



National Aeronautics and  
Space Administration



# **Simulated weightlessness alters cardiomyocyte structure and transcriptional regulation of mediators related to immunity and cardiovascular disease**

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# Acknowledgements

## **NASA Ames Research Center**

Ruth Globus

Sonette Steczina

Masahiro Terada

Metadel Abegaz

Yasaman Shirazi-Fard

Ann-Sofie Schreurs

## **HRP HHC Tissue Sharing Initiative**

April Ronca

Laura Lewis

Eric Moyer

Moniece Lowe

And the rest of the team

## **Icahn School of Medicine at Mount Sinai**

David Goukassian

Maria Cimini

## **Lawrence Livermore National Laboratory**

Gabriella Loots

Aimy Sebastian

Nicholas Hum

## **UC Davis**

Charles Fuller and Team

## **Funding**

HRP Human Health Countermeasures (Globus)

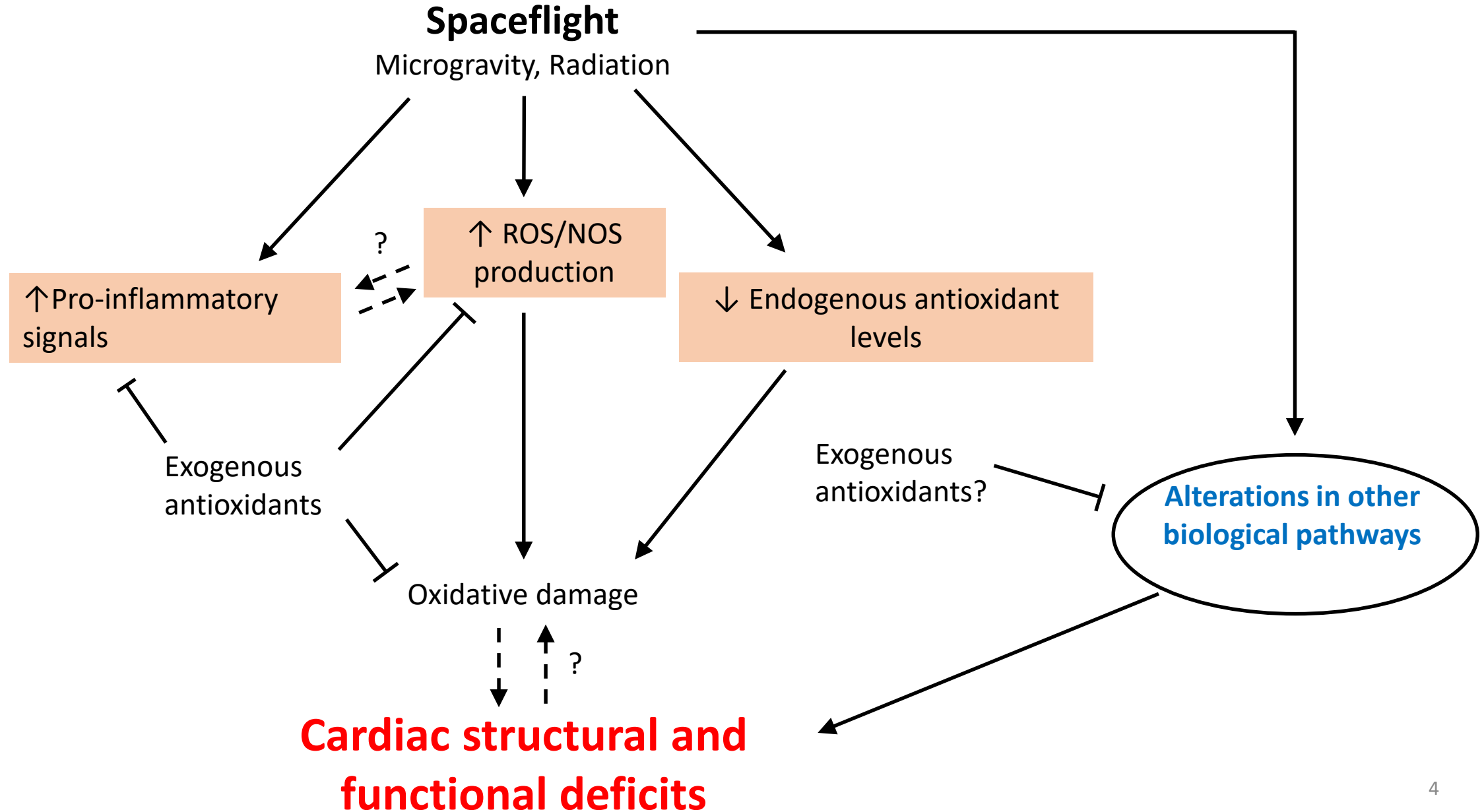
NASA Postdoctoral Program (NPP) Fellowship

(M. Terada, A.S. Schreurs)

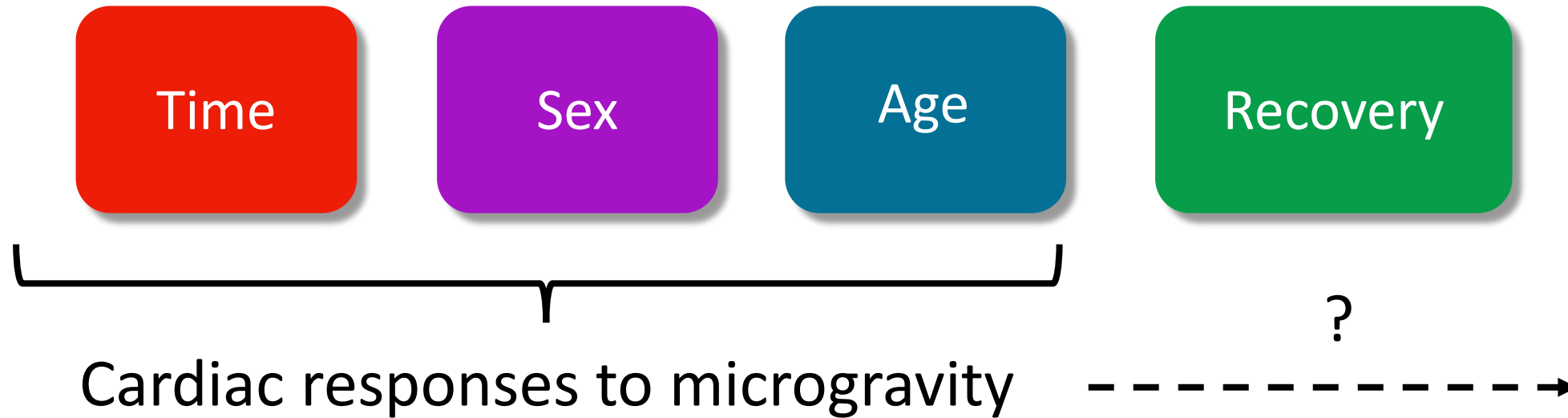
# Knowledge gaps: risk of CVD during spaceflight

- Time of exposure as a factor?
- Recovery possible?
- Age and sex-dependence?
- Mechanisms?

# Working model



# Study aims

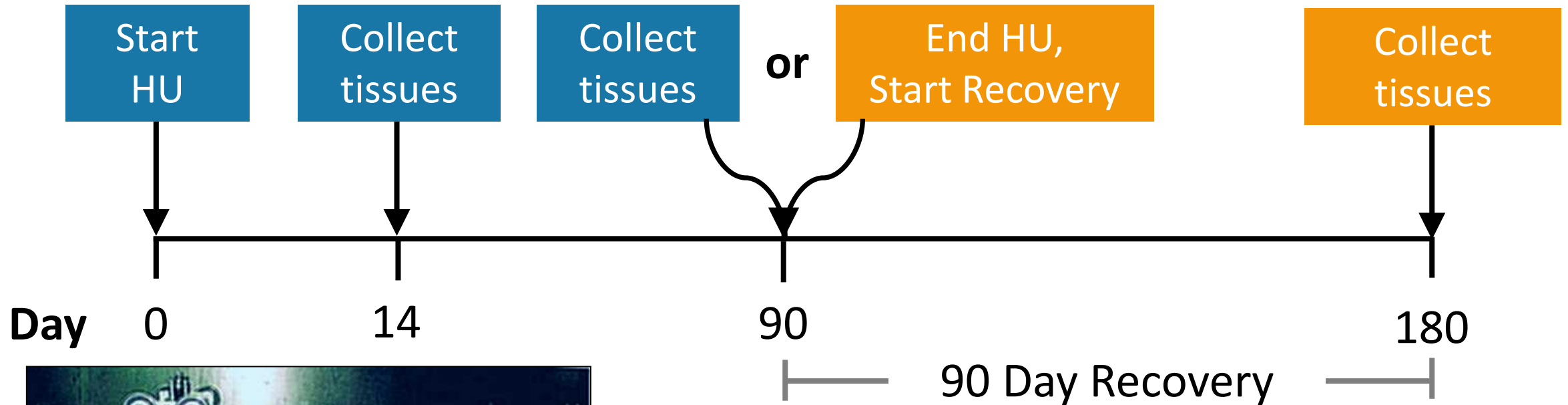


## Hypothesis

Prolonged exposure to simulated weightlessness and recovery  
→ changes in cardiac structure and expression of select genes  
including those involved in oxidative defense  
→ negatively impact cardiac tissue function

# Experiment design

## Hindlimb unloading (HU)



- Long Evans rats (not the strain in photo)
- Hearts collected and analyzed

# Experimental groups

NL: Control  
 HU: Hindlimb unloading  
 Rec: Reambulation

Sex

Age

Time

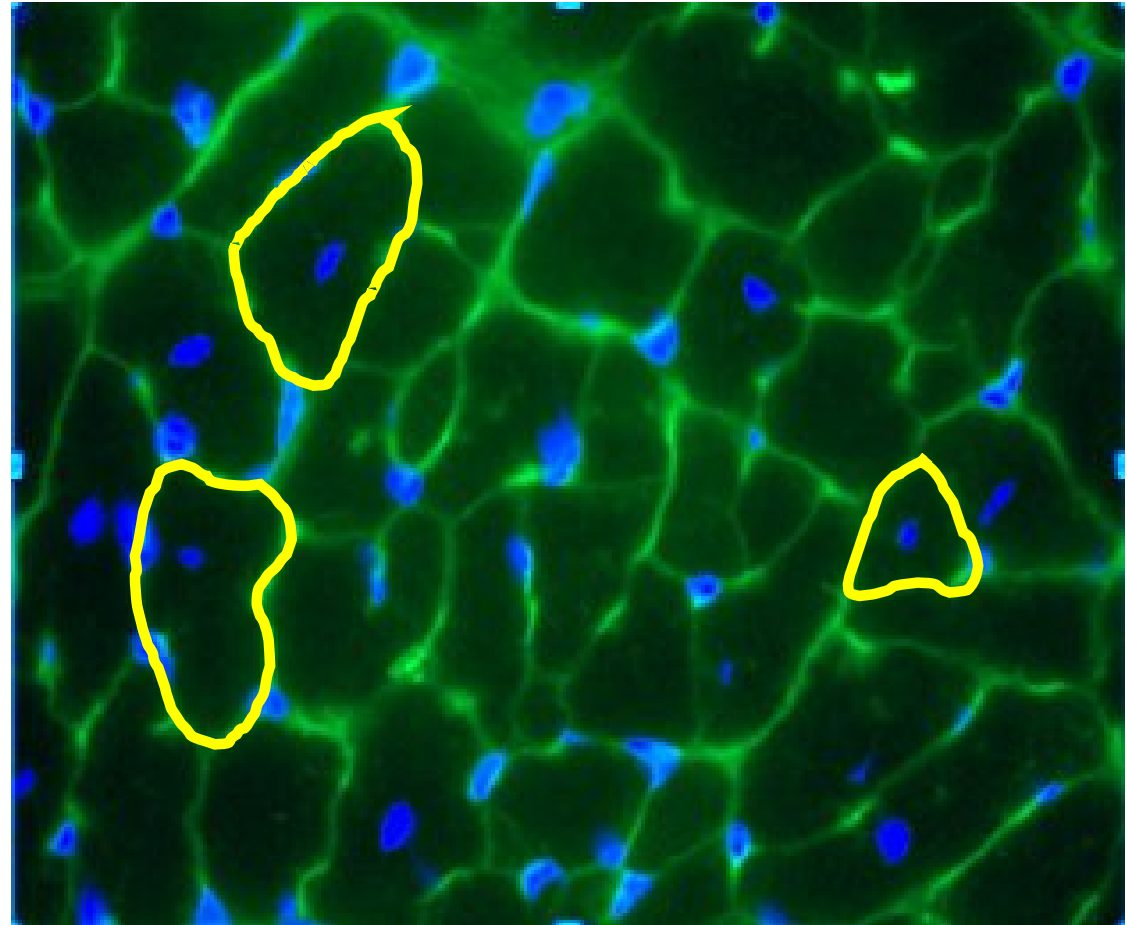
Recovery

Treatment and duration	Young female (3 months)	Young male (3 months)	Older male (9 months)
14 days NL	7	7	7
14 days HU	8	6	7
90 days NL	-	-	3
90 days HU	-	-	3
90 days NL + 90 days Recovery	-	-	4
90 days HU + 90 days Recovery	-	-	5

# Cardiomyocyte structural changes in response to spaceflight

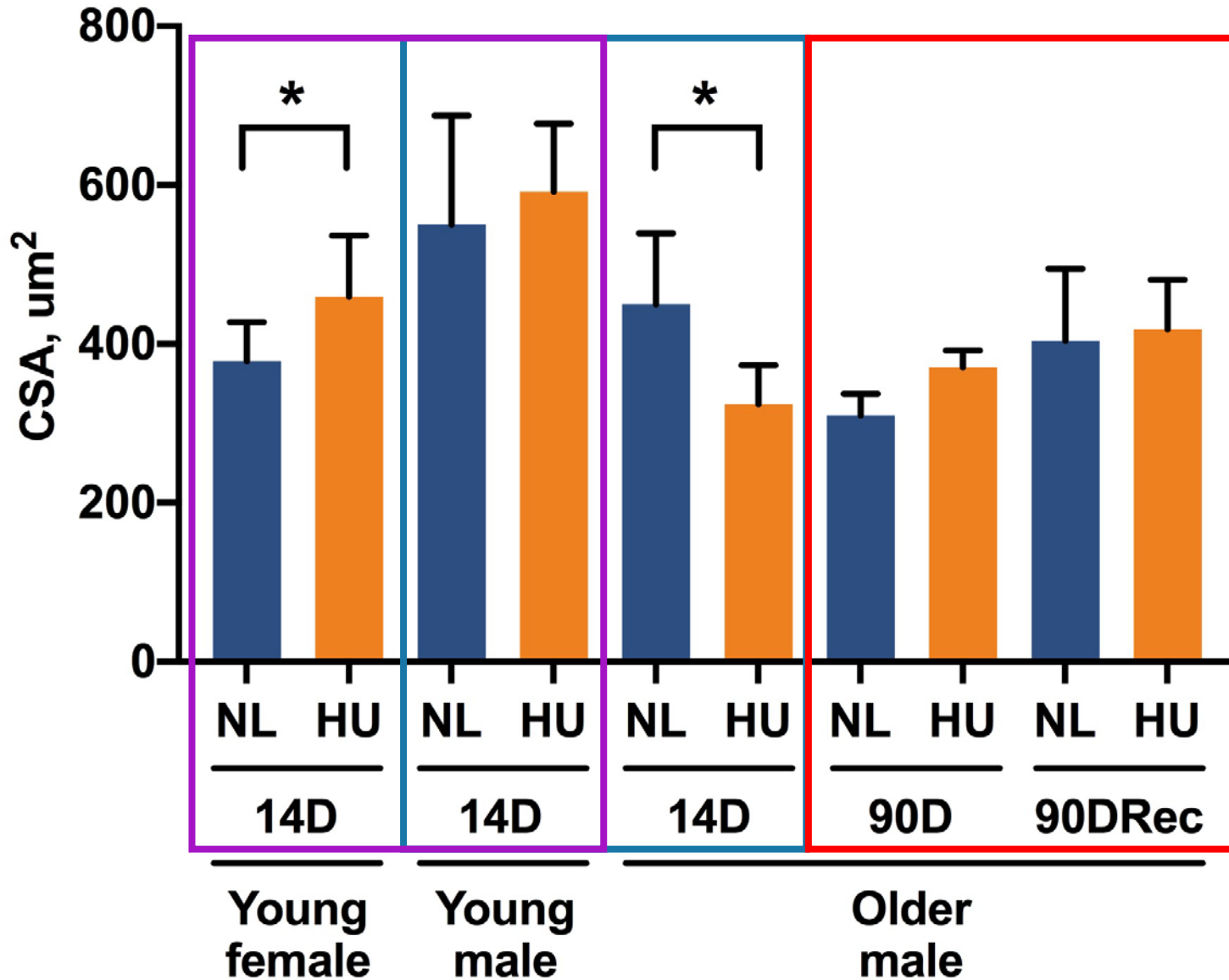
- Cell size changes seen in some heart pathologies
- In rats, 14 days of spaceflight leads to decreased cardiomyocyte size (Goldstein et al. 1992)
- Measure left ventricular wall cardiomyocyte cross sectional area (CSA)

WGA | DAPI





# HU-induced CSA changes are age- and sex-dependent, older males show no persistent deficits from prolonged HU



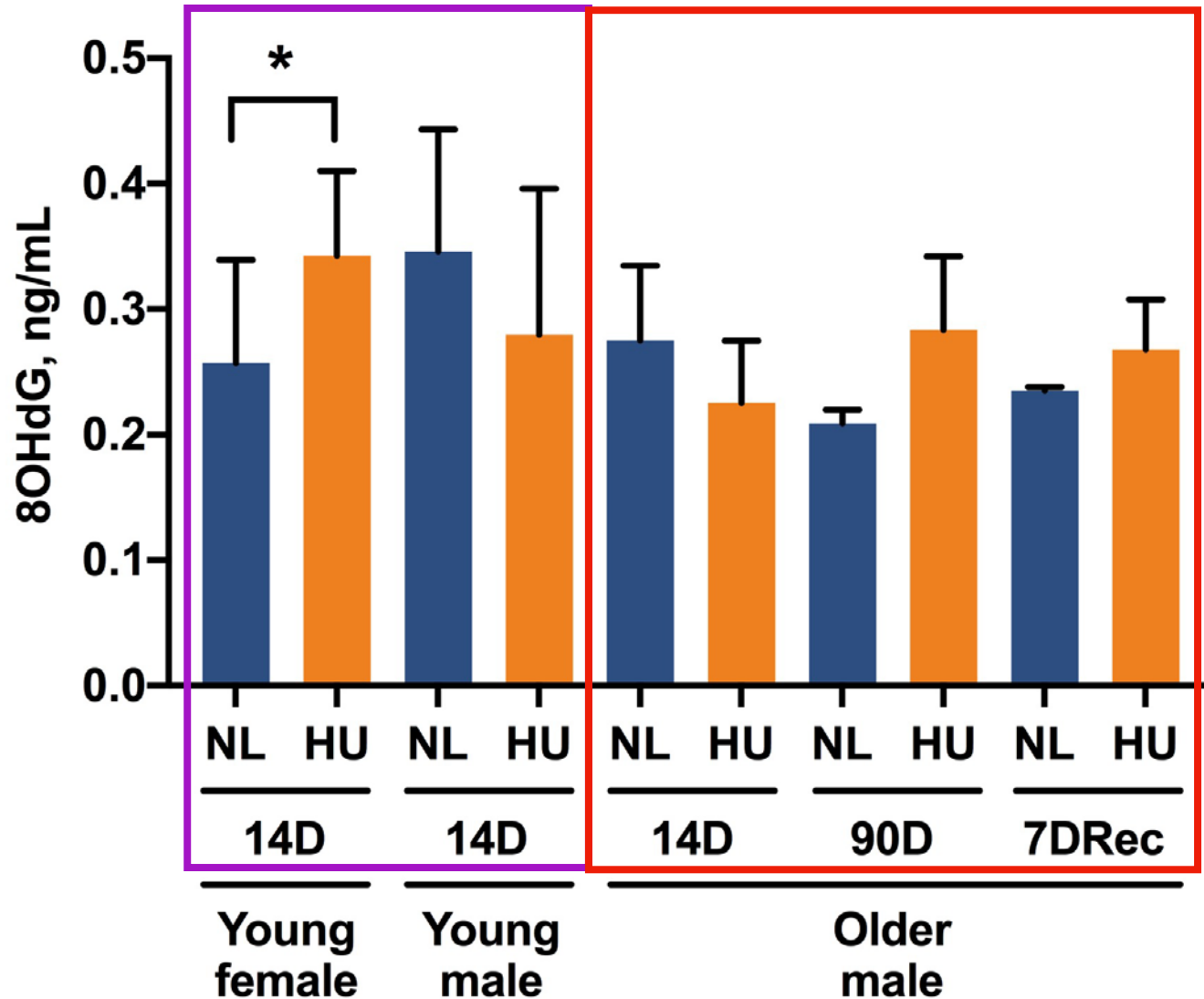
**Sex** Females respond to HU with increased CSA but no change in young males

**Age** Older males more sensitive to effects of HU than young males

**Time** No differences in NL and HU groups at 90 days and reambulation

\* $p < 0.05$ , means and SD shown

# Sex differences in levels of oxidative damage marker 8-OHdG during short-term HU



**Sex** In young females, HU increased 8-OHdG; No change in young males

**Age** No statistically significant changes caused by HU in males at all timepoints (although trends observed)

**Time**

**Recovery**

\*p < 0.05, means and SD shown

# Summary of findings: HU alters redox signaling

Groups, HU vs NL	8-OHdG	sod1, sod2
Young female, 14D	UP	-
Young male, 14D	-	UP
Older male, 14D	-	-
Older male, 90D	-	-
Older male, Rec	-	-

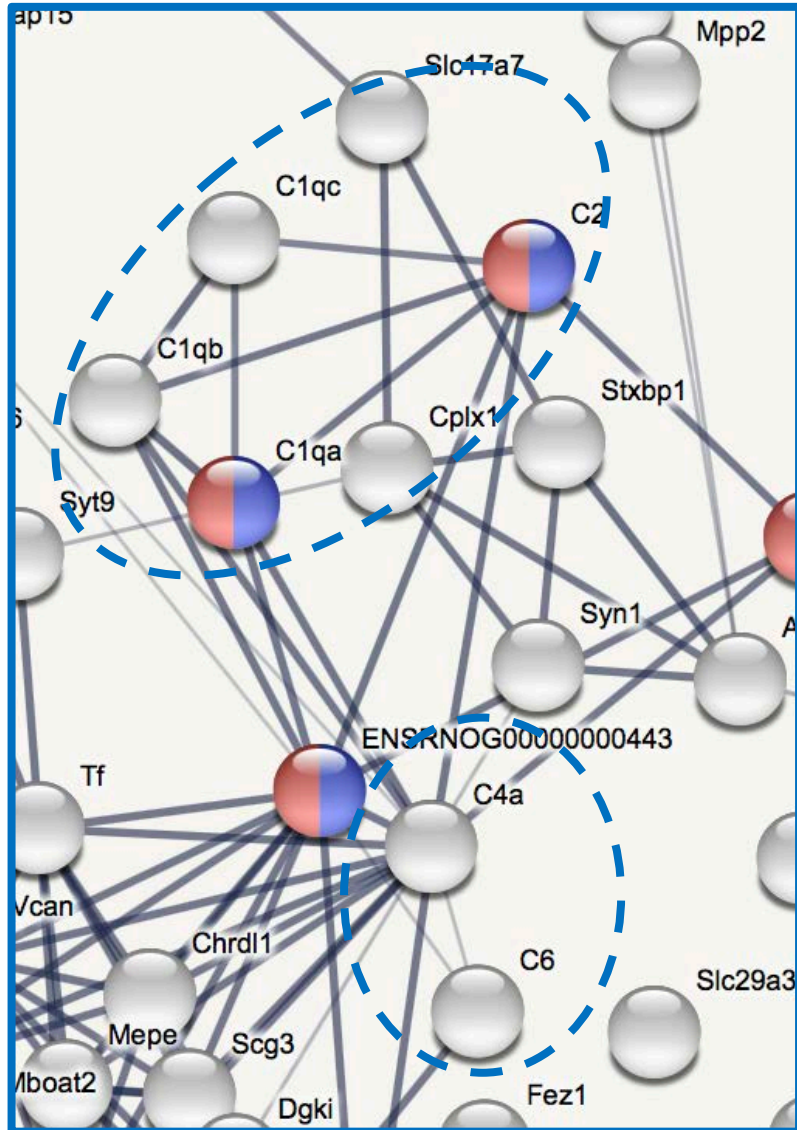
**SOD1 and SOD2:** Converts superoxide radicals to molecular oxygen and hydrogen peroxide → water + oxygen via catalase

**NFE2L2 (NRF2):** Master transcription factor with >100 antioxidant gene targets

# Summary of findings: HU alters redox signaling

Groups, HU vs NL	8-OHdG	sod1, sod2	nfe2l2
Young female, 14D	<b>UP</b>	-	-
Young male, 14D	-	<b>UP</b>	<b>DOWN</b>
Older male, 14D	-	-	<b>DOWN</b>
Older male, 90D	-	-	<b>DOWN</b>
Older male, Rec	-	-	<b>UP</b>

# RNAseq: Inflammatory processes and complement activation upregulated at 14D HU in older males vs controls



## Select top enriched processes

- ROS/RNS metabolic process
- Complement activation, classical pathway
- Inflammatory response
- Leukocyte migration

## Some enriched genes also immunomodulatory drug targets

**Rituximab:** Anti-CD20, Ab-based treatment for rheumatoid arthritis

**Alemtuzumab:** CLL drug; also immune suppressant, MS drug

# Summary: Relevance of rat HU findings for humans in deep space missions

## Structural changes in heart:

- In older males, decreased CSA during short term HU (14 days)
- With prolonged reambulation (90 days HU = ~7 human years in space), CSA can recover fully (*ventricular wall thickness too?*)
  - *Maybe humans, too?*

## Gene expression and RNAseq results:

- Females more susceptible to HU-induced oxidative damage at least in short-term
  - *Targeted countermeasure for females: higher dose of antioxidants?*
- Persistent downregulation of nfe2l2 in older males
  - *Decreased ROS production in HU? Downregulation of nfe2l2 a concern (i.e. SPE's)?*
- Upregulation of complement activation and inflammatory processes in short-term HU
  - *Dysregulation of local immune response? Needs mitigation?*
- Confirms value of rat HU model for human health and countermeasure research
  - *Shared genes regulated by HU and immunomodulating drugs*
  - *Animal-to-animal variability within group and between specific genes; like human crew*



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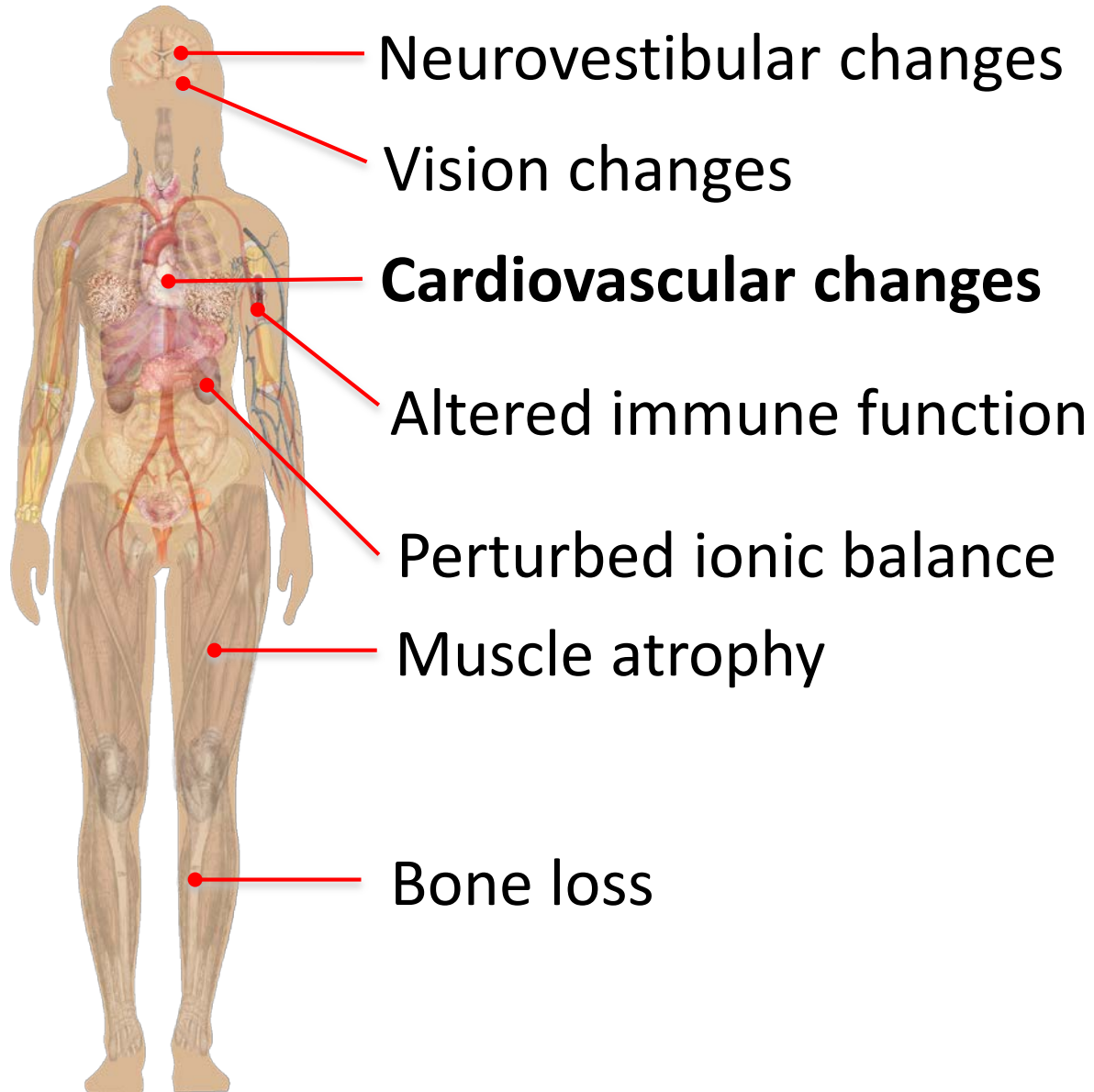
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# Spaceflight alters most organ systems

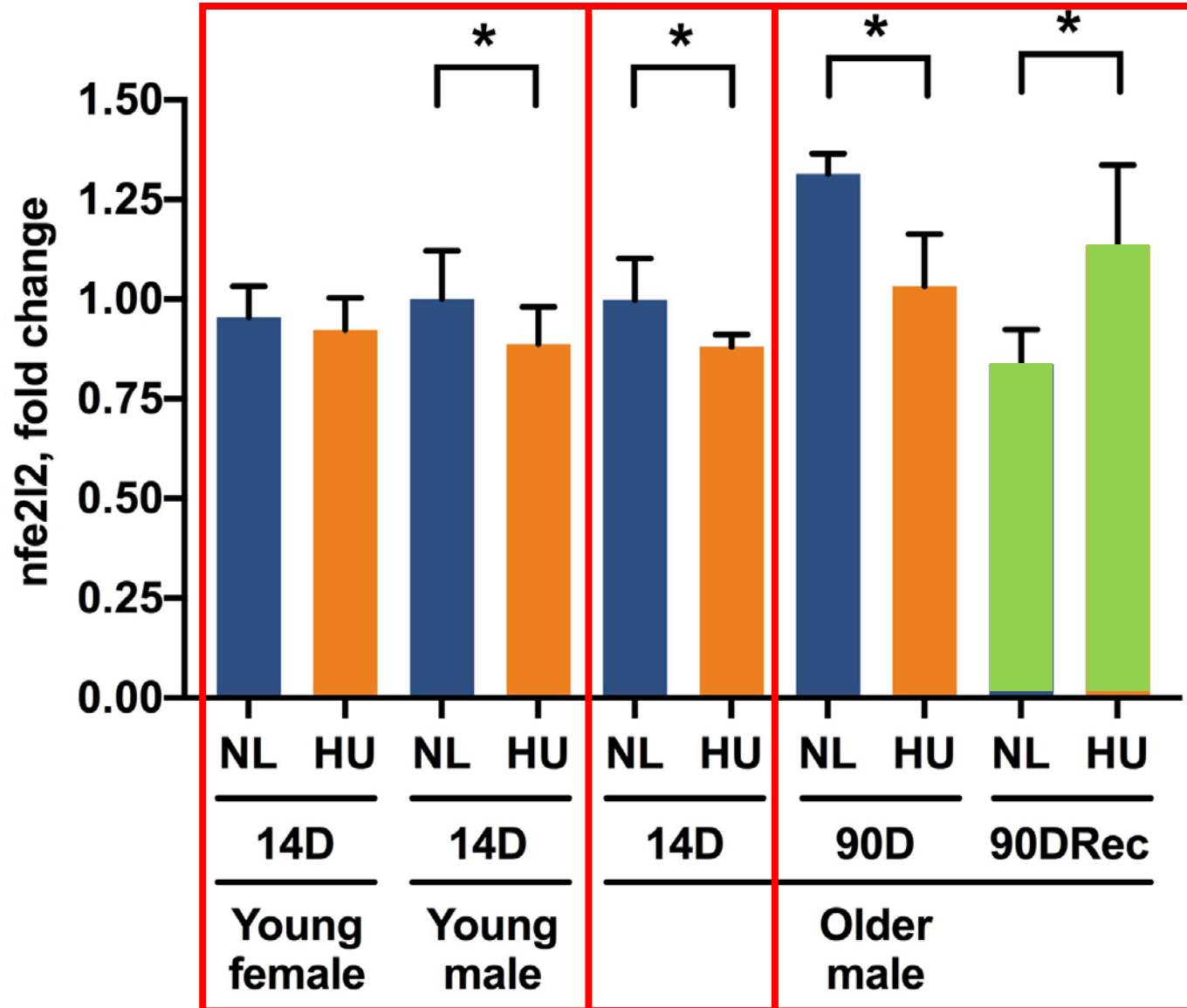


*Greater challenges during deep space missions:*

**prolonged exposure to microgravity + low dose radiation**



# HU downregulates key antioxidant transcriptional regulator *nfe2l2*; sex-dependent responses



**NFE2L2 (NRF2):** Master transcription factor with >100 antioxidant gene targets

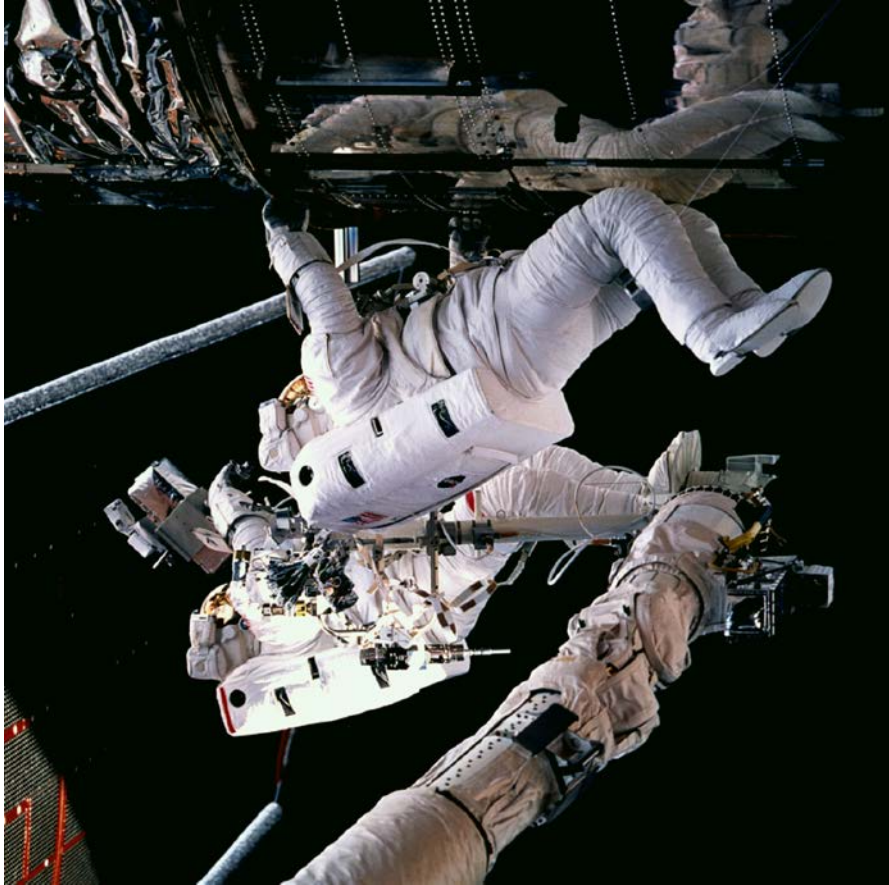
- Young and older males show decreased expression of *nfe2l2* after 14D HU; not in young females
- Downregulation persists at 90D HU in older males; reambulation reverses response

# HU can disrupt redox balance; disruption may persist through recovery phase in older males

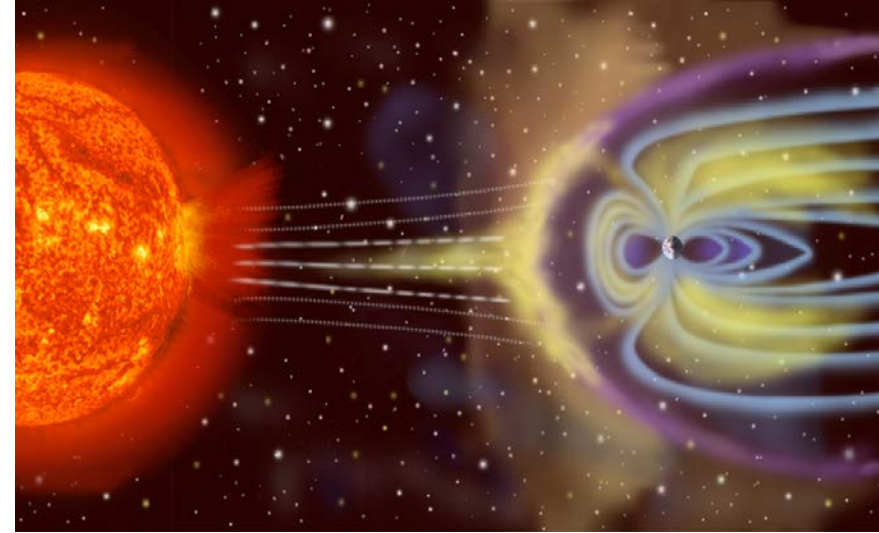
Groups, HU vs NL	8-OHdG	sod1, sod2	nfe2l2	CSA
Young female, 14D	UP	-	-	UP
Young male, 14D	-	UP	DOWN	-
Older male, 14D	-	-	DOWN	DOWN
Older male, 90D	-	-	DOWN	-
Older male, Rec	-	-	UP	-

# Components of the spaceflight environment

## Weightlessness



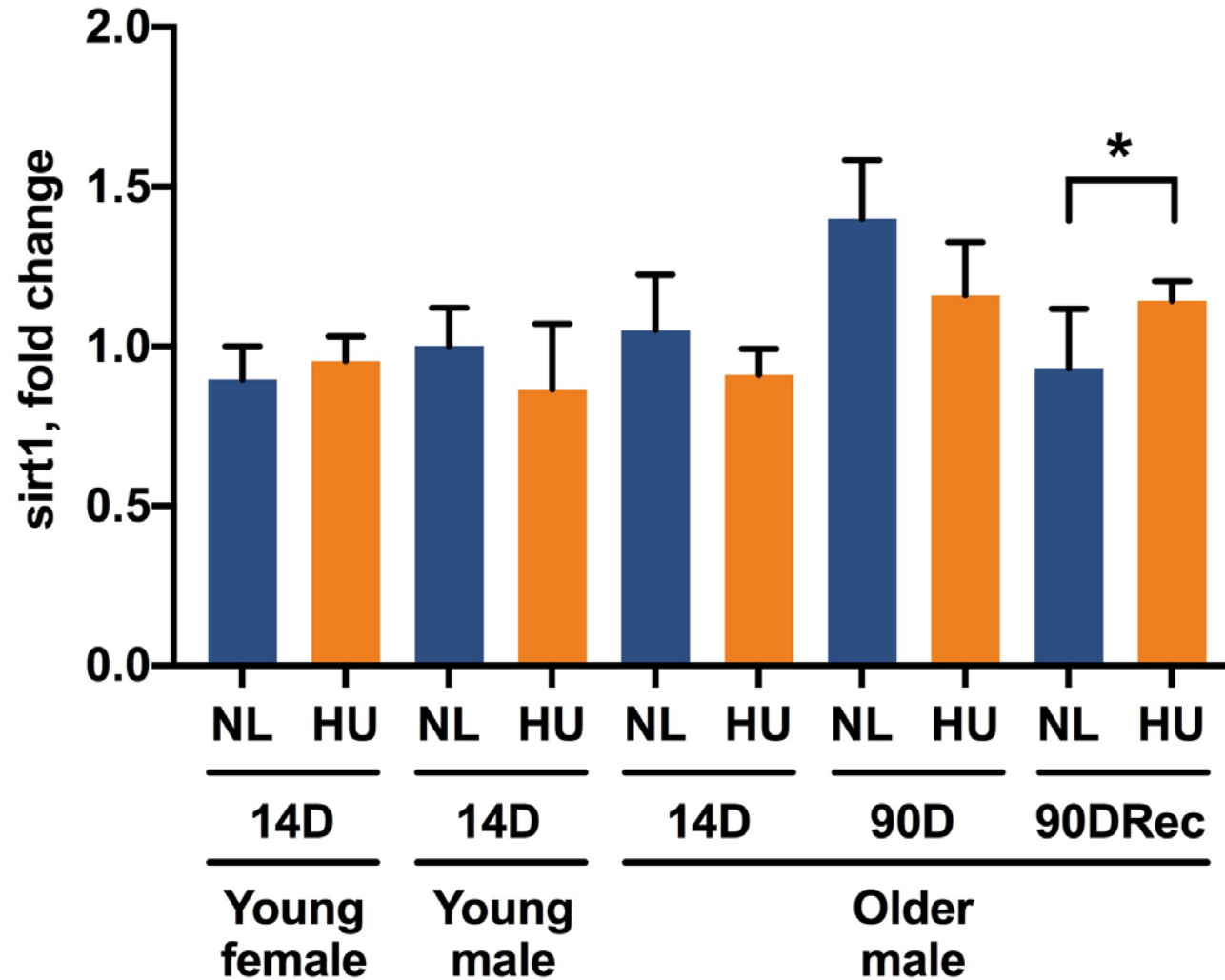
## Ionizing radiation



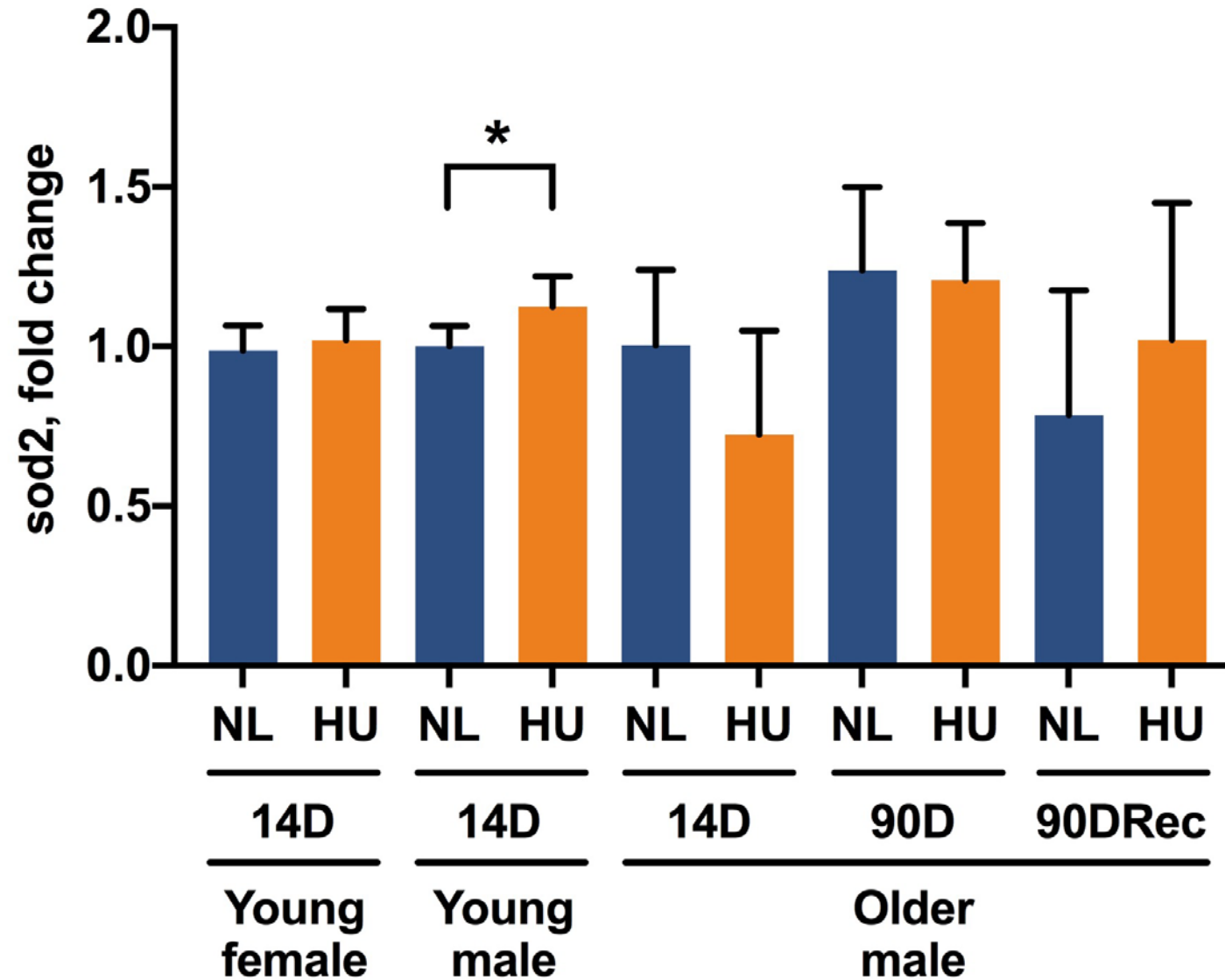
## Others

- Demanding workload
- Confined environment
- Elevated CO<sub>2</sub>
- Sleep disruption
- Dietary changes

# qPCR results, sirt1

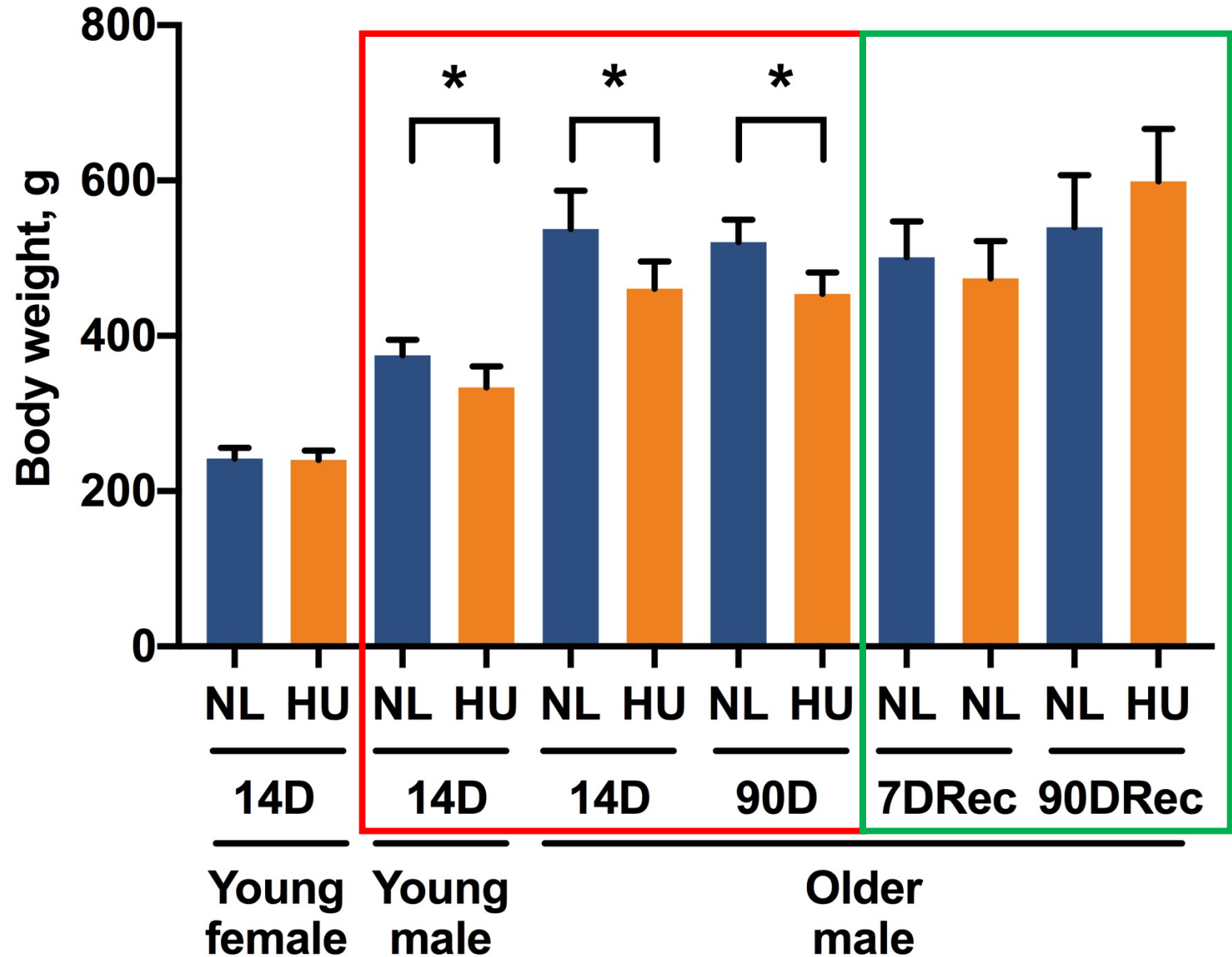


# qPCR results, sod2

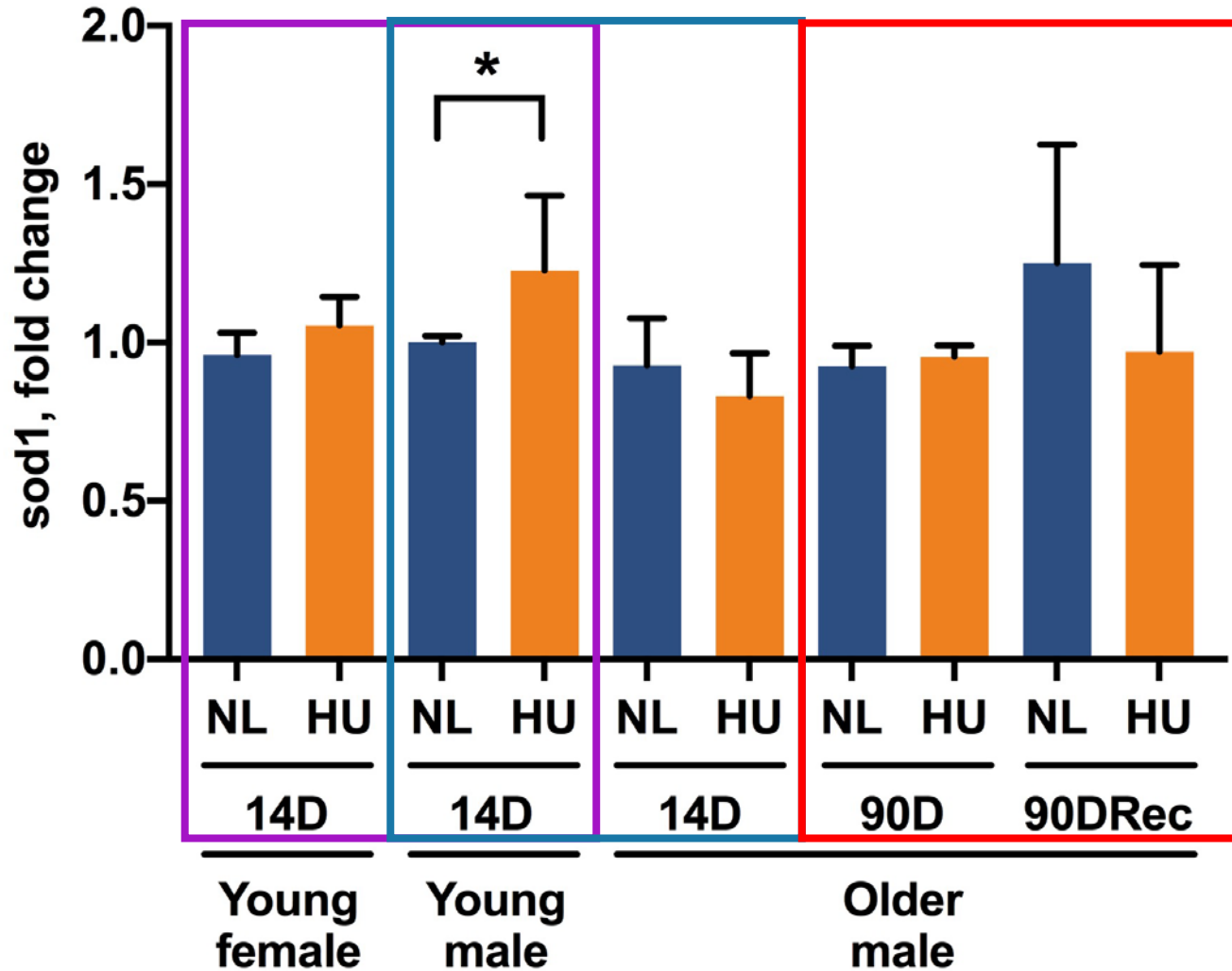


# Body weights

- Male HU groups (regardless of age) show modest decrease in body weights vs NL controls
- No differences in body weight between HU vs NL controls after reambulation



# Antioxidant responses to HU: sex and age differences



**SOD1:** converts superoxide radicals to molecular oxygen and hydrogen peroxide; cytosolic

Sex

Increased sod1 expression at 14 days HU in young males; not in young females and old males

Age

Time

No differences between HU and NL controls at 90 days and reambulation

Recovery

**Same results for Sod2 (mitochondrial)**

# In males, HU can alter the expression of nfe2l2, a major transcriptional regulator of the antioxidant response

Groups, HU vs NL	nfe2l2
Young female, 14D	-
Young male, 14D	<b>DOWN</b>
Older male, 14D	<b>DOWN</b>
Older male, 90D	<b>DOWN</b>
Older male, Rec	<b>UP</b>

**NFE2L2 (NRF2):** Master transcription factor with >100 antioxidant gene targets

- No change in HU females vs NL controls
- HU in males alters nfe2l2 expression