Fiscal Year:	FY 2024	**Task Last Updated:**	FY 04/12/2023
PI Name:	Rosa-Caldwell, Megan Ph.D.		
Project Title:	Influence of Sex Hormones on Nervous System and Musculoskeletal Health in Micro- and Martian Fractional Gravity in Rat Analogues		
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 (2) Neurobiology		
Space Biology Special Category:	None		
PI Email:	merosaca@bidmc.harvard.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	913-744-9019
Organization Name:	Beth Israel Deaconess Medical Center, Inc./Harvard Medical School		
PI Address 1:	330 Brookline Ave, TCC-810		
PI Address 2:			
PI Web Page:			
City:	Boston	**State:**	MA
Zip Code:	02215-5400	**Congressional District:**	7
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2018 Space Biology (ROSBio) NNH18ZTT001N-FG2. App D: Flight and Ground Space Biology Research
Start Date:	01/04/2021	**End Date:**	01/03/2023
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:	1	**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**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:	Per the PI: The collaboration of Ursula Kaiser, Ph.D. is greatly appreciated. Dr. Kaiser has contributed to the overall development and interpretation of data for the project. (Ed., 4/13/23). No other significant personnel changes. PI/Post-Doc: Megan Rosa-Caldwell PI/Mentor: Seward Rutkove		
COI Name (Institution):	Bouxsein, Mary Ph.D. (Beth Israel Deaconess Medical Center, Inc./Harvard Medical School) Rutkove, Seward M.D. (Mentor: Beth Israel Deaconess Medical Center, Inc./Harvard Medical School)		
Grant/Contract No.:	80NSSC21K0311		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	POSTDOCTORAL FELLOWSHIP In this postdoctoral fellowship, we propose to evaluate the impact of sex hormones on neuronal and musculoskeletal health in micro and Martian gravity environments by assessing naive and castrated/ovariectomized male and female rats. We hypothesize micro- and fractional gravity will result in differential aberrations to sex hormone status in female and male rats; these sex hormone aberrations will moderate neurological and musculoskeletal declines in micro- and fractional gravity environments. We will study these rats over a 4-week period and investigations will include a series of neurophysiological and functional measures, blood analyses, and a detailed series of post-mortem histological studies. With completion of this work, we will have a far deeper understanding of the relationship between hormonal status and neuronal and musculoskeletal function. This proposed work will be specifically responsive to the following subtopics in Appendix D: • (AB1-A): Behavior and underlying neural function, including circadian effects, controlling ability of animals to sense and respond to their environment. • (AB1-B): Studies to characterize interactions between multiple physiological systems. • (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.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This project investigates how sex hormones influence musculoskeletal health in both males and females during conditions that produce muscle loss. This work has impact on life on Earth because it is important to understand how different atrophic conditions (for example, bed rest) may differ between males and females. Additionally, understanding if, or how, sex hormones interact with muscle and bone health during atrophic conditions will develop our overall understanding of musculoskeletal biology, as well as facilitate the development of possible interventions to blunt muscle loss for patients on Earth.
Task Progress:	The goal of this proposal is to is to investigate the influence of sex hormones and biological sex on musculoskeletal changes in micro- and Martian gravity environments. Within this overall goal, we have three sub-goals: A. Establish the impact of sex hormones on neurological and musculoskeletal systems in these gravitational analogues. B. Identify potential sex differences in response to Martian fractional gravity. C. Determine the influence of micro- and partial-gravity environments on circulating hormonal status in males and females. In this proposal, we have male and female Fischer rats divided into different gravitational and hormonal conditions. Specifically, animals are divided into either ovariectomized/castrated or sham operated. Animals are then further sub-divided into three different gravitational loads 1g (control), 0.4g (simulating Martian gravity), or 0g (simulating micro-gravity). This design results in a total of 12 groups, including: Male-Sham-1g, Male-Sham-0.4g, Male-Sham-0g, Male-Castrated-1g, Male- Castrated-0.4g, Male-Castrated-0g, Female-Sham-1g, Female-Sham-0.4g, Female-Sham-0g, Female-Ovarectomized-1g, Female-Ovarectomized-0.4g, and Female-Ovarectomized-0g. Animals have respective surgeries and then have a 2 week recovery period. After the recovery period, animals had baseline testing of various musculoskeletal and neurological parameters. Animals were then assigned to their appropriate gravitational loads (1g, 0.4g, or 0g) and remain in those interventions for 28 days. After 28 days, animals again undergo testing for musculoskeletal and neurological parameters. Animals are then sacrificed and tissues collected for additional analysis. Additionally, in the female animals, after surgeries we also monitor estrous cycle in female rats through daily vaginal lavages. Vaginal cells are collected using sterile water and a pipette. Then cells are placed on a microscope slide and allowed to dry. Slides are then stained in crystal violet staining solution and visualized with a microscope. We are happy to note that we have completed all animal work for the proposed study. Upon receiving notification of selection in September 2020, we immediately started planning so the study could start as soon as funds became available. We originally planned to complete the surgeries in our own laboratory; however, we learned that Charles River could complete the surgeries before shipping animals. We opted for this option because completing the surgeries in our home laboratory would require borrowing shared surgical spaces with other researchers and this option would allow us to complete more animals in a shorter time period. In a minor change from the original proposal, animals had surgeries completed at 11 weeks of age and arrived at Beth Israel Deaconess Medical Center at 12 weeks of age (as opposed to surgeries at 12 weeks of age). After the appropriate acclimation period (48 hours), we then began estrous cycle monitoring of female rats. This two week measurement period allows for determination of baseline estrous cycle for each animal as well as confirms the ovariectomy surgery. Males still had this 2 week waiting period but were just gently handled without any additional monitoring/measurements. Since starting data collection in January of 2021, we have thus far completed 120 animals over the 42 day protocol. We completed animal data collection in May of 2022. Since completion of animal data collection, we have been analyzing data collected for 120 animals across > 20 outcome variables. We have also completed hormone analysis in the serum of the animals. However, due to the quality of the data we have opted not to present those results. Thus far, our data appears to suggest females tend to have greater relative muscle strength loss compared to males after exposure to simulated micro- or partial-gravity. These changes are also accompanied by greater overall muscle area loss in females compared to males. Interestingly, we note that sex differences between males and females tended to be greater during exposure to partial-gravity compared to micro-gravity. This may suggest both males and females reach a plateau in musculoskeletal losses with micro-gravity, possibly “hiding” sex difference in musculoskeletal alterations. However, with a slightly less aggressive atrophic stimuli (e.g., partial-gravity), there are gradations to muscle loss that can be detected between males and females. In essence, our results suggest aggressive stimuli such as micro-gravity elicit such dramatic muscle loss that potential sex differences may be lost. Though when there is more “wiggle room” in the degree of muscle loss with partial-gravity, sex differences between males and females become more apparent, with females tending to have greater muscle loss compared to males. It should be noted that both males and females experienced dramatic muscle loss at both micro- and partial-gravity and these sex differences were overall relatively subtle compared to the overall muscle loss detected across both males and females. With regards to hormonal status, there were no differences between castrated males and sham males on many components of muscle size and strength at either micro- or partial-gravity. In females, there were generally no

	differences in relative muscle strength losses between ovariectomized and sham animals after exposure to micro- or partial-gravity. However, we did find dramatic alterations in the female rat's estrous cycle after exposure to either simulated micro- or partial-gravity. Specifically, females spent more time in low estradiol phases of the estrous cycle (diestrus and metestrus). Moreover, these changes were detectable within one week of exposure to either micro- or partial-gravity. It is possible the clear alterations to estrous cycle in our females may have limited our ability to find differences between ovariectomized and sham females, though one of the goals of this project was to evaluate changes to the estrous cycle during exposure to micro- and partial-gravity. Overall, our data suggests either micro- or partial-gravity is capable of disrupting female estrous cycle and (presumably) estradiol secretion. To assess cognition, we used a novel object recognition assessment. To test this, animals were exposed to two small plastic toys of similar size and shape. After a 5 minute familiarization phase, one toy was removed and replaced by a new object that was a different color and shape. Animals were given 5 minutes to investigate the objects (one familiar, one novel). All videos were assessed by at least two investigators. Overall, the simulated gravity conditions (0.4g and 0g) appeared to negatively influence animals' cognitive function, though there were no obvious differences between either sexes or hormonal status. Finally, we assessed skeletal health in these animals using microCT. We are still actively working to complete data analysis and manuscript submission for these data. For global bone mineral density (BMD), as expected, castrated and ovariectomized animals, tended to have lower BMD compared to sham animals. However, when we normalized the data to percent difference from 1g control animals within hormonal status (sham or castrated/ovariectomized), it appears that castrated and ovariectomized rats had greater relative loss in BMD, despite (presumably) starting a lower BMD at the initiation of interventions. These data suggest that gonadal hormones are very important for skeletal health, in particular during exposure to simulated micro- or partial-gravity environments. Rats lacking gonadal hormones not only started at a lower BMD, but also had greater relative loss in BMD during micro- and partial-gravity interventions.
Bibliography Type:	Description: (Last Updated: 03/26/2025)
Abstracts for Journals and Proceedings	Rosa-Caldwell ME, Mortreux M, Sung D-M, Schreurs A-S, Bouxsein ML, Kaiser UB, Rutkove SB. "Sex differences in the etiology of micro-gravity induced musculoskeletal losses are not solely dependent on sex hormones." 2022 NASA Human Research Program Investigators' Workshop, Virtual, February 7-10, 2022. Abstracts. 2022 NASA Human Research Program Investigators' Workshop, Virtual, February 7-10, 2022. , Feb-2022
Articles in Peer-reviewed Journals	Rosa-Caldwell ME, Eddy KT, Rutkove SB, Breithaupt L. "Anorexia nervosa and muscle health: A systematic review of our current understanding and future recommendations for study." International Journal of Eating Disorders. 2022 Dec 18;1-18. Review. https://doi.org/10.1002/eat.23878 ; PMID: 36529682 , Dec-2022
Articles in Peer-reviewed Journals	Rosa-Caldwell ME, Mortreux M, Wadhwa A, Kaiser UB, Sung DM, Bouxsein ML, Rutkove SB. "Influence of gonadectomy on muscle health in micro- and partial-gravity environments in rats." J Appl Physiol (1985). 2023 May 23. https://doi.org/10.1152/japplphysiol.00023.2023 ; PMID: 37102698; PMCID: PMC10228673 , May-2023
Articles in Peer-reviewed Journals	Rosa-Caldwell ME, Mortreux M, Wadhwa A, Kaiser UB, Sung D-M, Bouxsein ML, Rutkove SB. "Sex differences in muscle health in simulated micro- and partial-gravity environments in rats." Sports Med Health Sci. 2023 Sep 12. Onine ahead of print. https://doi.org/10.1016/j.smhs.2023.09.002 , Sep-2023
Articles in Peer-reviewed Journals	Issertine M, Rosa-Calwell ME, Sung DM, Bouxsein ML, Rutkove SB, Mortreux M. "Adaptation to full weight-bearing following disuse in rats: The impact of biological sex on musculoskeletal recovery." Physiol Rep. 2024 Feb 21;12(4):e15938. https://doi.org/10.14814/phy2.15938 ; PMID: 38383049; PMCID: PMC10881285 , Feb-2024
Articles in Peer-reviewed Journals	Rosa-Caldwell ME, Pandeya S, Mortreux M, Rutkove SB. "Predicting muscle function and mass with electrical impedance myography: A study in rat analogs of micro- and partial gravity." Acta Astronaut. 2024 Oct 1;223:384-8. https://doi.org/10.1016/j.actaastro.2024.07.017 , Oct-2024
Awards	Rosa-Caldwell ME. "Second Place, Post-Doc Poster Competition, 2022 NASA Human Research Program Investigators' Workshop, Virtual, February 7-10, 2022." Nov-2022
Awards	Rosa-Caldwell ME. "APS Abstract Merit Award, Biennial Meeting of the American Physiological Society, 7th Conference on New Trends in Sex and Gender Medicine, Virtual, October 19-22, 2021." Oct-2021