Fiscal Year:	FY 2021	**Task Last Updated:**	FY 09/15/2022
PI Name:	Buettmann, Evan Ph.D.		
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--Element/Subdiscipline:	TRISH--TRISH		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	23284-9097	**Congressional District:**	4
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2020 TRISH-RFA-2001-PD: Translational Research Institute for Space Health (TRISH) Postdoctoral Fellowships
Start Date:	09/01/2020	**End Date:**	08/31/2023
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	TRISH
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: End date changed to 08/31/2023 per TRISH (Ed., 8/4/22).		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Donahue, Henry Ph.D. (MENTOR: Virginia Commonwealth University)		
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Task Description:	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. 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:	
Research Impact/Earth Benefits:	The outcomes of both of these research aims 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, it can be used to inform clinical treatment strategies on Earth in fracture patients experiencing bone and muscle loss due to extended bedrest, paralysis, or tumor resection. So far, we have demonstrated that simulated microgravity exposure leads to bone and muscle degradation and impairs fracture healing. However, weight-bearing reambulation (gravitational reloading) following simulated microgravity exposure can improve fracture healing by increasing cartilage and bone formation. Therefore, mechanical loading following disuse, as would occur with spaceflight, extended bedrest, and natural aging has the potential to improve bone repair. However, many individuals suffering from disuse-induced bone and muscle loss, such as found during spaceflight, may have difficulties exercising the injured limb due to locomotor impairments from pain, paralysis, or cardiovascular complications. Therefore, this proposal will identify whether possible therapeutic treatment strategies targeting Cx43, and or other biological targets, is an efficacious approach to augment bone healing during spaceflight conditions.
Task Progress:	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 extravehicular 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 remain 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 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. Furthermore, 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. 2. Characterization of a novel murine healing model undergoing different periods of unloading before and during fracture healing in wildtype mice has yielded key insights. Semi-automated micro-computed tomography (micro-CT) analysis has shown significantly reduced callus size and mineralized callus bone formation with continued hindlimb suspension (HLS) compared to ground controls after 14 days of fracture healing. In contrast, even with a period of HLS for 3 weeks, normal reambulation during bone healing fully restored callus bone formation and partially 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 trends toward reduced callus bone content, increased callus osteoclast activity, and reduced cartilage formation in HLS versus reambulated and ground control groups. 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 unloading. The normal osteochondral callus formation with reambulation, despite a prior history of unloading, suggests that normal gravitational loading immediately following fracture can overcome the negative aspects of extended unloading on bone healing. This demonstrates that small amounts of artificial loading at the fracture site during prolonged unloading may be beneficial to fracture healing. 4. Research for the upcoming year will be aimed at biomarker discovery for molecular targets regulating musculoskeletal

	mechanosensation, and/or regeneration, using our newly developed fracture healing model of bone unloading. Biomarkers will be assayed via high throughput functional gene expression modalities and correlated to fracture healing progression. Special attention to biological processes implicated in bone pathologies associated with disuse and aging, such as angiogenesis, oxidative stress, senescence, autophagy, wingless/integrated (WNT) signaling, and inflammation, will be assessed via callus gene expression during mechanical unloading and reambulation during the early stages of fracture repair. This will increase our knowledge of putative molecular targets for augmenting bone mass and fracture healing during prolonged unloading found during spaceflight. Furthermore, the role of Cx43 in mature osteoblasts and osteocytes using a transgenic mouse model (DMP1-Cre CX43 fl/fl) will be utilized to assess the role of bone targeted Cx43, as a potential countermeasure, in alleviating disuse and fracture healing associated with bone formation impairments during spaceflight.
Bibliography Type:	Description: (Last Updated: 01/11/2023)
Abstracts for Journals and Proceedings	Buettmann EG, DeNapoli RC, Abraham L, Denisco JA, Lorenz MR, Donahue HJ. "Reambulation protects against hindlimb suspension induced impairments in callus and woven bone formation during murine fracture healing." American Society of Bone and Mineral Research. San Diego, California. October 1-4 2021. Abstracts. American Society of Bone and Mineral Research. San Diego, California. October 1-4 2021. , Oct-2021
Abstracts for Journals and Proceedings	Buettmann EG, DeNapoli RC, Donahue HJ. "Investigating the effects of simulated microgravity by hindlimb suspension on murine bone fracture healing." 2021 NASA Human Research Program Investigators' Workshop, Virtual, February 1-4, 2021. Abstracts. 2021 NASA Human Research Program Investigators' Workshop, Virtual, February 1-4, 2021. , Feb-2021
Articles in Peer-reviewed Journals	Juhl OJ 4th, Buettmann EG, Friedman MA, DeNapoli RC, Hoppock GA, Donahue HJ. "Update on the effects of microgravity on the musculoskeletal system." npj Microgravity. 2021 Jul 23;7(1):28. https://doi.org/10.1038/s41526-021-00158-4 ; PMID: 34301942; PubMed Central PMCID: PMC8302614 , Jul-2021
Articles in Peer-reviewed Journals	Buettmann EG, Goldscheitter GM, Hoppock GA, Friedman MA, Suva LJ, Donahue HJ. "Similarities between disuse and age-induced bone loss." J Bone Miner Res. 2022 Jun 30;37(8):1417-34. Review. https://doi.org/10.1002/jbmr.4643 ; PubMed PMID: 35773785; PubMed Central PMCID: PMC9378610 , Jun-2022