Fiscal Year:	FY 2022	**Task Last Updated:**	FY 12/16/2021
PI Name:	McGee-Lawrence, Meghan Ph.D.		
Project Title:	Osteocyte Plasma Membrane Disruptions in Skeletal Adaptation to Loading and Unloading		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Animal Biology: Vertebrate		
Space Biology Cross-Element Discipline:	(1) Musculoskeletal Biology		
Space Biology Special Category:	None		
PI Email:	mmcgeelawrence@augusta.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	706-446-0128
Organization Name:	Augusta University Research Institute, Inc.		
PI Address 1:	Department of Cell Biology and Anatomy		
PI Address 2:	1460 Laney Walker Blvd, CB1101		
PI Web Page:			
City:	Augusta	**State:**	GA
Zip Code:	30912	**Congressional District:**	12
Comments:			
Project Type:	GROUND,New Investigation	**Solicitation / Funding Source:**	2018 Space Biology (ROSBio) NNH18ZTT001N-FG2. App D: Flight and Ground Space Biology Research
Start Date:	12/01/2020	**End Date:**	11/30/2023
No. of Post Docs:	1	**No. of PhD Degrees:**	1
No. of PhD Candidates:	4	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	2
No. of Bachelor's Candidates:	2	**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
Contact Email:	Yuri.V.Griko@nasa.gov		
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Hamrick, Mark Ph.D. (Augusta University Research Institute, Inc.) Johnson, Maribeth M.S. (Augusta University Research Institute, Inc.)		
Grant/Contract No.:	80NSSC21K0274		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	The skeleton's ability to adapt to mechanical loading is crucial for bone health, as exercise promotes hypertrophy but disuse (such as from spaceflight) leads to bone loss. We were the first to report that small, transient plasma membrane disruptions (PMD) develop with in vitro and in vivo mechanical loading in bone osteocytes. These disruptions initiate skeletal mechanotransduction, suggesting PMD are stimuli recognized by osteocytes to regulate bone adaptation to its loading environment. Importantly, we consistently observe that ~20% of long bone osteocytes develop PMD with routine cage activity in mice, suggesting that formation of osteocyte PMD may be essential to bone's sensation of and response to normal gravitational loads. Accordingly, our central hypothesis is that osteocyte PMD formation is impaired during skeletal disuse, leading to bone loss. Our goals are to test the effects of disuse on osteocyte PMD formation, to determine whether osteocytes become sensitized to PMD formation with impaired PMD repair or survival during reloading, and to determine whether modulating osteocyte PMD formation and/or repair affect these processes. Our strategy is to test these concepts in an in vivo murine model of hindlimb unloading, as well as with in vitro osteocyte models of unloading (rotating wall vessel bioreactor) and reloading (fluid shear stress). Our goals align with the NASA Space Biology program as they target Research Topic 3 (Animal Biology Studies in support of Human Space Exploration)/ Sub-Topic AH1-E (Effects of fractional gravity provided by spaceflight centrifugation or ground microgravity/partial gravity analogs to gain insights into mechanisms of how animals sense, respond, and adapt to gravity shifts that are less than 1G) by discovering the contribution of osteocyte PMD formation (and hypothesized impairment during disuse) to the skeleton's adaptation to its loading environment. This project will yield a new understanding of how complex organisms adapt to the space environment, using a ground-based analog for disuse from spaceflight; we anticipate that derived data will advance strategies for skeletal maintenance and prevention of bone fractures during disuse to promote and support human space exploration.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Disuse-induced bone loss, which occurs during prolonged exposure to microgravity during spaceflight and predisposes astronauts to risk of skeletal fractures, also occurs frequently on Earth in patients with spinal cord injuries, patients subjected to chronic/long-term bed rest, and in other cases of long-term decreased mobility. Furthermore, it is well understood that mechanical loading of the skeleton through physical exercise is beneficial for bone health across a wide spectrum of human patients, but there exists a substantial proportion of the population who cannot undertake regular vigorous exercise for a variety of reasons, including underlying health conditions, time constraints, or financial concerns. Therefore, understanding the fundamental mechanisms behind how bone senses and responds to changes in mechanical loading, and exploring ways to alter the skeletal response to a given level of mechanical loading (or withdrawal of loading), may lead to therapeutic interventions for improving bone health and reducing fracture risk.
Task Progress:	Despite this being the first year of this grant, we have made significant progress towards accomplishing the Specific Aims of the proposal. Major accomplishments include: establishment of a more robust disuse-induced bone model, beginning to define the molecular signature of mechanically loaded mechano-sensing bone cells (osteocytes) with plasma membrane disruptions (PMD), testing the effects of creating increased cell membrane fragility on bone adaptation to loading, testing the effects of creating delayed cell membrane repair on bone adaptation to loading, and testing the effects of enhancing cell membrane repair on bone adaptation to loading. These are detailed below. We initially proposed a two week single hindlimb immobilization model to stimulate disuse-induced loss of bone and muscle, as we had collected pilot data showing that mice would lose muscle and bone mass in this timeframe, and also show decreased evidence of osteocyte PMD in this window. This immobilization model has the advantage that one limb of the mouse is subjected to disuse, but the other remains mechanically loaded – allowing us to compare the effects of unloading within each animal, minimizing the number of animals needed for study and controlling for variability between mice. However, before embarking on our full studies, we first examined whether two weeks of immobilization was sufficient to properly cause bone loss. We were encouraged to find that an additional week of immobilization, three weeks in total, led to a more robust and reproducible loss of both muscle and bone mass than our originally proposed approach. Specifically, three weeks of immobilization significantly decreased muscle mass, cortical bone thickness, cortical bone area, and measurements of bone strength in the immobilized as compared to loaded limbs, whereas these trends were considerably weaker in the mice subjected to only two weeks of disuse. Therefore, we revised our experimental plans to focus on a 3 week immobilization model for all experiments moving forward, to ensure rigor and repeatability in our experiments. We are also interested in understanding what signals are specifically produced in mechanically loaded bone cells (osteocytes) that develop PMD (PMD+) as compared to cells that are loaded but do not develop PMD (PMD-). This will help us test and establish the importance of PMD in bone's sensation of mechanical loading, helping us to understand if this mechanism represents a viable target for modifying bone's adaptation to changes in loading. Over the last year, we have developed methods to mechanically load the osteocytes, sort them based on whether they developed a PMD during loading, and then analyze the molecular signature (gene expression trends) in the PMD+ as compared to PMD- cells. These studies are still ongoing, but preliminary results suggest that the PMD+ cells are critical for initiating the earliest responses to application of a mechanical load. In this first year of the grant, we have completed development of a genetic mouse model where we have made the osteocytes more susceptible to the development of PMD with loading (by knocking out a protein called Sptbn1,which is involved in membrane stability), and a model where we have slowed the rate of PMD repair in osteocytes (by knockout of a protein called Prkd1, which is involved in membrane repair). These models allow us to test the contribution of PMD-mediated events to bone adaptation to changes in mechanical loading. We have validated these models, showing enhanced fragility and impaired repair, respectively. Both of these models demonstrate impaired adaptation to loading, consistent with demonstrating an important role for PMD in bone mechanobiology. We intend to next subject these mice to disuse conditions, to test the effects of these genetic modifications on the response of bone to reduced mechanical loading and subsequent reloading during remobilization. Excitingly, we are also keenly interested in testing whether enhancing membrane stability or repair can have therapeutic implications in terms of modifying the skeleton's response to changes in mechanical loading. We have been treating mice and isolated osteocytes with an FDA (Food & Drug Administration) approved drug agent that enhances membrane stability; while this does not necessarily enhance the response of healthy mice or cells to mechanical loading, preliminary results suggest that this strategy can rescue the defects caused by impaired PMD repair identified in our genetic mouse models. While we have encountered some difficulty in personnel/staff recruitment for this project due to the ongoing pandemic,

	we are happy to report that we have involved four PhD students, four medical students, two undergraduate students, and a high school student in completion of our funded experiments over the past year. One PhD student successfully defended her PhD and graduated earlier this year, our two undergraduate students received their Bachelor's Degrees, and all of the students involved have received authorship on either journal manuscripts or conference abstracts stemming from their contributions. Therefore, this grant is supporting the career development of the next generation of scientists.
Bibliography Type:	Description: (Last Updated: 10/10/2023)
Abstracts for Journals and Proceedings	Tuladhar A, McGee WA, Yu K, Hamrick MW, McGee-Lawrence ME. "Prkd1 is critical for repair of plasma membrane disruptions (PMD) in osteocytes." ASBMR 2021 Annual Meeting, San Diego, CA, and Virtual, October 1-4, 2021. Abstracts. ASBMR 2021 Annual Meeting, San Diego, CA, and Virtual, October 1-4, 2021. Plenary Poster, Abstract ID #A21023817. , Oct-2021
Abstracts for Journals and Proceedings	Hagan M, Piedra V, Yu K, Roberts R, Dorn J, Balayan V, Cooley M, Hamrick MW, McGee-Lawrence ME. "Sptbn1 Deficiency Blunts Adaptation In Vivo and Alters Osteocyte Plasma Membrane Dynamics And Calcium Wave Propagation In Vitro Following Formation of Plasma Membrane Disruptions (PMD)." 2021 Orthopedic Research Society, Virtual, February 12-16, 2021. Abstracts. 2021 Orthopedic Research Society, Virtual, February 12-16, 2021. ORS Annual Meeting Oral Presentation #0358. , Feb-2021
Abstracts for Journals and Proceedings	Tuladhar A, Hagan M, Yu K, Awad M, Parker E, Hamrick MW, McGee-Lawrence ME. "Disuse from Immobilization Decreases Osteocyte Plasma Membrane Disruptions (PMD) and Causes Cortical Bone Loss." 2021 Orthopedic Research Society, Virtual, February 12-16, 2021. 2021 Orthopedic Research Society, Virtual, February 12-16, 2021.ORS Annual Meeting Poster #0531. , Feb-2021
Articles in Peer-reviewed Journals	Hagan ML, Balayan V, McGee-Lawrence ME. "Plasma membrane disruption (PMD) formation and repair in mechanosensitive tissues." Bone. 2021 Aug;149:115970. https://doi.org/10.1016/j.bone.2021.115970 ; PMID: 33892174; PMCID: PMC8217198 , Aug-2021