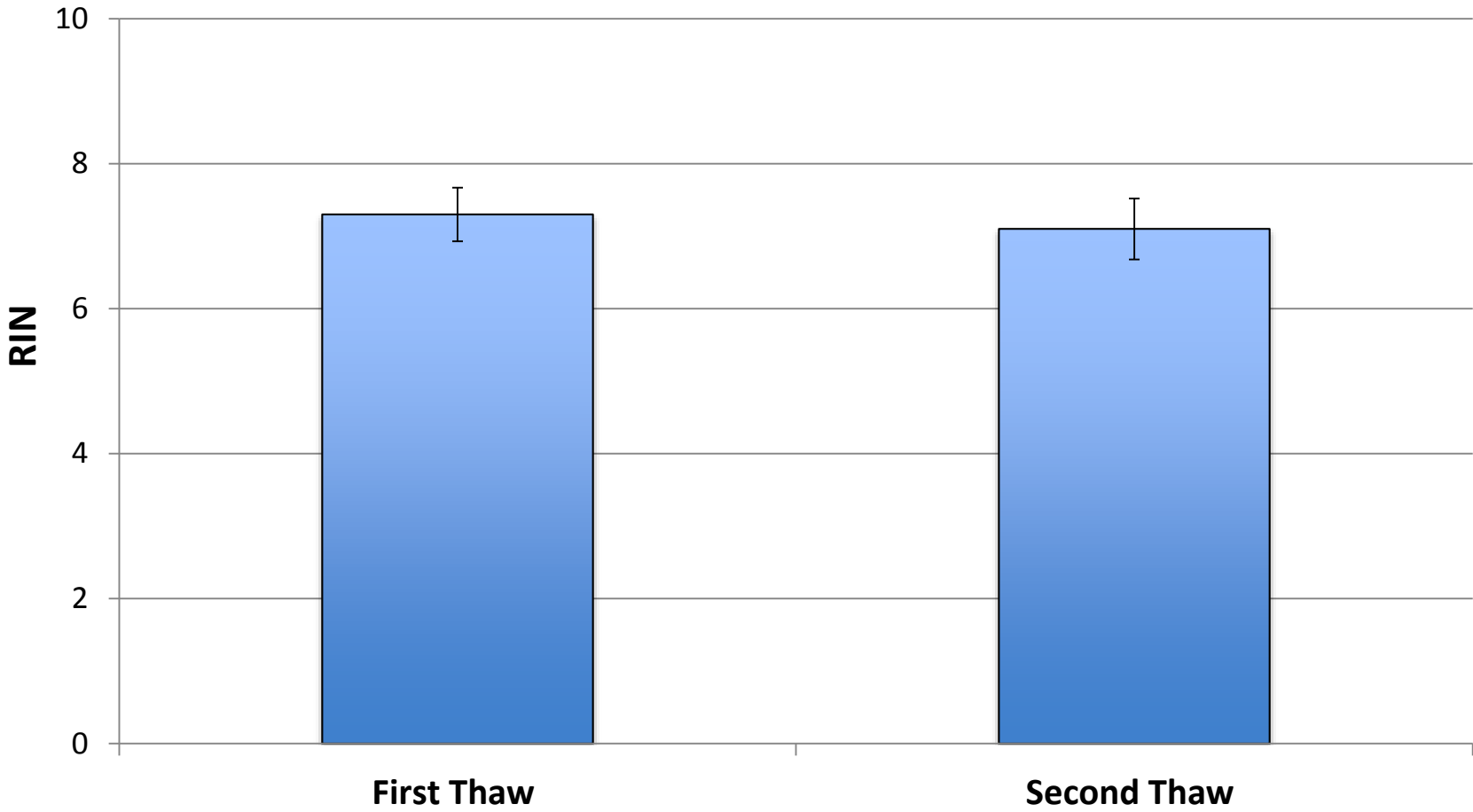
Mean $\pm$ SD (n=5)

# Kidneys





# Diaphragm



Mean+/- SD (n=5)

# Feedback from BSP PIs about the quality of tissues recovered from the second thaw

- Vaginal walls:
  - “The histology is fantastic - they look similar to freshly fixed tissue which is remarkable considering the vaginal walls were frozen, thawed, frozen again and then thawed before fixation” - Dr. Lane Christenson, University of Kansas Medical Center
- Aorta:
  - “RNA isolated from the second thaw aorta were not of high quality for RNA-seq but were suitable for microarray analysis using the FFPE (formalin-fixed, paraffin-embedded) method” -Dr. Sonja Schrepfer, UCSF
- Brown adipose tissue:
  - “PCR array targeted for adipogenic genes were successfully performed using RNA samples isolated from brown adipose tissues” -Dr. Russel Turner, Oregon State University

# Summary and Conclusion

- Select tissues can be utilized for gene expression and histology studies despite being retrieved from carcasses subjected to at least two freezing and thawing cycles
- These results expand science return from valuable and limited rodent experiments in space

# Acknowledgements

- Rodent Research Project Team, ARC
- Space Biology Program
- ISS Program

**EXTRA**

# Rodent Research-3

- Launched on SpX8: April 8, 2016
- 20 female adult for Eli Lilly/US National Laboratory
- Mission duration: 6 weeks on the ISS
- Animals euthanized and stored at  $<-80^{\circ}\text{C}$  on the ISS until return to Earth on SpX9 (August 2016)
- **BSP dissection:** Sept 2016



# RR3 BSP dissection

- Over 25 types of tissues were retrieved from 30 non-treated RR3 mice for NASA BSP
- ~1800 vials of tissues were transferred to Ames Life Science Data Archive for the scientific community (not including the tissues processed by the RR3 PIs and SLPS PI team)
- Tissues were snap frozen, preserved in RNAlater or fixed for histology.