Fiscal Year:	FY 2023	**Task Last Updated:**	FY 11/10/2022
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:	In the second year of this grant, we have made significant progress towards accomplishing the Specific Aims of the proposal. Major accomplishments include: repeating our disuse-induced in vivo bone studies, initiating our in vitro disuse (rotating wall vessel bioreactor) experiments with promising preliminary results, continuing to define the molecular signature of mechanically loaded mechano-sensing bone cells (osteocytes) with plasma membrane disruptions (PMD), and initiating experiments designed to rescue repair defects associated with delayed membrane repair. These are detailed below. We have developed and validated a method to successfully induce disuse in vitro, using the Synthecon slow turning lateral vessel (rotating wall vessel) bioreactor system. In pilot experiments earlier this year, we developed cell culture protocols to seed osteocytes onto scaffold materials, expose the osteocytes to several days of disuse, and then subject them to varying levels of mechanical shear stress to simulate re-loading after disuse. We have validated this disuse model, showing that three days of culture in the bioreactor consistently up-regulates disuse-associated genes like sclerostin in these osteocyte populations. Moreover, we have developed a successful protocol to expose these cells to re-loading via fluid shear. We had initially proposed to re-seed the cells from the disuse-exposed scaffolds into flow chambers to determine effects of re-loading, but instead employed our more recently developed turbulent fluid shear stress model, finding that we are able to effectively wound the cells in situ on the scaffolds. We are excited to report that our initial hypotheses were confirmed – i.e., that exposure to disuse does appear to sensitize osteocytes to the formation of PMD during re-loading. These results are consistent with our earlier in vivo studies, and will serve as a launching point for our upcoming experiments aimed at helping osteocytes better survive and respond to the stress of re-loading after exposure to prolonged disuse. 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-), and understanding how disuse impacts these signaling pathways. This will help us test and establish the importance of PMD in bone's sensation of load, 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 continued experiments that 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 the first year of the grant, we developed several genetic mouse models, one of which slowed the rate of PMD repair in osteocytes by knocking out a protein called Prkd1 which is involved in membrane repair. This model demonstrated impaired adaptation to loading, consistent with a critical role for PMD-mediated mechanisms in bone mechanobiology. In the second year of the grant, we have tested whether enhancing membrane stability / 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-approved drug agent that enhances membrane stability, and have completed experiments demonstrating that this drug fully rescues the defects caused by impaired PMD repair identified in our genetic mouse models. In particular, we have found that treatment of cells with Poloxamer 188 rescues PMD repair rates in Prkd1-inhibitired or Prkd1-knockout osteocytes to control levels, even though it does not appear to further enhance repair behavior in wildtype (control) cells. We are now in the middle of testing the in vivo effects of Poloxamer 188, administering it to Prkd1-deficient mice to determine if this treatment rescues bone adaptation in these mice. We are also investigating the effects of disuse on membrane repair rate and stability, using the bioreactor system mentioned at the beginning of this report. We continue to support the professional development of students, having now supported a total of four PhD students, five medical students, three undergraduate students, and a high school student in completion of our funded experiments over the past two years. One PhD student has successfully advanced to candidacy and intends to defend his PhD within

	the next year. All of the students involved have received authorship on either journal manuscripts or conference abstracts stemming from their contributions. Therefore, this grant continues to support the career development of the next generation of scientists.
Bibliography Type:	Description: (Last Updated: 10/10/2023)
Abstracts for Journals and Proceedings	Tuladhar A, Shaver J, McGee WA, Yu K, Bensreti H, Bollag WB, Hamrick MW, McGee-Lawrence ME. "Poloxamer-188 treatment rescues osteocytes with an impaired ability to repair plasma membrane disruptions caused by mechanical loading." ORS 2023 Annual Meeting, Dallas, TX, February 10-14, 2023. Abstracts. ORS 2023 Annual Meeting, Dallas, TX, February 10-14, 2023. Oral Presentation Paper #0010. , Feb-2023
Abstracts for Journals and Proceedings	Tuladhar A, Shaver JC, McGee WA, Yu K, Bollag WB, Hamrick MW, Johnson M, McGee-Lawrence ME. "Prkd1 (PKC-mu) regulates the formation and repair of plasma membrane disruptions (PMD) in osteocytes." ASBMR 2022 Annual Meeting, Austin, TX, September 9-12, 2022. Abstracts. ASBMR 2022 Annual Meeting, Austin, TX, September 9-12, 2022. American Society for Bone and Mineral Research. Plenary Poster #FRI-278/SAT-278. , Sep-2022
Abstracts for Journals and Proceedings	Dorn J, Bensreti HE, Yu K, Zhong R, Zhan-Moodie S, Faith H, Hamrick MW, McGee-Lawrence ME. "Poloxamer 188 treatment enhances osteocyte PMD development but blunts bone adaptation to mechanical loading." ORS 2022 Annual Meeting, Tampa, FL, February 4-8, 2022. Abstracts. ORS 2022 Annual Meeting, Tampa, FL, February 4-8, 2022. Poster PS-500. , Feb-2022
Abstracts for Journals and Proceedings	Tuladhar A, Shaver J, McGee WA, Hagan ML, Yu K, Bollag WB, Hamrick MW, McGee-Lawrence ME. "Prkd1 (PKC-mu) regulates the formation and repair of plasma membrane disruptions (PMD) in osteocytes." ORS 2022 Annual Meeting, Tampa, FL, February 4-8, 2022. Abstracts. ORS 2022 Annual Meeting, Tampa, FL, February 4-8, 2022. Poster PS-499. , Feb-2022