Aging and Oxidative Stress: Insights from Spaceflight

NASA HQ

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Ruth K. Globus, Ph.D.

Space Biosciences Division

NASA-Ames Research Center

A Big Goal

Long duration, human habitation in space - stay healthy during and after

Topic today: how living in space causes changes in the human body that resemble age-related diseases on earth (like osteoporosis), and how we experimentally explore coping responses.

Big goal calls for big questions: for long duration habitation

- **What** biological changes (relevant to human health) occur and **when**?
  - how far do adverse changes progress?
  - what (if anything) to do about them?

- **How** do these changes come about?
  - what are the fundamental mechanisms at the molecular, cellular and physiological, levels?
    - to better understand human biology and disease on Earth
    - can we now better predict and identify interventions?
      - taking the guess work out

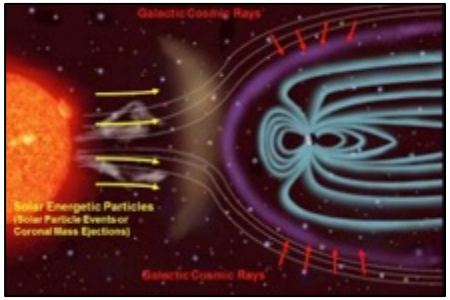
- **What** is the relationship between mechanisms and effects of aging vs. spaceflight?

Outline

- Spaceflight challenges and consequences
- Oxidative stress and aging: close relations
- Rodents as analogues for humans
- Skeletal biology
  - Molecules to cells to tissue health
- Experimental approaches & results
  - Groundbased models
  - Spaceflight
- Conclusions and directions

Challenges to human biology of living in space

Microgravity
- Fluid shifts toward upper body
- Loss mechanical stimulation; relative disuse



Sources of ionizing radiation in space
- Solar Particle Events
- Galactic Cosmic Radiation
- Van Allen belts

Species
- Predominantly protons
- High-Z, high-energy (HZE particles)
- Secondary (primarily gamma)

- Nutrition
- Demanding workload
- Sleep disruption/circadian
- Confined environment
- Elevated carbon dioxide

Some responses to these challenges on earth understood, but not well enough to predict consequences with lengthy, deep space missions.

Consequences

Bone

Muscle

Cardiovascular

Balance (vestibular)

Sensory

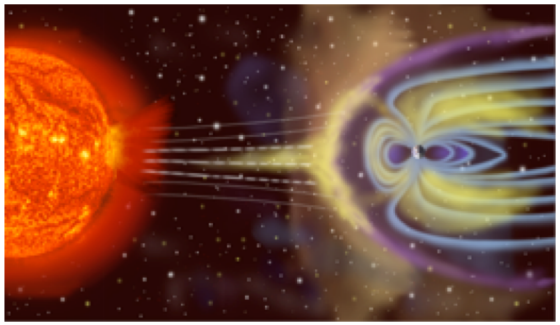
Blood

Immune

Vision

Hormones

Hypothesis



Reactive Oxygen Species **(ROS)** in excess
e.g. O_2^-, OH^-, H_2O_2

Oxidative damage
DNA, proteins, lipids

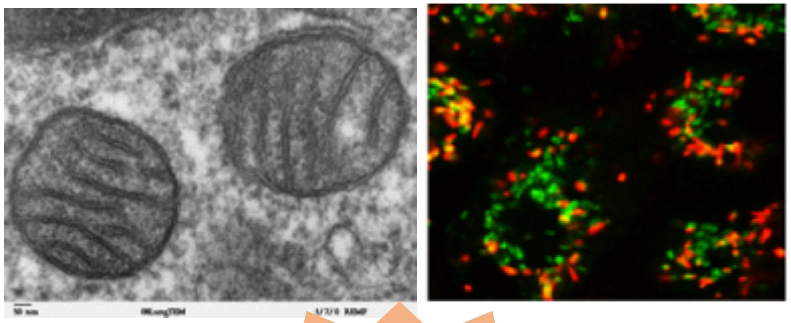
Tissue degeneration
Inflammation

Disrupt cell-cell and intracellular signaling

Accelerated aging of tissues?
- Skeletal tissue
- Cardiovascular
- Central Nervous System

Oxidative stress and aging: close relations

Mitochondria
the 'powerhouse of the cell'



Reactive Species
NO, H_2O_2
$O{\cdot}2^-$, $OH^{\cdot}$, H_2O_2

Harlan 1950's: Free Radical Theory of Aging

"Eustress"

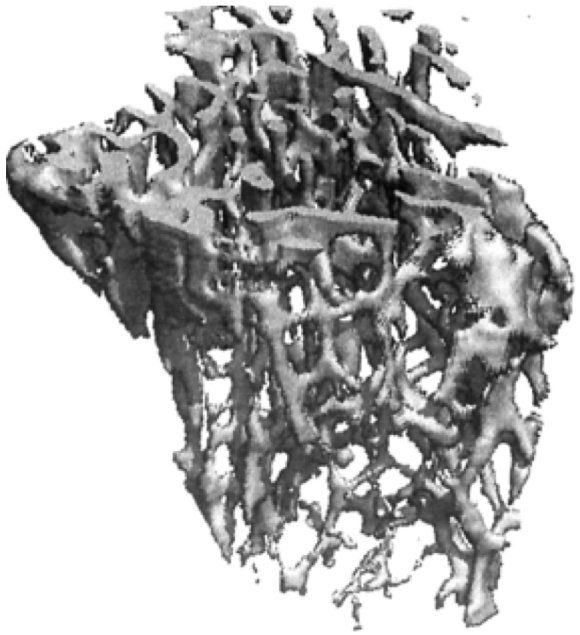
Tissue Maintenance: Nominal function

"Distress"

Tissue Breakdown: Failed renewal

Stem cells---Mature cells

Challenges leading to bone loss in humans and rodents

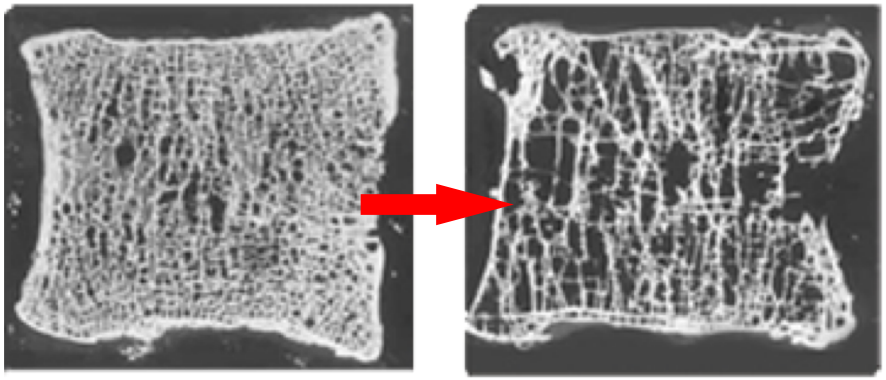


Challenges:

Aging
Microgravity
Radiation
Disuse
Hormonal changes

Mouse

Human



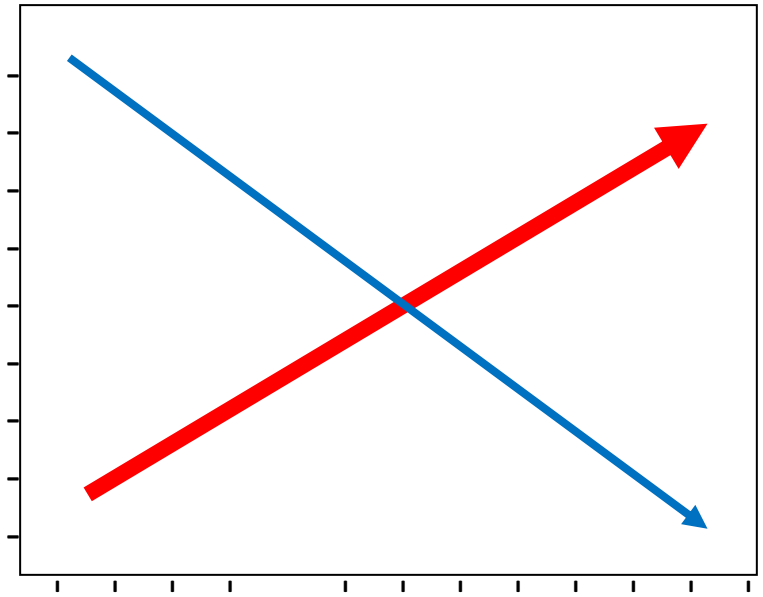
Normal Bone

Osteoporotic Bone

* permission to use image granted by Turner Biomechanics Laboratory.

Excess reactive oxygen species in skeletal disease

ROS Production (marrow)



Vertebral Strength (mPa)

Mouse Age: Growing Adult Advanced Age

Excess production of free radical species contributes to osteoporosis

Quenching mitochondrial H_2O_2 selectively in cells responsible for bone resorption (osteoclasts) protects from ovariectomy-induced osteoporosis (Bartell et al. Nature Comm 2014).

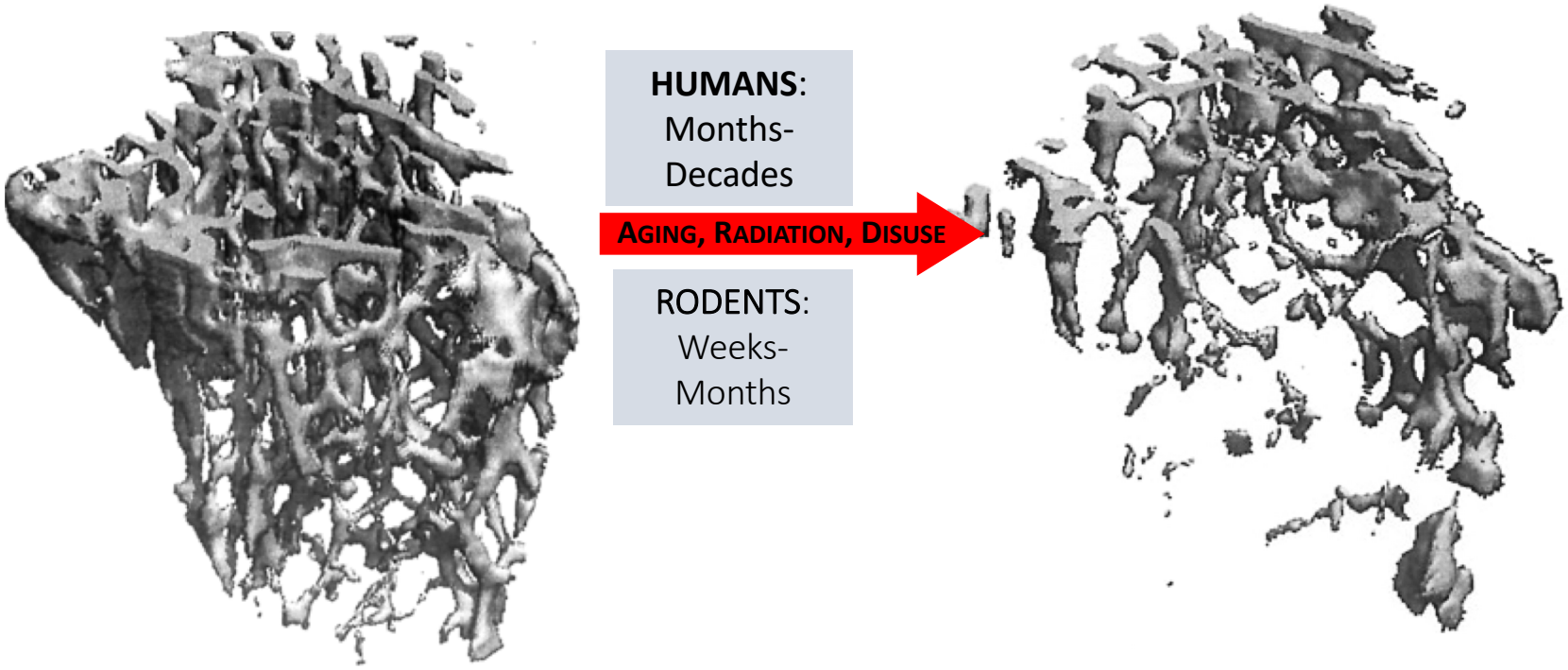
Why rodents? Compressed timescale

Lifespan

Human: ~70-90yr Mouse: ~2yr

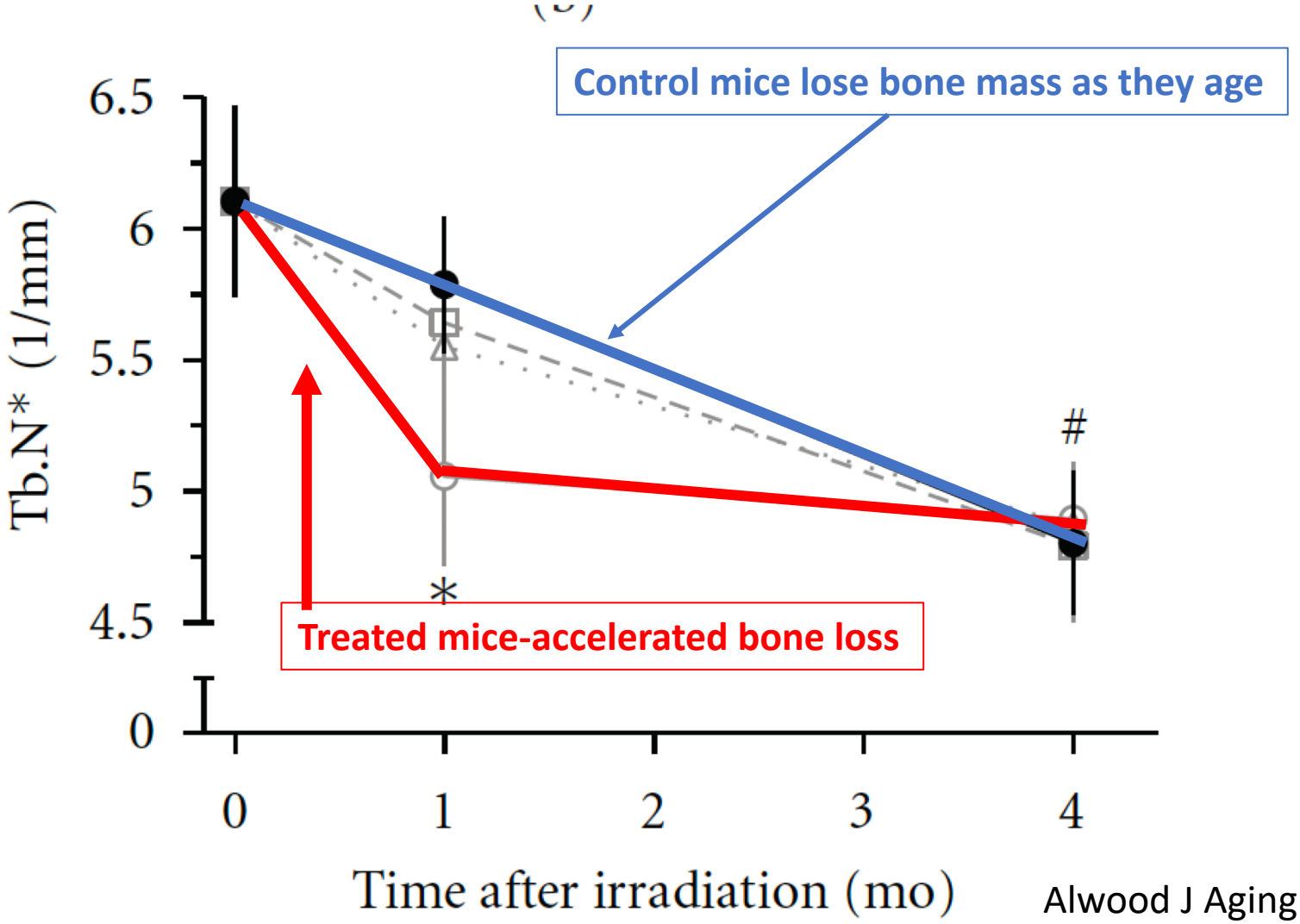
Despite big difference in lifespan- get similar age-related diseases.

Comparing rates of bone loss (osteoporosis) in humans and rodents



So, a few months rodent flight experiment ≈ years of astronaut time in space

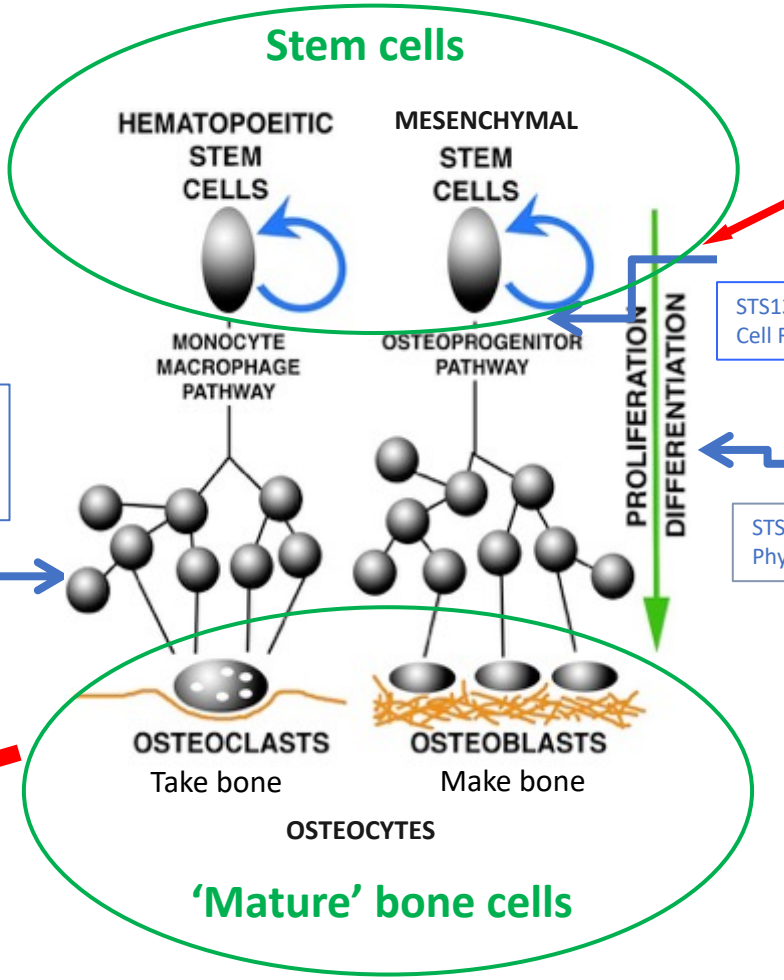
Bone loss with aging



Alwood J Aging Res

Cellular mechanisms for controlling bone health: rodent spaceflight experiments

Spaceflight affects cells at various stages during growth and maturation



STS131: Blaber et al. Stem Cell Res 2014. 12:181

Foton M3: Tamma et al.,: FASEB J. 2009 Aug; 23(8):2549-54. Epub 2009 Mar 27.

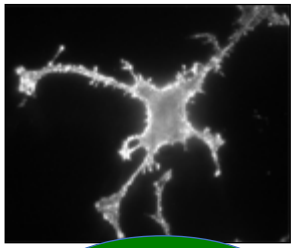
STS3: Roberts et al. Am J Physiol 1987; 252:R247-51.

BONE LOSS

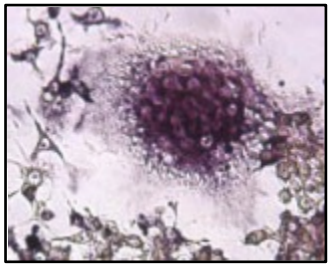
Endogenous ROS/RNS signaling in health

BONE

OSTEOBLAST LINEAGE
(mesenchymal stem cells)



NO/ROS

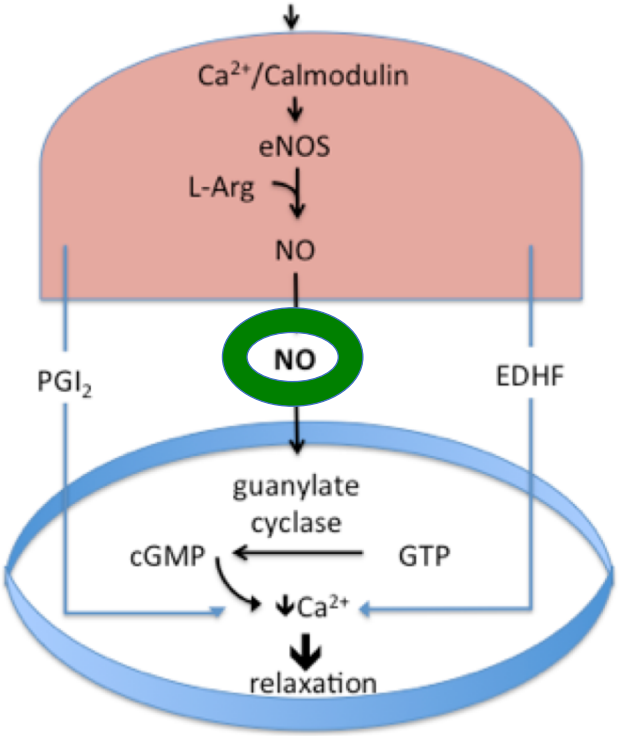


OSTEOCLASTS
(haematopoetic-myeloid)

ROS

MICROVESSEL

VASCULAR ENDOTHELIUM



VASCULAR SMOOTH MUSCLE

Together, defining bone structure

Together, defining vessel function

Groundbased Analogues

To simulate weightlessness and space radiation

To define mechanisms- manipulate expression of specific molecules

To test potential interventions

Our toolbox: simulated spaceflight using ground-based analogs for rodents

Hindlimb unloading (HU) to simulate weightlessness

-fluid shift

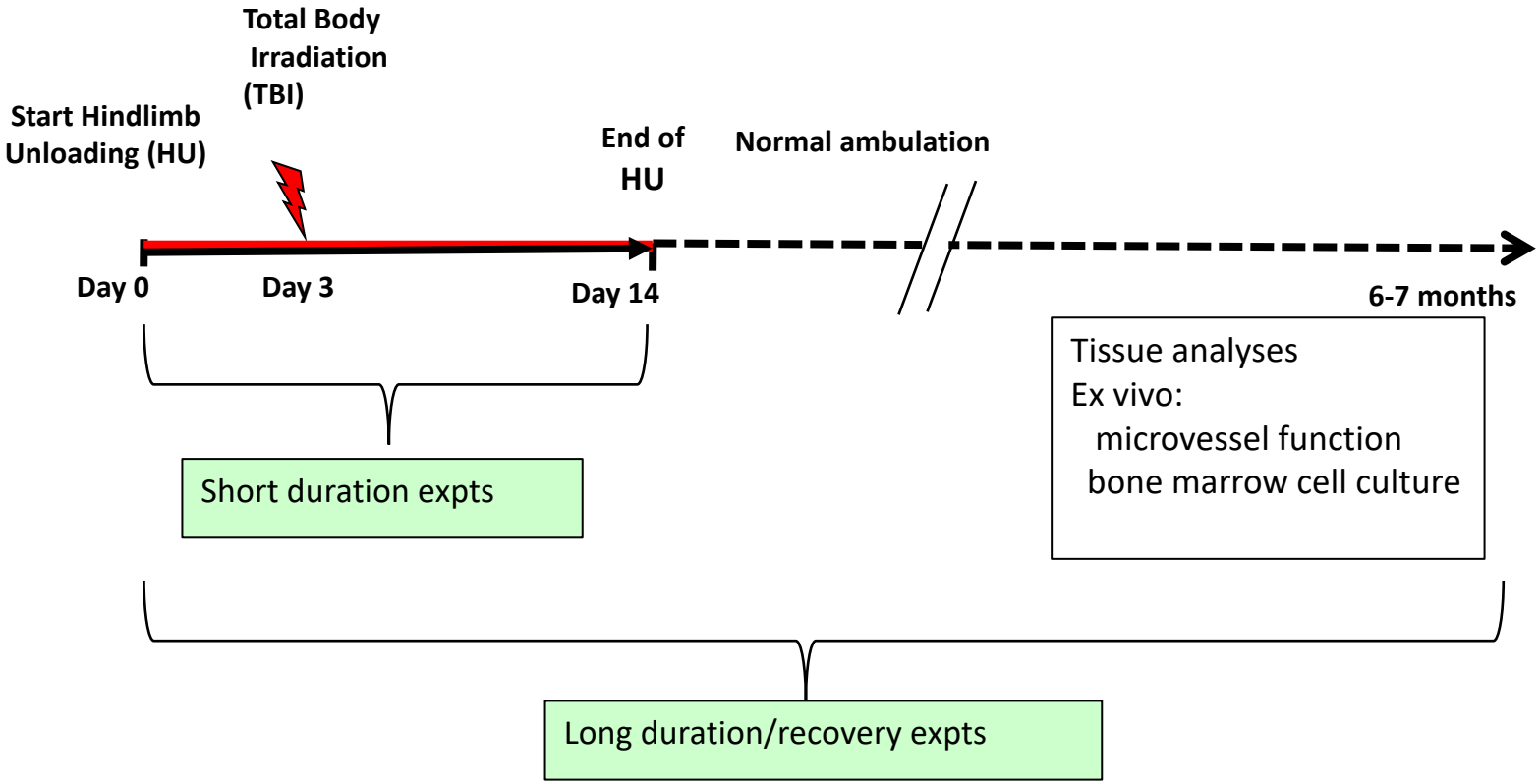
-musculoskeletal disuse

Space radiation simulations

Low LETz/High LET

NASA Space Radiation Lab or gamma/x-ray

Experimental design



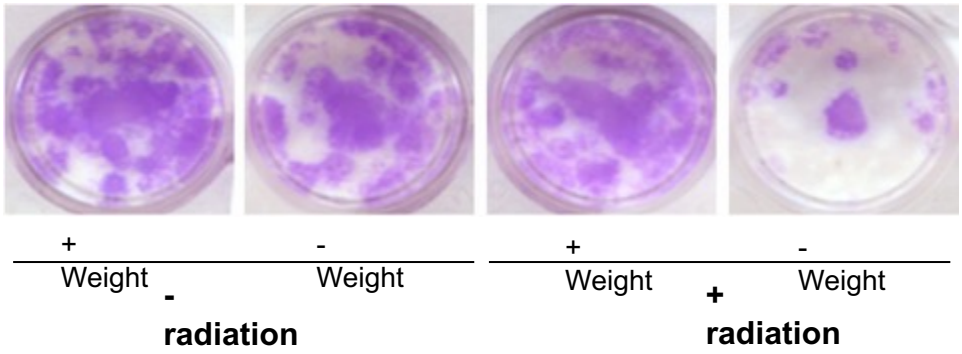
Experimental Groups: 2X2

- Normally loaded (NL)
- Hindlimb Unloaded (HU)
- Irradiated (TBI)
- Hindlimb Unloaded + Irradiated

Total body irradiation:

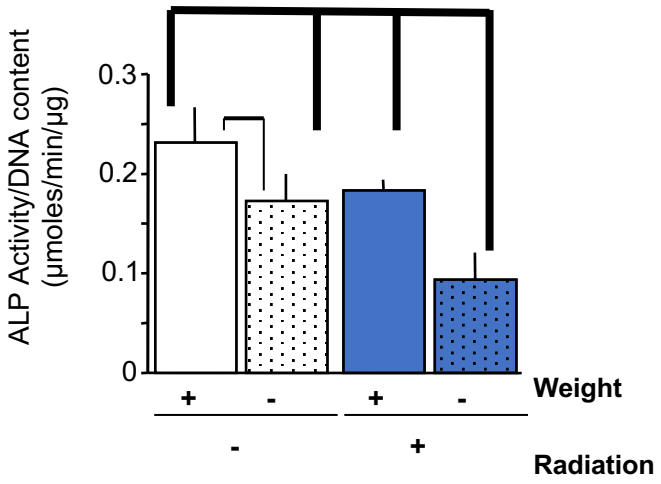
- Gamma ^{137}Cs (1-2Gy)
- HZE ^{56}Fe (0.5-2Gy, 600MeV/n)
- Protons ^{1}H (0.5-2Gy/150,MeV/n)
- Dual: ^{1}H (0.5Gy)/^{56}Fe (0.5Gy)

Radiation and simulated weightlessness inhibit growth and differentiation of bone-forming stem cells



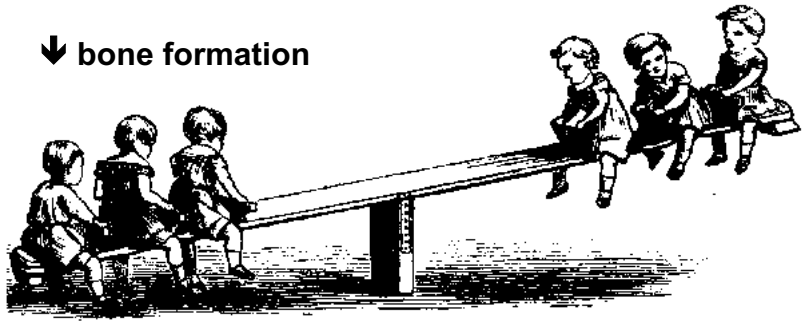
SUMMARY

DIFFERENTIATION

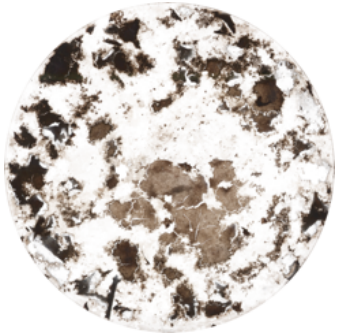


bone resorption

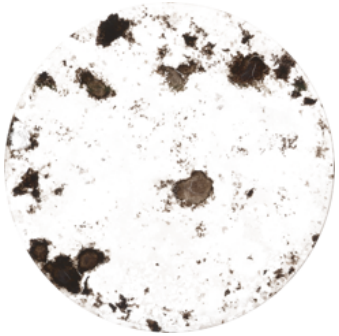
bone formation



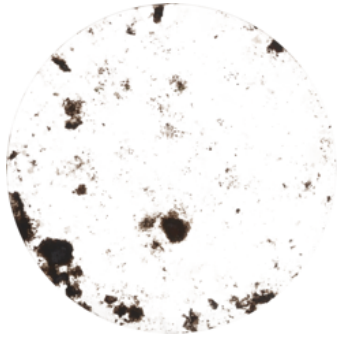
Heavy ion radiation persistently damages bone-forming stem cells



Sham (0Gy)

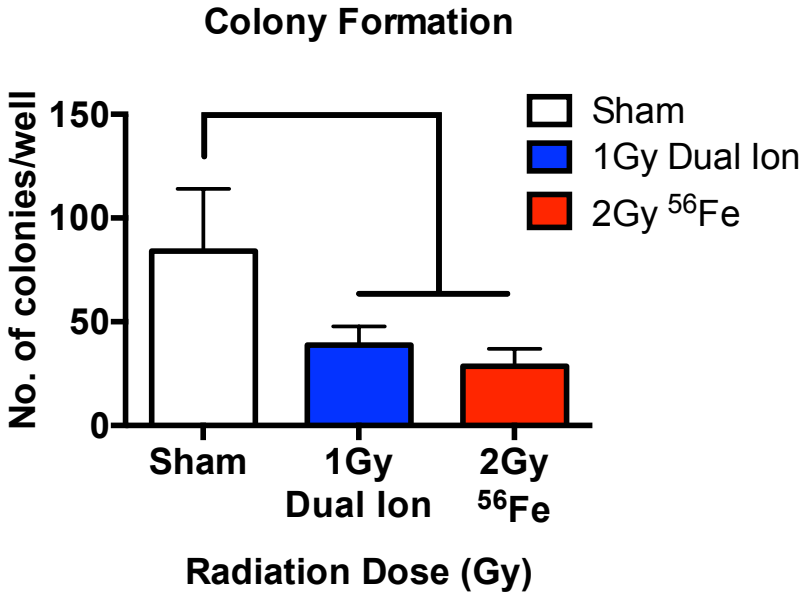


Dual Ion (1Gy)



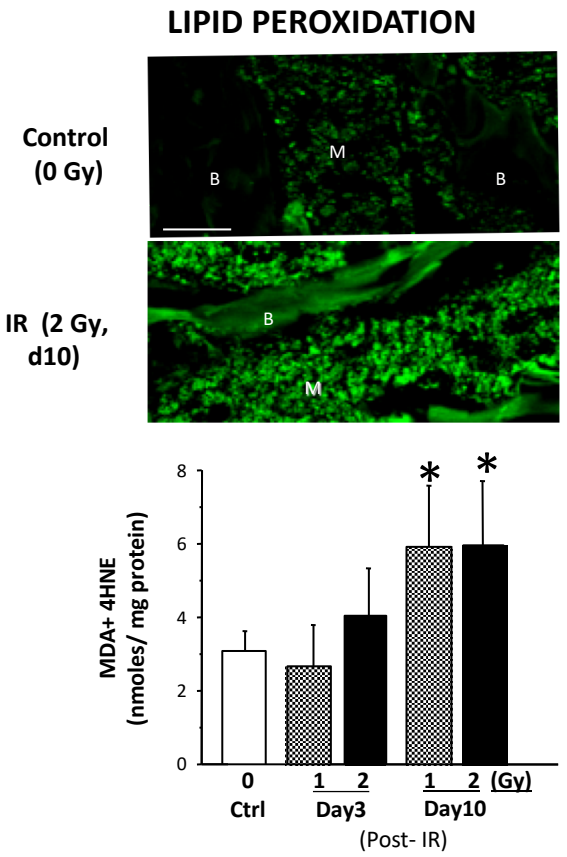
^{56}Fe (2Gy)

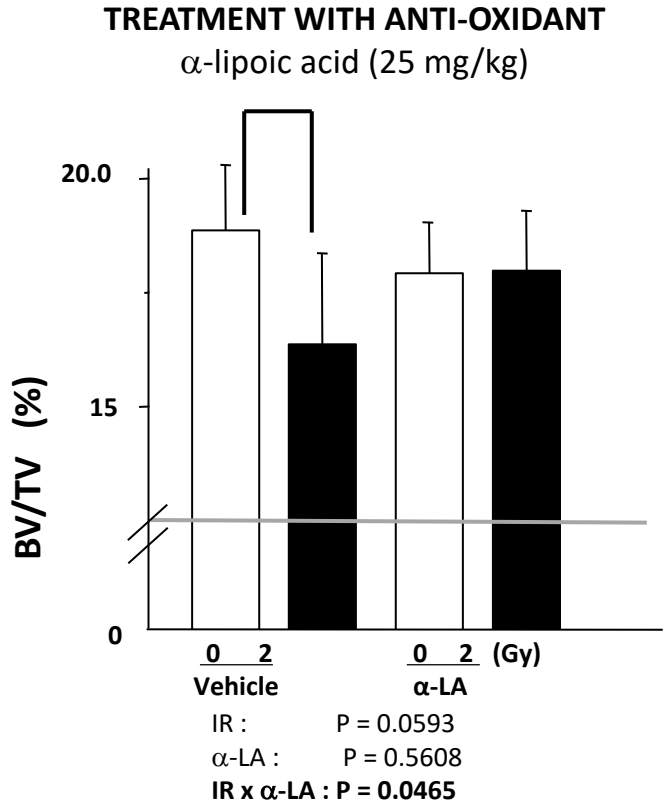
6-months after exposure



Oxidative stress in acute radiation-induced bone loss (3d)

Kondo et al. J Appl Physiol 2010

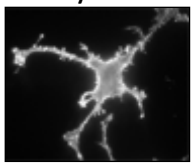




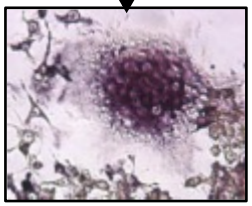
Endogenous ROS/RNS signaling for adaptive responses

BONE

OSTEOBLAST LINEAGE
(mesenchymal stem cells)



NO/ROS



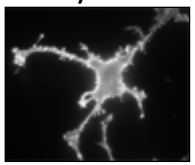
OSTEOCLASTS
(haematopoetic-myeloid)

Together, defining bone structure

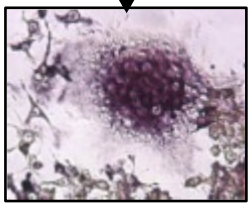
Endogenous ROS/RNS signaling for adaptive responses

BONE

OSTEOBLAST LINEAGE
(mesenchymal stem cells)



NO/ROS

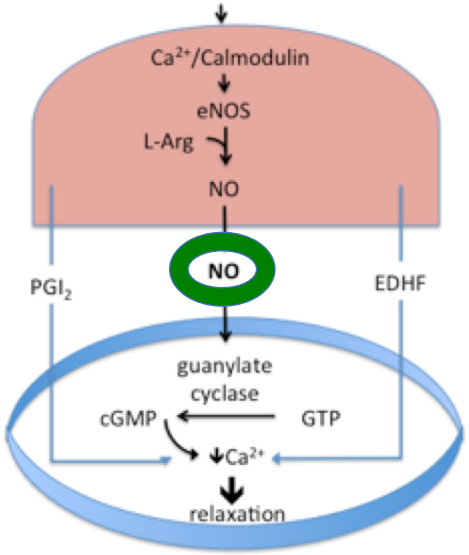


OSTEOCLASTS
(haematopoetic-myeloid)

Together, defining bone structure

MICROVESSEL

VASCULAR ENDOTHELIUM



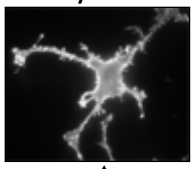
VASCULAR SMOOTH MUSCLE

Together, defining vessel function

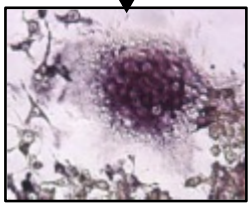
Endogenous ROS/RNS signaling for adaptive responses

BONE

OSTEOBLAST LINEAGE
(mesenchymal stem cells)



NO/ROS



OSTEOCLASTS
(haematopoetic-myeloid)

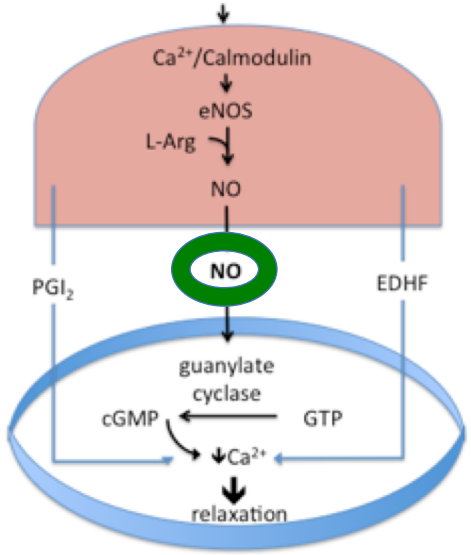
↑ROS

Bone:
Morikawa
Mundy
Manolagas
+

CV:
Berkowitz
Kucik
Goukassian
Connor&Hood
+

MICROVESSEL

VASCULAR ENDOTHELIUM

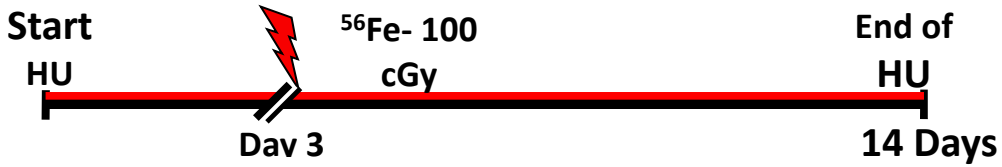


VASCULAR SMOOTH MUSCLE

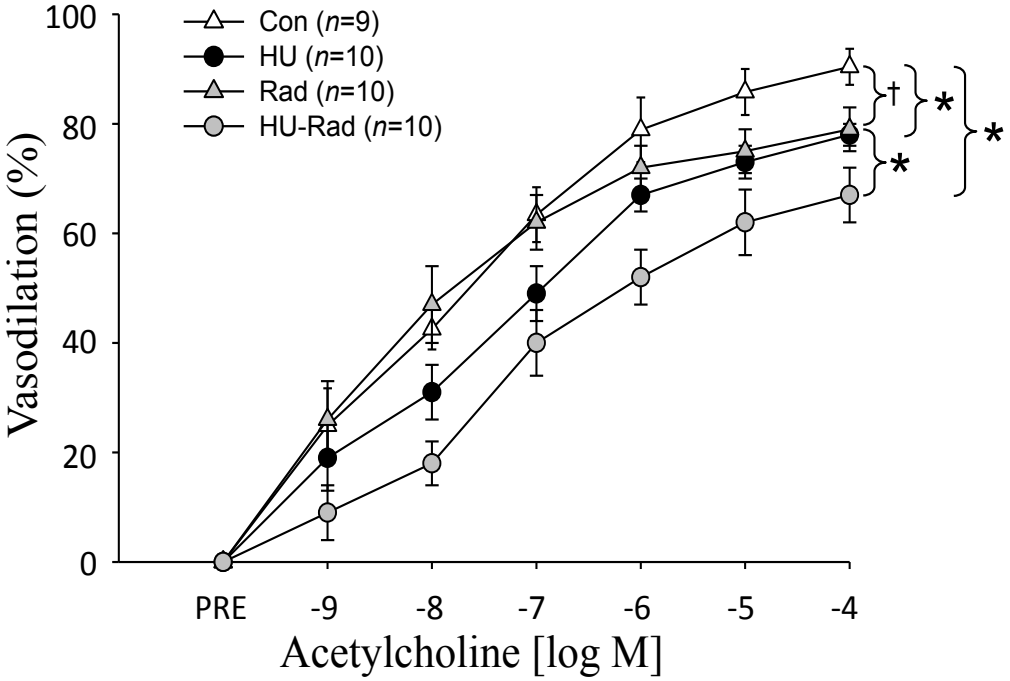
Together, defining bone structure

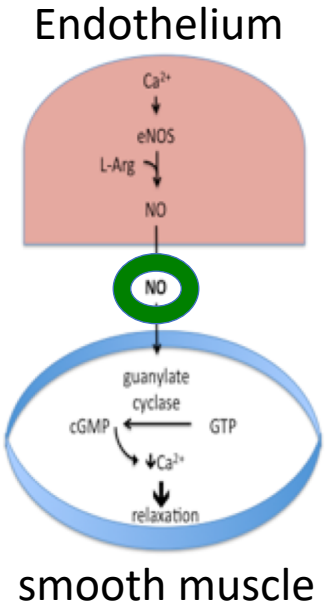
Together, defining vessel function

Vascular dysfunction: ^{56}Fe and HU impair peak endothelium-mediated vasodilation in the gastrocnemius feed artery



Mike Delp
Brad Behnke

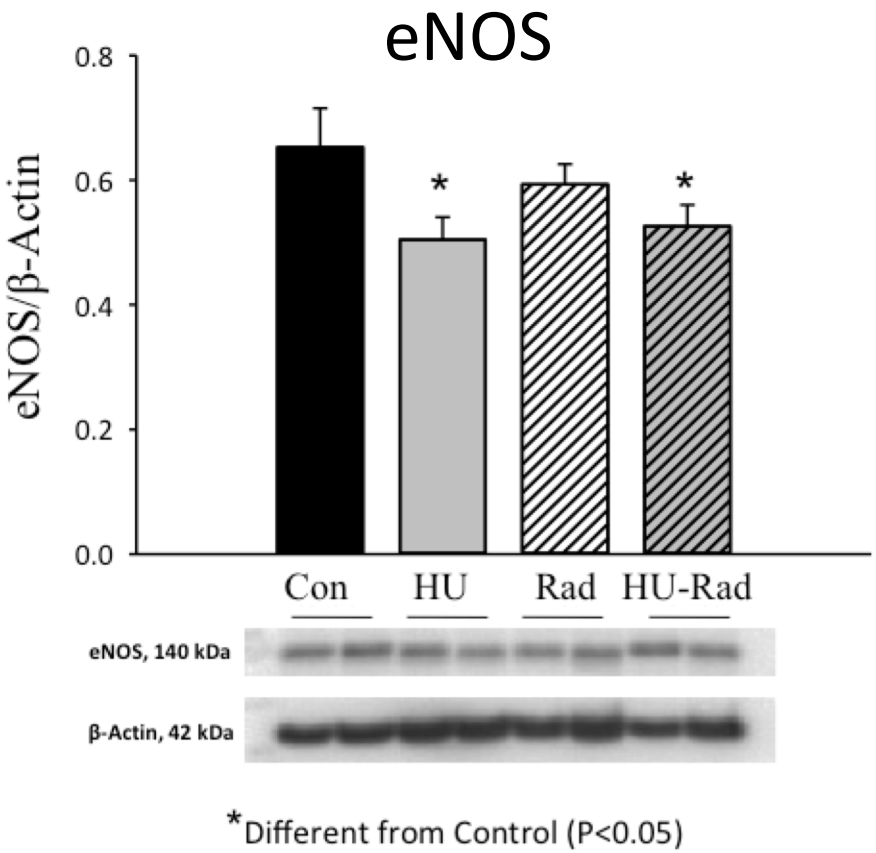


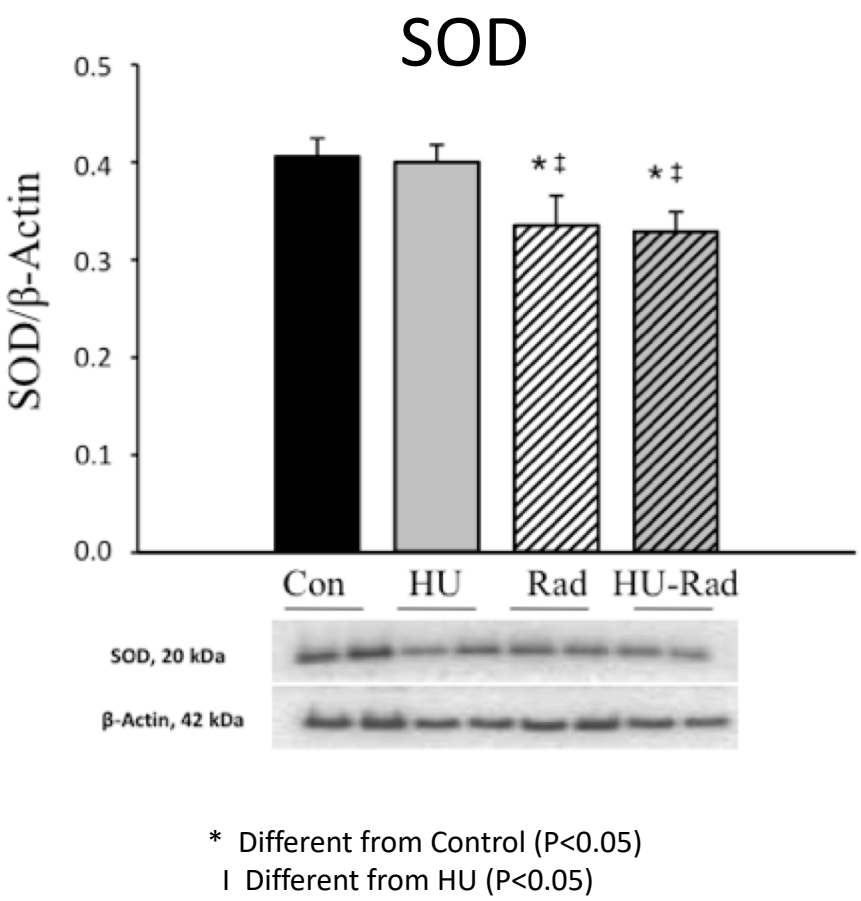


Conclude: IR or HU each causes vascular dysfunction; greater effect when combined (NO-mediated)

- -differences abolished by inhibitors of NO and PGI2 signaling
- -no adverse effects on smooth muscle cell constriction or pressure dynamics
- -good correlation between peak vasodilation cancellous bone volume

Selective regulation of HU vs ^{56}Fe-IR of protein expression in gastrocnemius feed artery





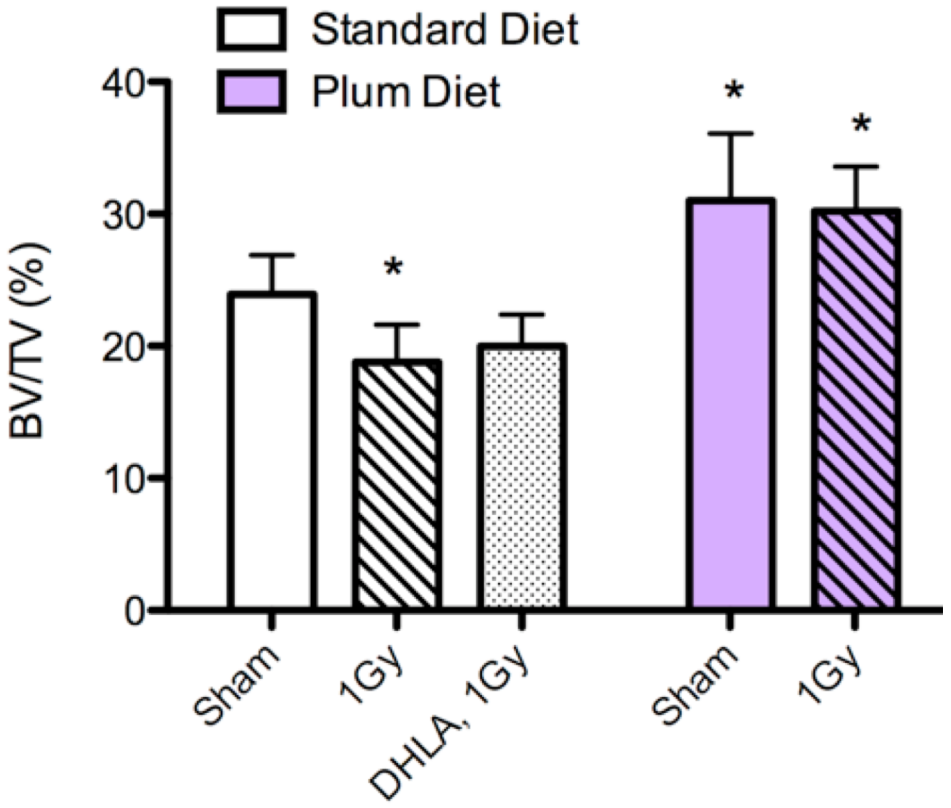
Conclude: Different effects of IR vs HU on ROS/NO-related pathways may contribute to greater dysfunction when treatments combined.

Conclude blood vessel-bone story

- Antioxidant enzymes in the cell work together
  - Simulated weightlessness and radiation adversely affect different enzymes.
  - May account for worse combined effect than single challenge in some cases

- Others organ systems:
  - Intestinal barrier function- immune responses (Weissman)
  - Brain and cardiovascular system (Mao)

Tested selected treatments for ability to prevent radiation-induced bone loss

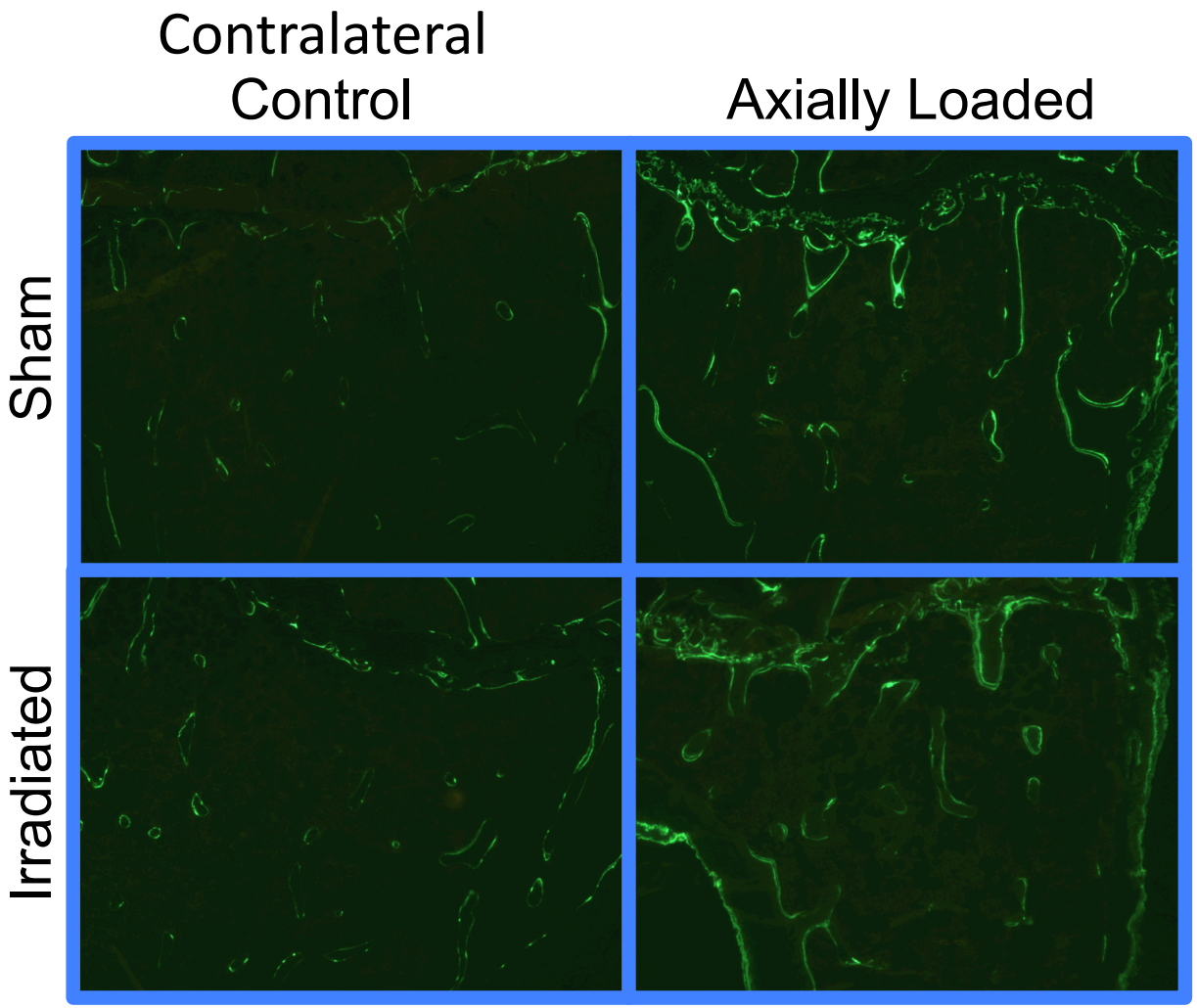


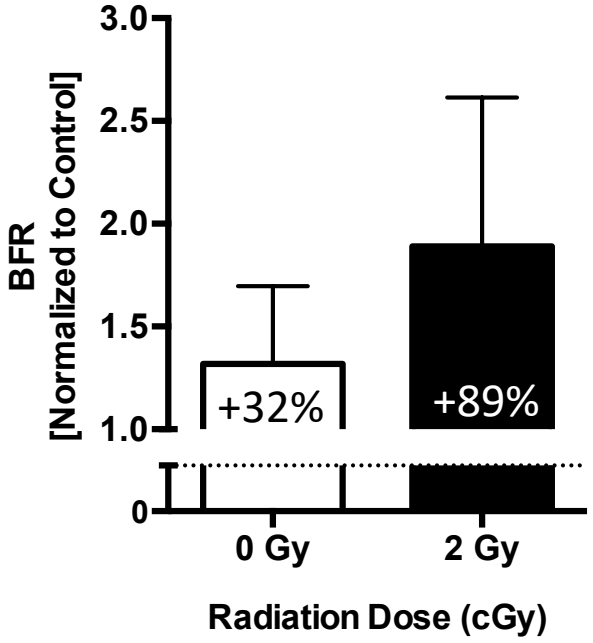
Mean±S.D.
*P<0.05 Sham

Preliminary results: dried plum diet (but not lipoic acid) increased cancellous BV/TV and prevented acute radiation-induced bone loss

-Protective effects from HZE noted by other polyphenol-rich diets, (eg Poulouse et al. Brain Res 2014)

Axial loading stimulates endosteal bone formation in cancellous tissue despite prior ^{56}Fe IR





Radiation:
p< 0.81
Axial Loading:
p < 0.04
Radiation*Loading: p < 0.26

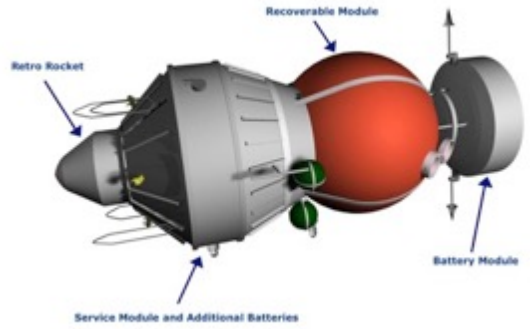
Emerging from mechanistic studies: potential prevention & treatment regimens: some surprises

- Screening potential anti-oxidant countermeasures
  - Complex dietary supplement worked better than single anti-oxidants
- Protect even from radiation-induced bone loss.
- Late radiation effects- possible ‘treatment’ by mechanical stimulation

From groundbased analogues to spaceflight: challenges and opportunities

Spaceflight experiments: rodents

SPACE PLATFORMS



BIOCOSMOS 1971-present BION M1

SPACE SHUTTLE
1981-2011

INTERNATIONAL SPACE STATION
2000-present

Rodent research: Shuttle Era (knowledge gaps)

- Yielded new insights; growth in knowledge made possible by frequent access
  - Responses to spaceflight
  - Treatments
- Relatively short duration: all <3 weeks
- Mostly studied growing, not adult rats
  - What about adults?
- All but 2 of 27 rodent experiments entailed landing with several hour delay before tissue recovery
  - Additional variables of landing and delay, even if brief (~3hr) may impact some (not all) outcomes
    - e.g. Muscle experiment showed a given measured response was due to landing, not microgravity (muscle micro-tears; Riley et al.)
  - This points to science value of on-orbit sample recovery

Main objectives of Rodent Research project

Filling the gaps in knowledge to achieve new scientific discoveries:

- Provide reliable, long duration habitat for rodents (mice and rats) on the ISS
  - Group or individually housed;
    - potential for future modifications to support multiple generations
  - Low maintenance on orbit (minimize crew time)
    - daily animal health checks by specialists on the ground

- Perform multiple missions
  - Current plan: two per year

- Provide capability to apply cutting-edge technologies to samples recovered on-orbit

Technical advances:
Usher in new opportunities for discovery

Apply techniques to problems in space biology, e.g.

- genetically modified animals:
  - reveals mechanism, directs research for intervention/treatment
    - Flight Example: Rodent Research 1-Novartis experiment with MuRF-1 knock animals to study muscle wasting (in progress)
- 'omics:
  - "..the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms" (Wikipedia)

    e.g. genomics, proteomics, metabolomics.
    - Flight Example: Wilson et al. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq.

      (Wilson et al. PNAS. 2007 ;10416299)

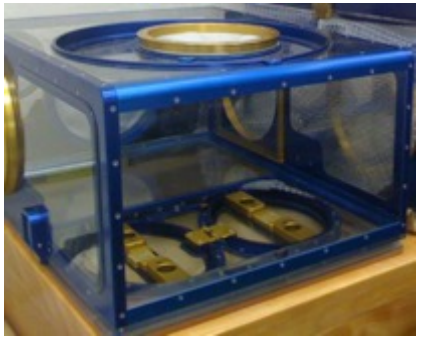
Challenges

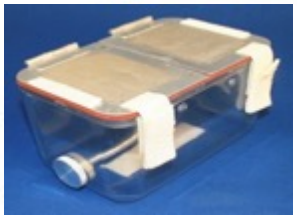
Now and future: Rodent Research experiments on the ISS

Basic equipment needed to conduct a rodent experiment on Earth

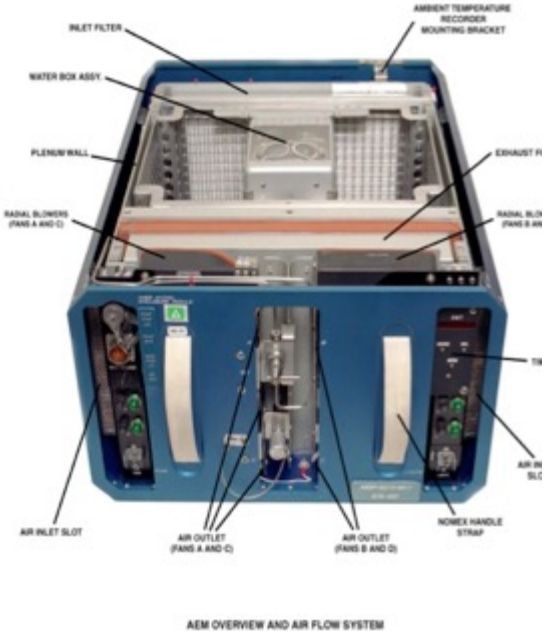
… pretty straightforward with the help of Earth's gravity…

Basic equipment needed to conduct a rodent experiment on orbit

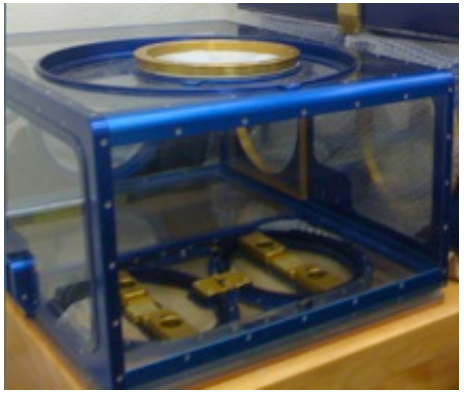




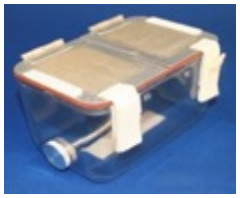
The RR Hardware



Transporter



Animal Access Unit



Mouse Transfer Box

Kits (many)

Habitat

Rodents inside Habitat on Earth

Mice in Habitat on Earth

-5 mice per compartment,

10 per Habitat

-Grating on all sides

-Air flow to entrap waste in filters

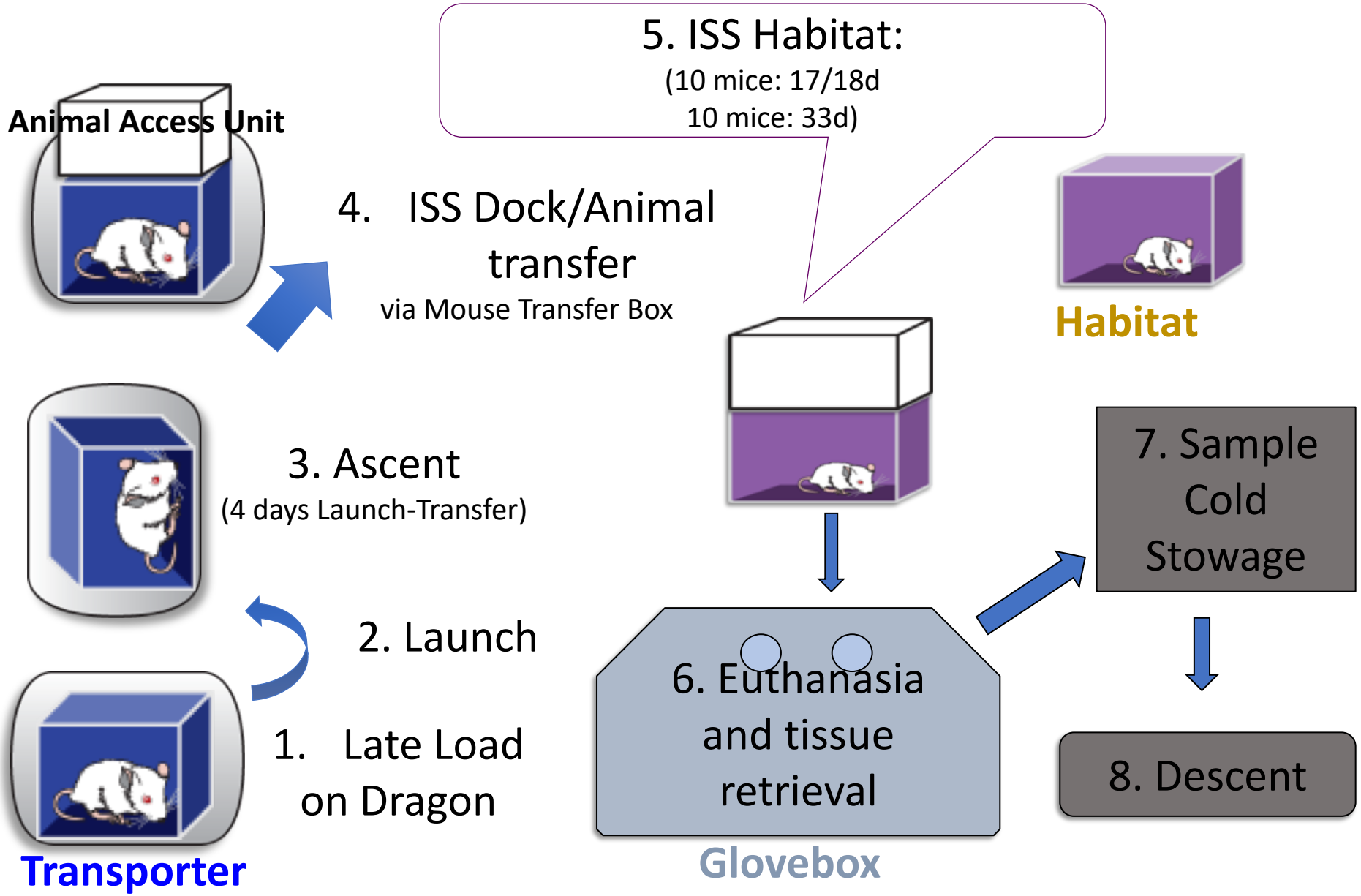
-Food supplied in form of bars

-Water supply (not in image

-Lighting (dark-light

-Video cameras (infrare

RR1: Concept of Operations



RR1: Validation Results
Experimental groups of mice

- 4 separate groups to better understand observed responses to this unique habitat and environment.

Independent Variable	Group
Time	**Basal** (time of launch= starting, in standard cages)
	Vivarium (in standard cages)
Cage	**Ground Controls** (in flight hardware on Earth; matched to ISS)
Space	**Flight** (flight hardware on ISS)

RR1: Validation- Body and tissue weights

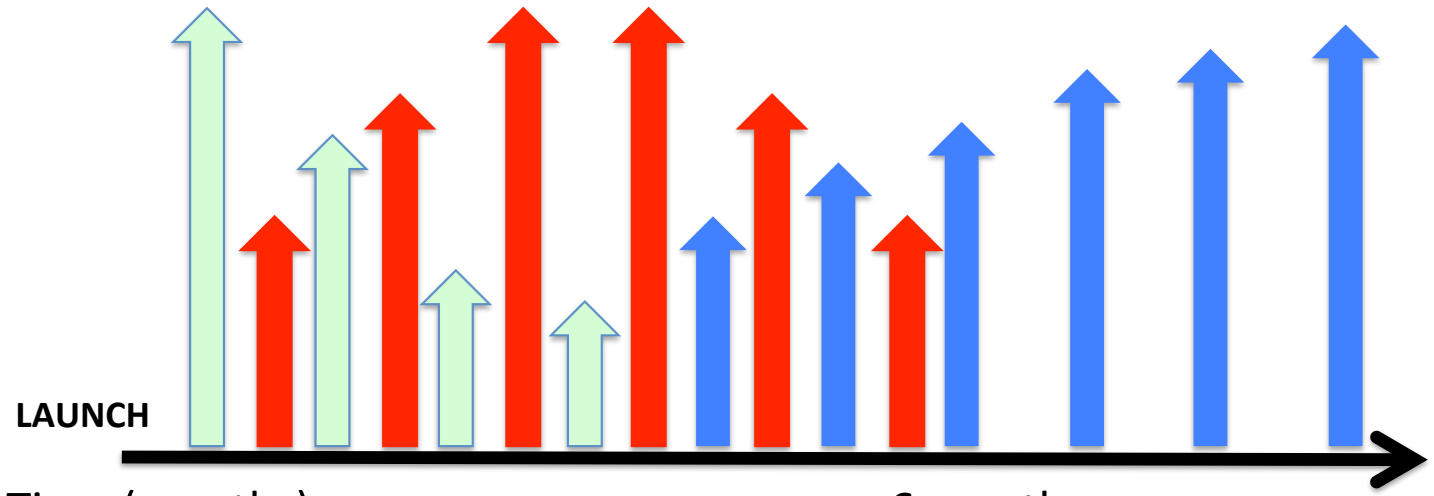
Changes:	Body	Liver	Adrenal	Thymus	Spleen	Soleus muscle*
Time (BAS to VIV)	No change	No change	No change	No change	↑	No change
Cage (VIV to GC)	No change	No change	No change	No change	No change	No change
Space (GC to FLT)	No change	↑	No change	↑	↓	↓

- Some surprises: findings differ from those of short duration rodent experiments (green)
- No significant changes in masses of other muscles (e.g. gastrocnemius)

Summary RR1-continued

- These findings suggest at least two phases of physiological changes after entry into the spaceflight environment.
  - Further progression?

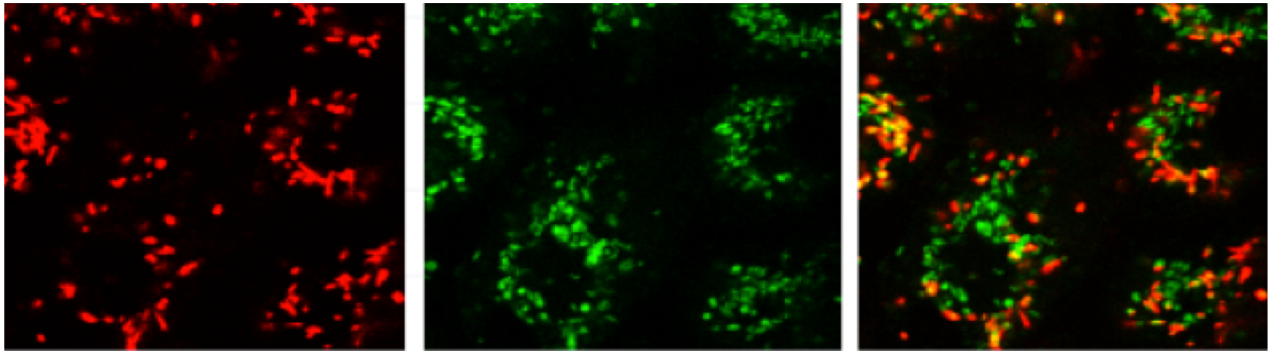
- Inviting all hypotheses…



Physiological responses to spaceflight- various time dependencies

After Nicogossian

mCAT mice: a model for quenching Reactive Oxygen Species in mitochondria



- Overexpresses human catalase transgene in mitochondria
- Longer lifespan
- Protected from cardiovascular deficits
- Protected from neurodegeneration
- Appear to be resistant to age-related tissue degeneration

To live in space:

- Multiple challenges are posed by the space environment
- Multiple physiological systems are affected
- Resulting complexity is such that the consequences over a lifetime on adult human health and reproduction simply cannot be predicted at this time
  - Insight into responses and mechanisms improves prediction and mitigation

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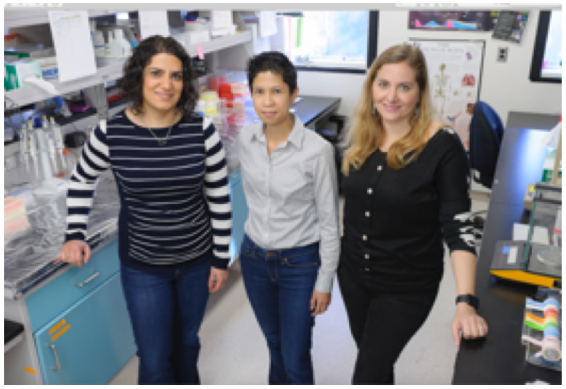

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