

# Group housing during hindlimb unloading to simulate weightlessness

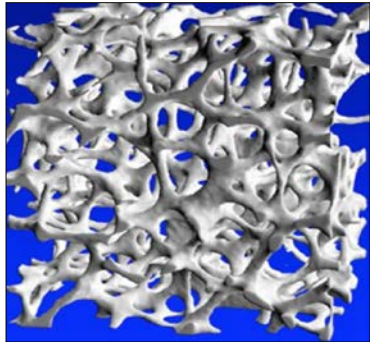
**Candice Tahimic**<sup>1,2</sup>, Moniece Lowe<sup>1,3</sup>, Sonette Steczina<sup>1,3</sup>, Samantha Torres<sup>1,3</sup>, Masahiro Terada<sup>1,4</sup>, Ann-Sofie Schreurs<sup>1,5</sup>, April Ronca<sup>1</sup>, Joshua Alwood<sup>1</sup>, Ruth K. Globus<sup>1</sup>

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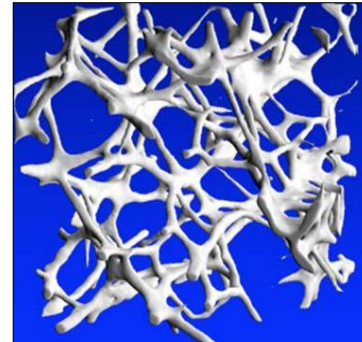
Disclosure: No conflict of interest

# Rodent hindlimb unloading (HU) as a ground-based analog for weightlessness

- Analog for musculoskeletal disuse and cephalad fluid shift observed in spaceflight (Morey-Holton, Bikle and Globus 1984)



**Human: months to decades**  
**Rodent: days to months**



Images courtesy of Dr. Ralph Muller



- Tail traction along a pulley system to elevate hindlimbs; single housing
- Variations
  - Partial unloading (Spatz et al 2017)
  - Single or group housing; tail piercing at intervertebral disc space (Ferreira, Crissy and Brown 2011)

# Rationale for group housing during HU

- Rodents are social animals
- Long-term social isolation → altered behavior and organ-system responses
  - Single vs group housed mice*
    - Altered synaptic plasticity; ↑ voluntary ethanol intake (Talani et al 2014)
    - Immune system changes (Ref)
    - Smaller; ↓ soft-lean tissue mass, ↓ BMC and BMD (Nagy et al 2002)
    - ↑ variance in % body fat, BMC and BMD (Nagy et al 2002)
- Better simulation of housing conditions in ISS (AEM)

## **Key topics**

1. Group (paired housing) HU design
2. Group vs single housing HU: effects on spaceflight-relevant tissues
3. Behavioral analysis: preliminary results

# Group vs single housing HU

## *Hypotheses*

- Long-duration HU in group housing  
→ deficits in musculoskeletal structure; comparable to single housing HU
- Group housing will provide opportunities for olfactory, visual, auditory and physical stimulation and interaction  
→ improved animal well-being; minimize effects of stress-induced social isolation

# Experiment design

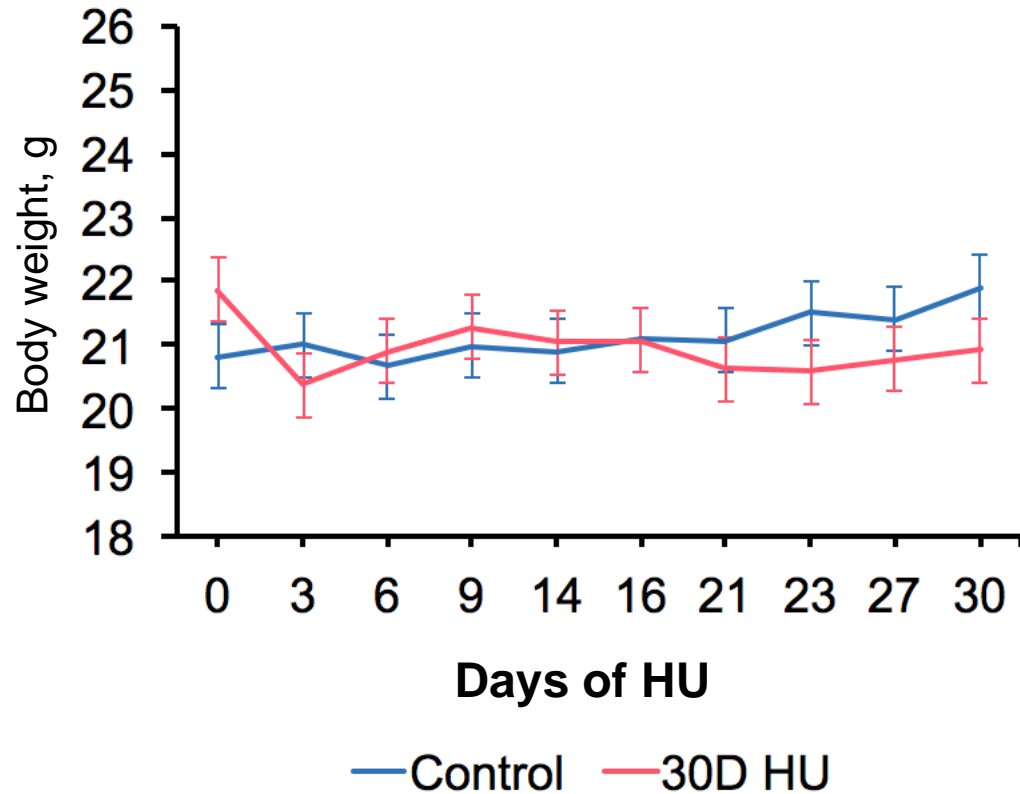
Experiment Parameters	Study 1	Study 2
Groups	Group housing HU Group housing control	Single housing HU Single housing control Group housing HU Group housing control
Sex	Female	Female
Strain	C57BL/6J	C57BL/6NJ
Age at start of HU	16-17 weeks	16 weeks
Duration of HU	30 days	30 days
Measurements	Soleus, adrenal and spleen weights; body weights; food consumption	Soleus, adrenal and spleen weights; body weights; food consumption; behavior

IACUC approval obtained prior to any animal experiments.

# No differences in body weights in single vs group housing HU

## Study 1

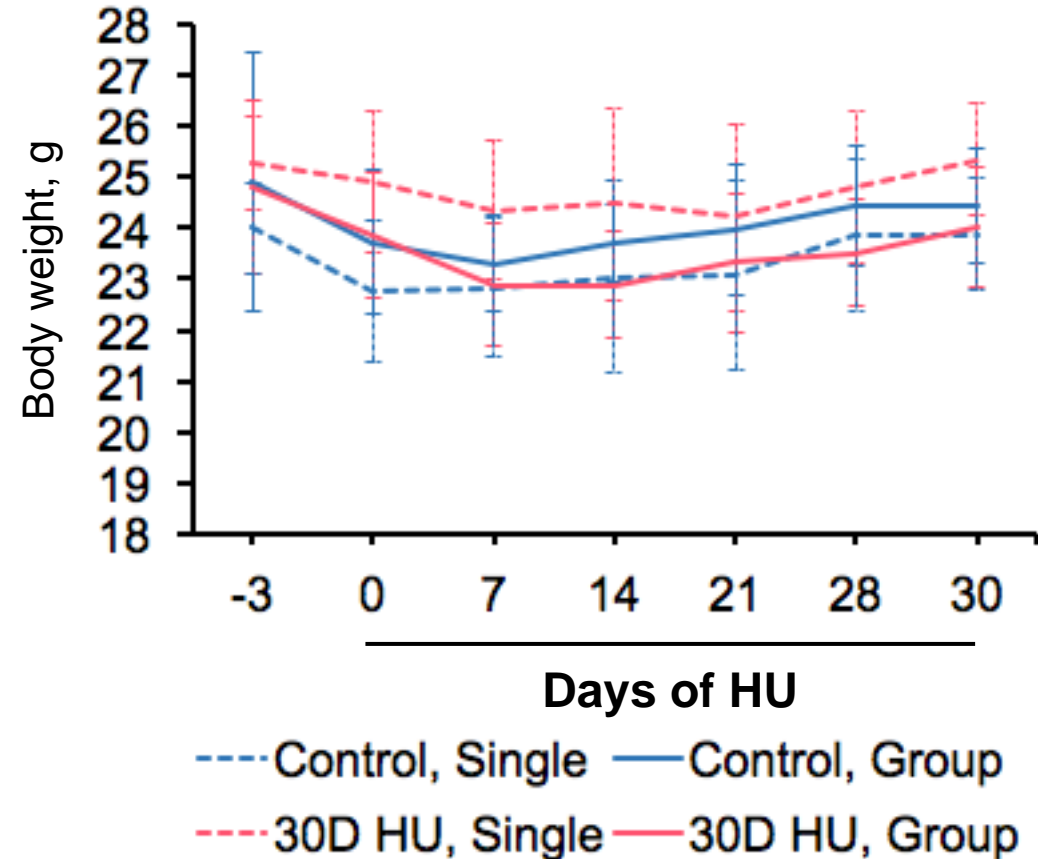
Group housing, C57BL/6J



Stats: t-test at  $p < 0.05$

## Study 2

Single vs Group housing, C57BL/6NJ



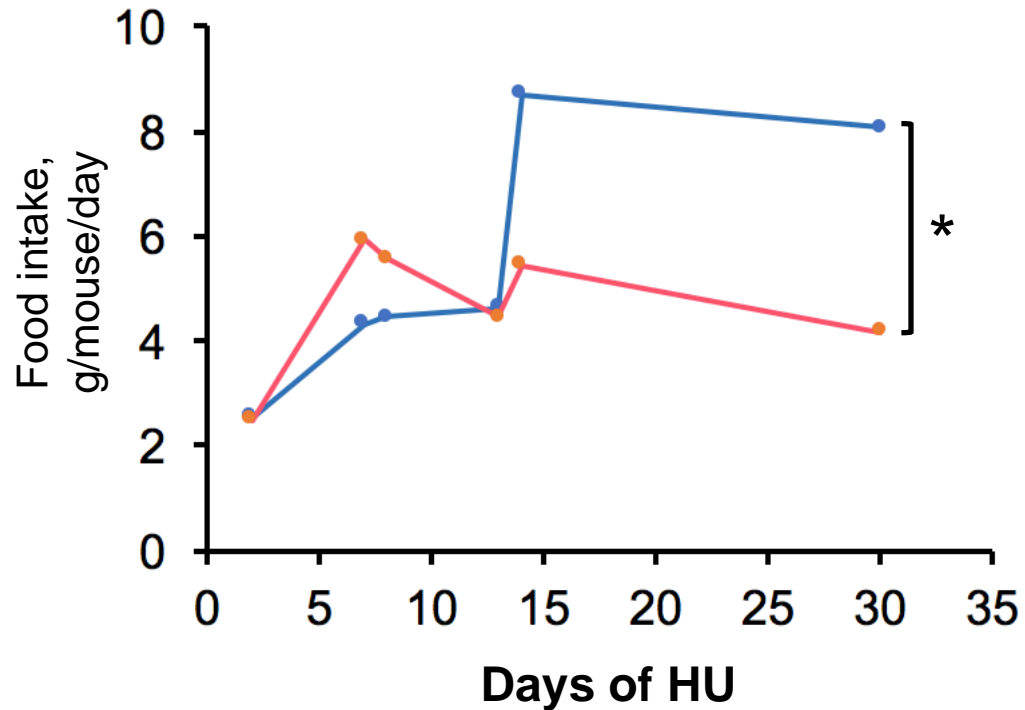
Stats: One-way ANOVA at  $p < 0.05$



# Increased food consumption in single vs group housing HU animals; strain differences in response to HU

## Study 1

Group housing, C57BL/6J

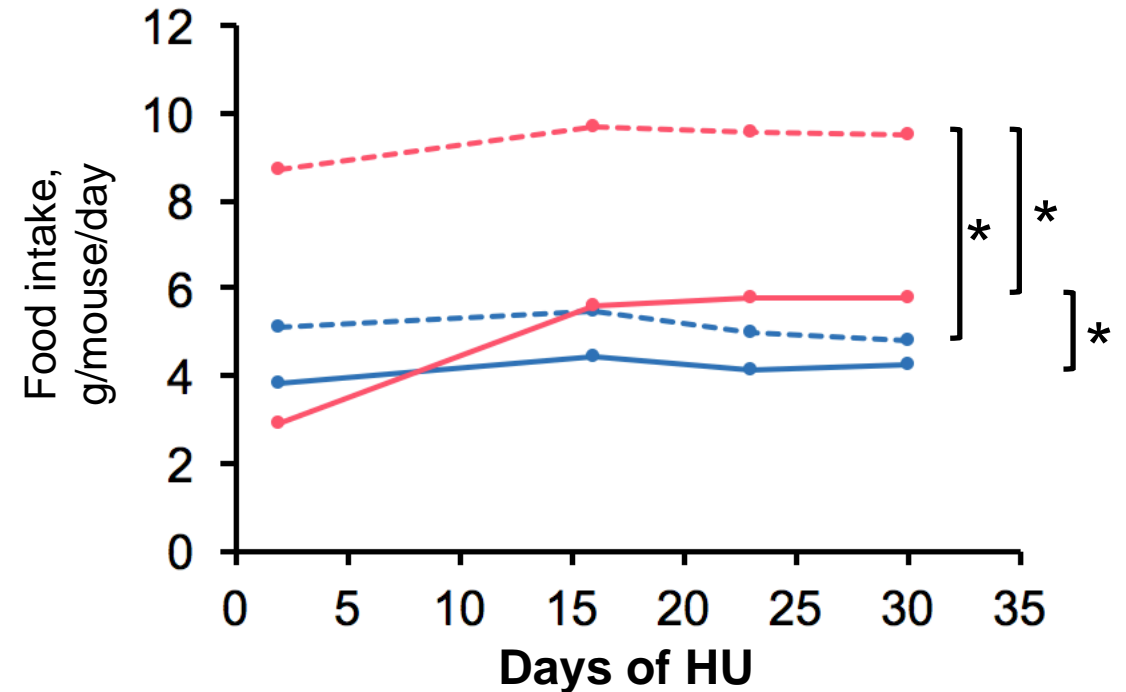


—●— Control —●— 30D HU

\*Significant by t-test at  $p < 0.05$ ;  
Means and SD shown

## Study 2

Single vs Group housing, C57BL/6NJ



—●— Control, Single —●— Control, Group  
- - -●- - 30D HU, Single —●— 30D HU, Group

\*Significant by one-way ANOVA followed by  
Tukey-Kramer post-hoc test at  $p < 0.05$ ;  
Means and SD shown

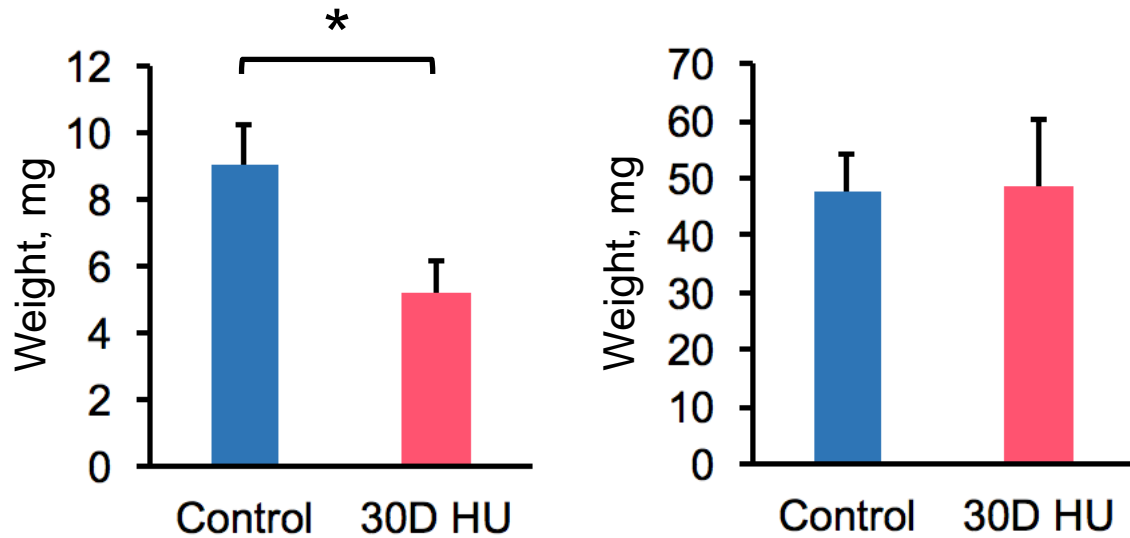
# Group housing HU leads to expected responses in postural and type II fiber-enriched muscles; response not different from response to single housing HU

## Study 1

Group housing, C57BL/6J

**Soleus**

**Tibialis anterior**

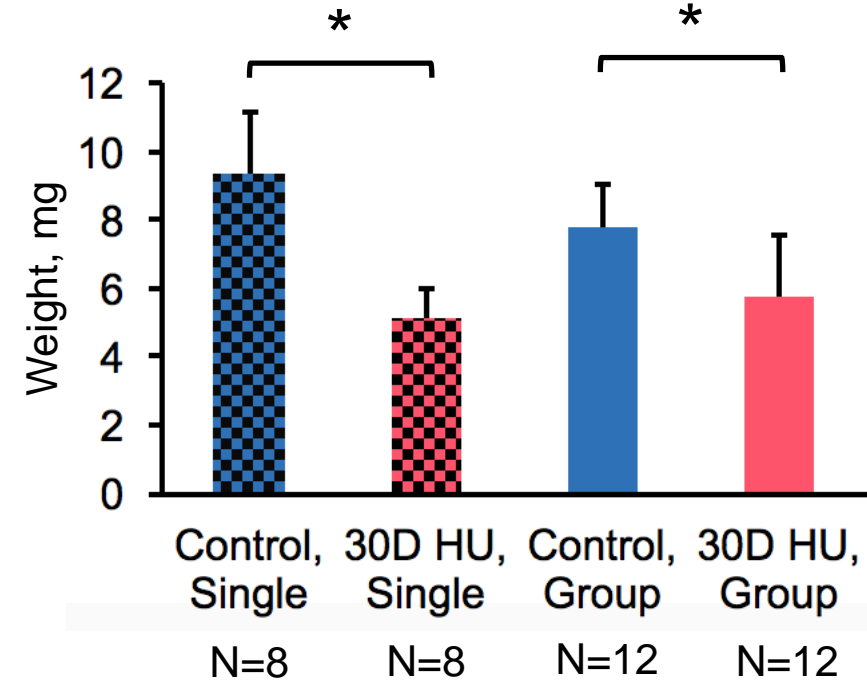


\*p<0.05 by t-test; Means and SD shown; N=8/group

## Study 2

Single vs Group housing, C57BL/6NJ

**Soleus**

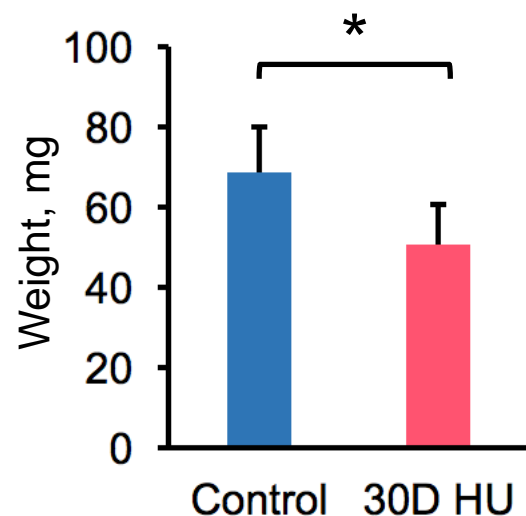


\*One-way ANOVA and Tukey-Kramer post-hoc test at p<0.05; Means and SD shown

# No differences in spleen weights in single vs group housing HU

## Study 1

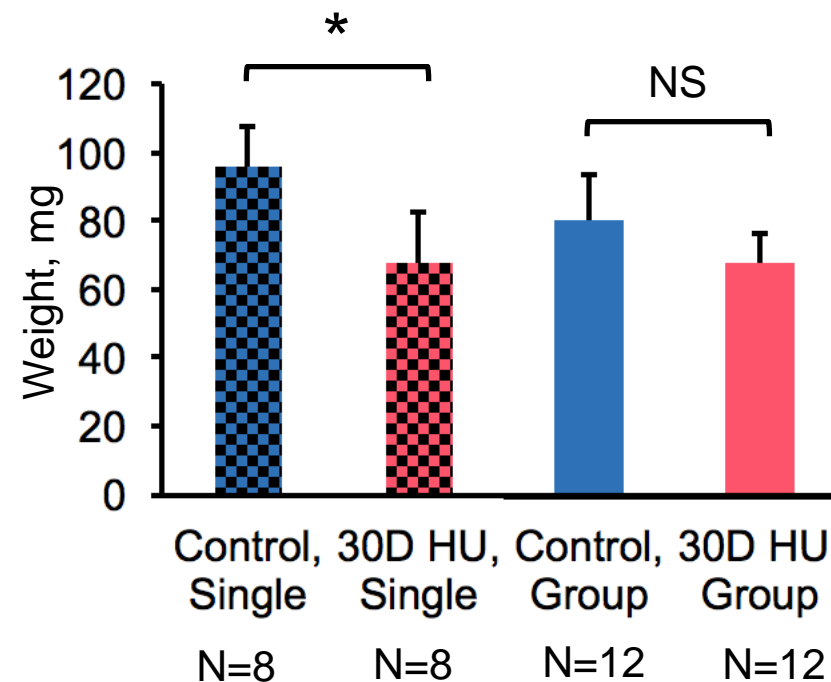
Group housing, C57BL/6J



\* $p < 0.05$  by t-test;  $N = 8$ /group

## Study 2

Single vs Group housing, C57BL/6NJ

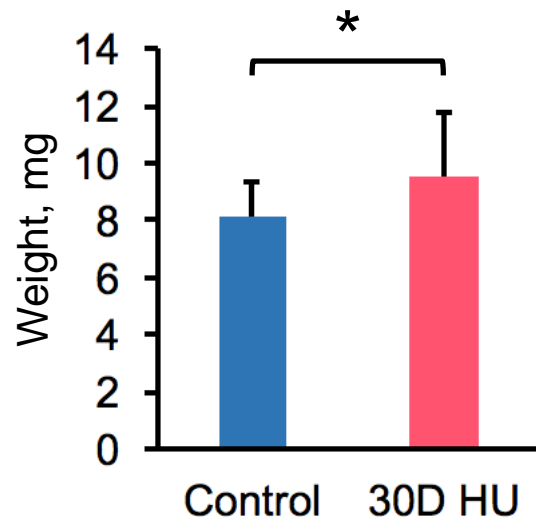


\*One-way ANOVA and Tukey-Kramer post-hoc test at  $p < 0.05$ ;  
NS: Not significant;

# Group housing HU leads to a modest increase in adrenal weights; not different from response to single housing HU

## Study 1

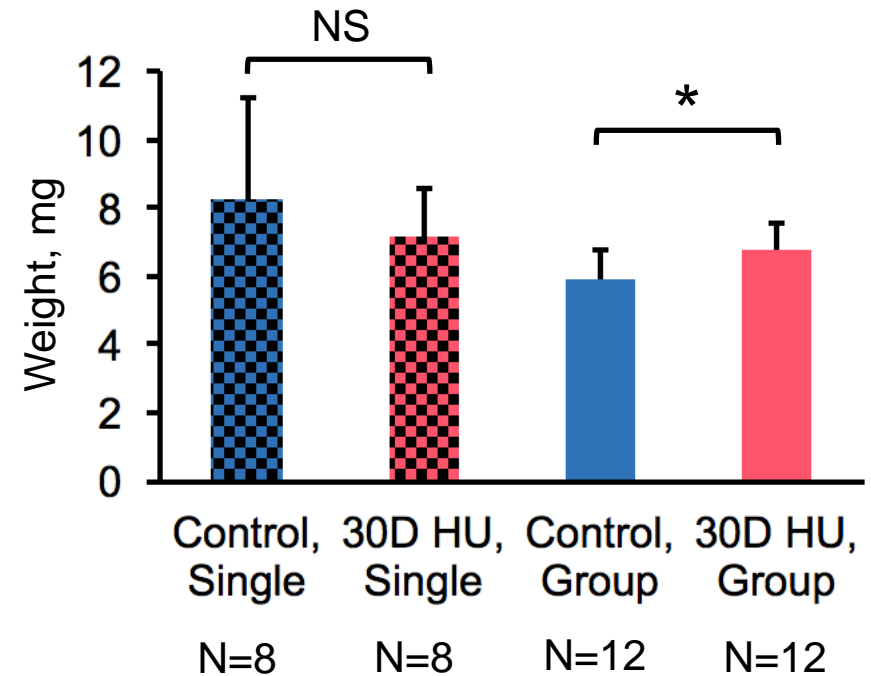
Group housing, C57BL/6J



\* $p < 0.05$  by t-test;  $N = 8$ /group

## Study 2

Single vs Group housing, C57BL/6NJ



\*Wilcoxon test at  $p < 0.05$ ;

NS: Not significant;

a: Not significant vs 30D HU Single

# Group and single housing HU: preliminary results from behavioral analysis

## Methods

- 24hr video acquisition (5 frame/sec) started on unloading day 28
- 15min samples analyzed within 3 hrs of lights on/off (12hr light:dark cycle)

## Qualitative observations

- Group-housed HU mice did not exhibit physical contact, although occasionally observed to ambulate toward cagemate
- Singly-housed HU mice look similar to group-housed in terms of behavioral repertoire
- Singly-housed control mice appeared fairly inactive

# Summary

- Group (paired housing HU) design
  - Portable, low footprint for storage
  - Compatible with automated vivarium cleaning equipment
  - Source materials readily available
  - Building requires standard tools
- Group housing HU (vs controls)
  - ↓ soleus mass, no change mass of type II fiber-enriched muscle (TA)
  - ↓ spleen mass; consistent with findings from 13-day spaceflight mission (Gridley et al 2013)
  - Slight increase in adrenal weights
- Single vs group housing HU differences: only in food consumption
- Group housing HU well tolerated

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