Fiscal Year:	FY 2022	**Task Last Updated:**	FY 05/18/2022
PI Name:	Lawler, John Ph.D.		
Project Title:	Upstream Regulation of Nox2 and Skeletal Muscle Atrophy During Microgravity and Countermeasure Development		
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) Cell & Molecular Biology (2) Animal Biology: Vertebrate		
Space Biology Cross-Element Discipline:	(1) Musculoskeletal Biology		
Space Biology Special Category:	(1) Translational (Countermeasure) Potential		
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Organization Name:	Texas A&M University		
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City:	College Station	**State:**	TX
Zip Code:	77843	**Congressional District:**	17
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2016-17 Space Biology (ROSBio) NNH16ZTT001N-FG. App G: Flight and Ground Space Biology Research
Start Date:	06/01/2019	**End Date:**	05/31/2023
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:	2	**No. of Master' Degrees:**	
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	3
No. of Bachelor's Candidates:	6	**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 05/31/2023 per F. Hernandez/ARC (Ed., 6/20/22). NOTE: Start/end dates changes to 6/1/2019-7/31/2022 (originally 3/1/2019-5/31/2022) per NSSC award documents per F. Hernandez/ARC (Ed., 12/3/2020)		
Key Personnel Changes/Previous PI:	August 2020 report: Dr. Khaled Kamal - Assistant Research Scientist ; Amin Mohajeri - PhD student; Mariam Othman - PhD student (add) Adding Joo Kim (PhD student) Jordyn Johnson - MS student. (remove - left because of COVID) Add: Aggie Research Scholars: Devon Roeming, Samhitha Harvey, Danielle DeCastro, Gracie Barrow, Yasmin Bagheri Graduated: Myles McFarland (Aggie Research Scholar) 2020- ; Mollie Linder (Aggie Research Scholar) 2020- ; Hallie Harris (Aggie Research Scholar) 2020 - ; Francisco Melesio (Aggie Research Scholar) 2020 - ; Sonny Rodriguez (Aggie Research Scholar) 2020- ; Mia Ngyuen (Aggie Research Scholar) 2020- ; Lorrie Hill 2018-2020 (Kinesiology Research Scholar).		
COI Name (Institution):	Fluckey, James Ph.D. (Texas A & M, College Station)		
Grant/Contract No.:	80NSSC19K0432		
Performance Goal No.:			

Performance Goal Text:	
Task Description:	Skeletal muscles are dynamic mechanical and metabolic machines that drive body movement and energy expenditure. Skeletal muscles are dynamic tissues that can adapt quickly to alterations in mechanical unloading by altering their mass and muscle fiber cross-sectional area. Skeletal muscle strength and endurance are essential to the health, well-being, and performance of astronauts during spaceflight and upon return to a gravitational environment. The mechanical unloading due to the microgravity (μG) of spaceflight causes muscle fiber atrophy and fiber-type shift of postural muscles in the lower extremities and flexors in the upper extremities. Microgravity also increases the risk of skeletal muscle damage, weakness, and thus the risk of injury upon reloading (e.g., extravehicular activities, Mars). Mechanotransduction is the ability to sense and regulate adaptive responses to increased or decreased loading. New paradigms have emerged from ground analogs of μG that have contributed to a leap of our understanding of mechanotransduction and muscle atrophy. Specifically, the mu-splice variant of neuronal nitric oxide synthase (nNOSμ) was discovered as causal in both muscle hypertrophy with overloading and atrophy with unloading. Our laboratory has found that reactive oxygen species (ROS) directly contribute to both muscle fiber atrophy and fiber-type shift from slow to fast. Pilot Data and cutting-edge research have identified mitochondria, the Nox2 isoform of NADPH oxidase, and upstream angiotensin II receptor 1 (AT1R) as sources of ROS during mechanical unloading. Preliminary Data show that inhibition of Nox2 translocation of nNOSμ away from the sarcolemma, muscle fiber atrophy, and fiber-type shift. However, the upstream mechanisms that regulate Nox2 during μG are poorly understood, impeding progress in space biology and novel countermeasure development. The lack of such knowledge impedes our development in understanding the mechanisms that underlie redox regulation of mechanotransduction in skeletal muscle. This grant application serves as a renewal and extension and Renewal of our research team's NNX13AE45G award, particularly stretching our horizons in understanding how Nox2 assembly is enhanced during microgravity in skeletal muscle. New studies have identified novel inhibitors for proteins recently govern assembly of the Nox2 complex at the cell membrane acid—sphingomyelinase (ASMase) and cyclophilin A. We hypothesize that the following novel countermeasures will protect against nNOSμ translocation and the spaceflight phenotype— (a) the ASMase inhibitor etidronate (Didronel) and (b) cyclophilin A inhibitor TMN-355. We further postulate that Nox2 is causal in ROS-induced suppression of anabolic signaling. The efficacy and specificity of the above countermeasures will be confirmed with gene knockdown experiments. Texas A&M is a rich research environment for NASA research, including the Space Life Science Program. We will use the latest molecular and image analysis tools in the development of highly novel countermeasures against spaceflight sarcopenia during microgravity. Dr. Lawler and Dr. Fluckey's laboratories have continued to be supported by NASA, and are dedicated to finding targeted, antioxidant countermeasures against spaceflight sarcopenia. The ground hindlimb unloading model will be used in short and long-term experiments. Our research will also directly translate to skeletal muscle wasting in clinical setting on Earth, an important mission of the Space Biology program. For example, hospitalization, particularly in an ICU (intensive care unit) can reduce skeletal muscle by 25%. Cast immobilization can decrease affected muscle mass by 30% as well. In addition, mechanical unloading due to disuse (e.g., bedrest) and illness (e.g., sepsis, chronic obstructive pulmonary disease, chronic heart failure) exacerbates atrophy, weakness, and impedes recovery.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Our research will also directly translate to skeletal muscle wasting in clinical setting on Earth, an important mission of the Space Biology program. For example, hospitalization, particularly in an ICU (intensive care unit) can reduce skeletal muscle mass by 25%. Cast immobilization can decrease affected muscle mass by 30% as well. In addition, mechanical unloading due to disuse (e.g., bedrest) and illness (e.g., sepsis, chronic obstructive pulmonary disease, chronic heart failure) exacerbates atrophy, weakness, and impedes recovery.
Task Progress:	During this year, we settled into our Gilchrist Building laboratory facilities in the 3rd floor. We purchased a new Nikon Ti2-A fluorescence microscope. We also have continued to manage and reboot operations during COVID waves. • We found that microgravity depresses protective proteins, i.e., heat shock protein 70 (HSP70), sirtuin-1 (SIRT1), nuclear factor erythroid 2–related factor 2 (Nrf2), and Manganese – specific superoxide dismutase (MnSOD) were depressed with 7 days of unloading, allowing excessive oxidative stress that led to muscle atrophy (Lawler 2021, 2022). • Mitigation of elevated assembly of the NADPH oxidase-2 complex by using the peptide inhibition of gp91ds-tat significantly protected against unloading-induced reduction of HSP70 in skeletal muscle. • Nox2 was found to be causal in skeletal muscle atrophy associated with microgravity. • Nox2 peptide inhibition protected against loss of nNOS at the muscle cell membrane. • Angiotensin II receptor 1 (AT1R) inhibition protected against Nox2 elevation, oxidative stress, and loss of sarcolemmal nNOS (Hord 2021). • Demonstrated development and proof of concept of a new Bioreactor to simulate overloading and microgravity in skeletal muscle cells. • Translation to Duchenne muscular dystrophy (DMD): Nox2 was elevated in both Duchenne muscular dystrophy and spaceflight, leading to skeletal muscle myopathy. • Mice with DMD had significant muscle damage and elevated levels of pro-inflammatory proteins RANKL, Cyclophilin A, and Acid Sphingomyelinase compared with normal, healthy mice. • Inhibition of Nox2 protected against upregulation of RANKL in skeletal muscle of dystrophic mice. • Stress protective proteins SIRT1, HSP70, Nrf2, and MnSOD were depressed in mice with Duchenne muscular dystrophy, similar to spaceflight -- thus contributing to oxidative stress and myopathy. • Our research team also found elevated levels of mRNA transcripts (gene expression) for Cyclophilin A and Acid Sphingomyelinase in dystrophic muscle -- golden retriever muscular dystrophy (GRMD) muscle. • We demonstrated proof of concept of systemic AAV9 delivery to skeletal muscle and heart. This new technology

	development and application holds great promise for SAFE gene therapy against inflammation, oxidase stress, and impaired stress proteins.
Bibliography Type:	Description: (Last Updated: 11/16/2023)
Abstracts for Journals and Proceedings	Roeming D, Ramanuja S, Kamal KY, Othman M, Lawler JM. "AAV9/shRNA Knockdown of RANKL protein expression utilizing systemic drug delivery: Proof of Concept. " Texas A&M Student Research Week, College Station, Texas, March 3-4, 2022. Abstracts. Texas A&M Student Research Week, College Station, Texas, March 3-4, 2022. , Mar-2022
Abstracts for Journals and Proceedings	Ramanuja S, Roeming D, Kamal KY, Othman M, Lawler JM. "AAV9/shRNA Knockdown of RANKL protein expression via systemic drug delivery." 17th Texas A&M Pathways Student Research Symposium. Abstracts. 17th Texas A&M Pathways Student Research Symposium, College Station, Texas, March 3-4, 2022. , Mar-2022
Abstracts for Journals and Proceedings	Lawler JM. "Spaceflight sarcopenia: Solutions in redox biology." 2022 Texas Chapter ACSM Annual Meeting, Waco, Texas, February 24 -25, 2022. Abstracts. 2022 Texas Chapter ACSM Annual Meeting, Waco, Texas, February 24 -25, 2022. , Feb-2022
Abstracts for Journals and Proceedings	Kamal KY, Hord JM, Wu C, Talcott S, Janini Gomes M, Fluckey JF, Ford JF, Turner ND, Lawler JM. "Combination nutrition interventions against spaceflight sarcopenia. " 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
Abstracts for Journals and Proceedings	Kamal KY, Mohajeri A, Lawler JM. "Towards mitigating skeletal muscle atrophy: Peptidyl inhibition of Nox2 enhances stress response signaling during mechanical unloading." 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. Abstracts. 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. , Nov-2021
Abstracts for Journals and Proceedings	Lawler JM, Kamal KY, Mohajeri A. "I hear you kNOX-ing: The emerging role of NADPH oxidase-2 in spaceflight sarcopenia. " 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. Abstracts. 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. , Nov-2021
Abstracts for Journals and Proceedings	Mohajeri A, Kamal KY, Othman MA, Lawler JM. "Translating lessons from the microgravity of spaceflight to Duchenne muscular dystrophy: Elevation of Nox2 signaling and impaired stress protection. " 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. Abstracts. 37th Annual Meeting of the American Society for Gravitational and Space Research, Baltimore, MD, November 3-6, 2021. , Nov-2022