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Project Title: Investigating the Effects of Simulated Microgravity Duration and Connexin 43 Deficiency on Bone Fracture Healing Division Name: Human Research Program/Discipline: Program/Discipline: Program/Discipline: Program/Discipline: Program/Discipline: Program/Discipline: Program/Discipline: Program Riberton State Sta			rask Last Opuateu.	11 01/01/2024
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POSTDOCTORAL FELLOWSHIP

Astronauts exposed to long periods of unloading due to extended spaceflight experience on average a decrease in bone strength at 2.0 - 2.5% per month. This sharp decline in bone strength can predispose astronauts to fragility fractures, especially when re-entering a gravity-based loading environment due to extra-vehicular activities, extraterrestrial exploration, off-nominal spacecraft landings, and or upon return to Earth. While emerging evidence suggests that unloading, as would occur in microgravity during spaceflight, impairs fracture healing, the cellular and molecular mechanisms by which this occurs largely remains unknown due to a lack of ground-based rodent analog models mimicking spaceflight conditions. Understanding the mechanisms underlying the microgravity-induced impairment in bone regeneration following fracture will lead to the development of new countermeasure targets. One potential countermeasure target is Connexin 43 (Cx43), the primary gap junction protein in bone. Gap junctions facilitate intercellular communication between neighboring bone cells such as osteoblasts and osteocytes and have been strongly implicated in bone fracture healing and bone adaptation to the mechanical environment.

Task Description:

In order to study how the duration of microgravity and Cx43 affect fracture healing outcomes, a novel murine-healing model undergoing different periods of unloading before and during fracture healing, will be developed and characterized. This model will be created by combining the ground-based microgravity analog, hindlimb tail unloading, in conjunction with an established mouse endochondral bone healing model, the stabilized open surgical femoral fracture model. Bone healing outcomes via molecular, histological, mechanical and cellular techniques, will be evaluated in wildtype and Cx43 transgenic mice. Biomarker characterization of healing progression will be evaluated. The outcomes of this research will provide better mechanistic insight into how microgravity and gravitational reloading such as that found during spaceflight and terrestrial exploration, respectively, affects bone healing. Furthermore, this proposal will identify whether possible treatment strategies targeting Cx43, and or other biological targets, is an efficacious approach to augment bone healing during microgravity.

Rationale for HRP Directed Research:

This project has the capability to address the major NASA Human Research Program (HRP) Gaps in Osteo 1 (Risk of Bone Fracture due to Spaceflight-induced Changes to Bone) and Fracture 1 (We Don't Understand how Spaceflight Affects Fracture Healing).

Research Impact/Earth Benefits:

This is the first project to look at mechanical loading interventions and targeted gene deletions using transgenic mice to increase fracture healing during simulated microgravity. If successful, we can combat multiple musculoskeletal health risks (bone, muscle) of spaceflight using a single countermeasure (mechanical loading, Cx43 deletion, and or exercise).

- 1. Astronauts exposed to long periods of unloading due to extended spaceflight experience on average a decrease in bone strength at 2.0 – 2.5% per month. This sharp decline in bone strength can predispose astronauts to fragility fractures, especially when re-entering a gravity-based loading environment due to extra-vehicular activities, extraterrestrial exploration, off-nominal spacecraft landings, and or upon return to Earth. While emerging evidence suggests that unloading, as would occur in microgravity during spaceflight, impairs fracture healing, the cellular and molecular mechanisms by which this occurs largely remains unknown due to a lack of ground based rodent analog models mimicking spaceflight conditions. Understanding the mechanisms underlying the microgravity-induced impairment in bone regeneration following fracture will lead to the development of new countermeasure targets. One potential countermeasure target is Connexin 43 (Cx43), the primary gap junction protein in bone. Gap junctions facilitate intercellular communication between neighboring bone cells such as osteoblasts and osteocytes and have been strongly implicated in bone fracture healing and bone adaptation to the mechanical environment. In order to study how the duration of microgravity and Cx43 affect fracture healing outcomes, a novel murine healing model undergoing different periods of unloading before and during fracture healing will be developed and characterized. This model will be created by combining the ground-based microgravity analog, hindlimb unloading by tail suspension (HLU), in conjunction with an established mouse endochondral bone healing model, the stabilized open surgical femoral fracture model. Bone healing outcomes via molecular, histological, mechanical and cellular techniques will be evaluated in wildtype (Aim 1) and Cx43 transgenic mice (Aim 2). The outcomes of this research will provide better mechanistic insight into how microgravity and gravitational reloading such as that found during spaceflight and terrestrial exploration respectively affects bone healing. Furthermore, this proposal will identify whether possible treatment strategies targeting Cx43 and or other biological targets is an efficacious approach to augment bone healing during microgravity. 2. Characterization of bone muscle and callus formation in a novel murine healing model undergoing different periods
- 2. Characterization of bone muscle and callus formation in a novel murine healing model undergoing different periods of unloading before and during fracture healing in male and female wildtype mice has yielded key insights. First, we demonstrated that gravitational reloading for 2 weeks during fracture healing following spaceflight-like conditions resulted in similar gastrocnemius (hybrid mainly fast twitch) but not soleus (slow twitch) muscle mass to control levels. Second, following these two weeks post-fracture, diaphyseal (cortical) and epiphyseal (cancellous) bone volume fraction increased from HLS but didn't reach control levels. Looking at the injured limb, semi-automated micro-CT analysis showed significantly reduced callus size and mineralized femoral callus bone formation with continued HLS compared to ground controls after 14 days of fracture healing. In addition, even with a period of HLS for 3 weeks, normal reambulation during bone healing partially restored callus bone formation and fully restored callus volume to control levels. Automated histological analysis using a proprietary machine learning algorithm from these same femurs reinforced micro-CT results by showing significantly reduced callus cross-sectional area and bone formation accompanied by increased callus osteoclast activity and reduced cartilage formation in HLS versus reambulated and ground control groups. Trends suggest that DMP1 Cx43 may protect from metaphyseal but not cortical bone loss or muscle atrophy with HLS. Trends suggest that DMP1 Cx43 may decrease fracture healing on ground but lead to improved healing during HLS.
- 3. These findings support our hypotheses and mirror the poor/delayed hard and soft callus formation seen in rodent models of spaceflight and the ground based analog hindlimb suspension. The improved osteochondral callus formation with gravitational reloading, despite a prior history of simulated microgravity exposure, suggests that normal gravitational loading immediately following fracture can overcome some of the negative aspects of extended unloading on bone healing. This demonstrates that small amounts of artificial loading at the fracture sight during prolonged unloading may be beneficial to fracture healing. This knowledge has broadened the scope of the project to now include direct mechanical loading and or exercise as potential countermeasures for impaired fracture healing as well during spaceflight. Furthermore, Cx43 deficiency in DMP1 cells (osteocytes) may preserve bone mass and improve fracture healing at certain anatomical sites during simulated microgravity but more data is needed for validation before Cx43's

Task Progress:

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	4. Research for the upcoming year will be aimed at further interrogation of the mechanistic role of Cx43 in altering fracture repair due to simulated microgravity in mature osteoblasts and osteocytes using a noninducible transgenic mouse model (DMP1-Cre CX43 fl/fl) and now inducible model (DMP1-CreERT2 CX43 fl/fl). This will allow us to determine if our Cx43-deficient fracture phenotype during simulated microgravity is due to confounding bone developmental changes or rather changes in immediate mechanosensation during healing. We will also determine the optimal loading regimen for normal bone repair during HLS using direct bone loading and Optogenetics to stimulate muscle contraction during simulated microgravity exposure. This work will inform exercise interventions and the potential of optogenetics as effective countermeasures for improving muscle function and bone healing during spaceflight.
Bibliography Type:	Description: (Last Updated: 01/11/2023)
Awards	Buettmann EG. "American Society for Bone and Mineral Research (ASBMR) 2021 Meeting Research Travel Award, San Diego, October 1-4, 2021." Oct-2021
Awards	Buettmann EG. "ISFR Biennial Meeting 2022 Travel Award, Edinburgh, Scotland, September 5 – 7, 2022." Sep-2022
Awards	Buettmann EG. "ISFR Biennial Meeting 2022 Poster Award, Edinburgh, Scotland, September 5 – 7, 2022." Sep-2022
Awards	Buettmann EG. "Virginia Commonwealth University (VCU) Postdoctoral Travel Award, May 2022." May-2022
Books/Book Chapters	DeNapoli RC, Buettmann EG, Donahue HJ. "Cellular and Molecular Biology in Bone Remodeling." in "Osteoporotic Fracture and Systemic Skeletal Disorders." Ed. Takahashi, H.E., Burr, D.B., Yamamoto, N. (eds). Springer, Singapore. https://doi.org/10.1007/978-981-16-5613-2_1 , Sep-2021