Fiscal Year:	FY 2021	**Task Last Updated:**	FY 08/25/2021
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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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:**	07/31/2022
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:	2	**No. of Master' Degrees:**	
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	2
No. of Bachelor's Candidates:	7	**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
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Flight Program:			
Flight Assignment:	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 - Post-doctoral fellow ; Amin Mohajeri - PhD student; Jordyn Johnson - MS student. 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:	We have two new publications: Lawler JM, Hord JM, Ryan P, Holly D, Janini Gomes M, Rodriguez D, Guzzoni V, Garcia-Villatoro E, Green C, Lee Y, Little S, Garcia M, Hill L, Brooks MC, Lawler MS, Keys N, Mohajeri A, Kamal KY. "Nox2 inhibition regulates stress response and mitigates skeletal muscle fiber atrophy during simulated microgravity." Int J Mol Sci. 2021 Mar;22(6):3252. https:// Hord JM, Garcia MM, Farris KR, Guzzoni V, Lee Y, Lawler MS, Lawler JM. "Nox2 signaling and muscle fiber remodeling are attenuated by losartan administration during skeletal muscle unloading." Physiol Rep. 2021 Jan;9(1):e14606. https:// Nox2 Inhibition Regulates Stress Response and Mitigates Skeletal Muscle Fiber Atrophy during Simulated Microgravity: Insufficient stress response and elevated oxidative stress can contribute to skeletal muscle atrophy during mechanical unloading (e.g., spaceflight, bedrest). Perturbations in heat shock proteins (e.g., HSP70), antioxidant enzymes, and sarcolemmal neuronal nitric oxidase synthase (nNOS) have been linked to unloading-induced atrophy. We recently discovered that the sarcolemmal NADPH oxidase-2 complex (Nox2) is elevated during unloading, downstream of then angiotensin II receptor 1, and concomitant with atrophy. Here, we hypothesized that peptidyl inhibition of Nox2 would attenuate disruption of HSP70, MnSOD, and sarcolemmal nNOS during unloading, and thus muscle fiber atrophy. F344 rats were divided into control (CON), hindlimb unloaded (HU), and hindlimb unloaded + 7.5 mg/kg/day gp91ds-tat (HUG) groups. Unloading-induced elevation of the Nox2 subunit p67phox+ staining was mitigated by gp91ds-tat. HSP70 protein abundance was significantly lower in HU muscles, but not HUG. MnSOD decreased with unloading; however, MnSOD was not rescued by gp91ds-tat. In contrast, Nox2 inhibition protected against unloading suppression of the antioxidant transcription factor Nrf2. nNOS bioactivity was reduced by HU, an effect abrogated by Nox2 inhibition. Unloading-induced soleus fiber atrophy was significantly attenuated by gp91ds-tat. These data establish a causal role for Nox2 in unloading-induced muscle atrophy, linked to preservation of HSP70, Nrf2, and sarcolemmal nNOS. Nox2 Signaling and Muscle Fiber Remodeling are Attenuated by Losartan Administration during Skeletal Muscle Unloading: Reduced mechanical loading results in atrophy of skeletal muscle fibers. Increased reactive oxygen species (ROS) are causal in sarcolemmal dislocation of nNOS and FoxO3a activation. The Nox2 isoform of NADPH oxidase and mitochondria release ROS during disuse in skeletal muscle. Activation of the angiotensin II type 1 receptor (AT1R) can

elicit Nox2 complex formation. The AT1R blocker losartan was used to test the hypothesis that AT1R activation drives Nox2 assembly, nNOS dislocation, FoxO3a activation, and thus alterations in morphology in the unloaded rat soleus. Male Fischer 344 rats were divided into 4 groups: ambulatory control (CON), ambulatory + losartan (40 mg/kg/day) (CONL), 7-days of tail-traction hindlimb unloading (HU), and HU + losartan (HUL). Losartan attenuated unloading-induced loss of muscle fiber cross-sectional area (CSA) and fiber-type shift. Losartan mitigated unloading-induced elevation of ROS levels and upregulation of Nox2. Furthermore, AT1R blockade abrogated nNOS dislocation away from the sarcolemma and elevation of nuclear FoxO3a. We conclude that AT1R blockade attenuates disuse remodeling by inhibiting Nox2, thereby lessening nNOS dislocation and activation of FoxO3a.

ABSTRACTS

Lawler JM, Hord JM, P. Ryan, D. Holly, Guzzoni, V, Janini Gomes M, D. Rodriguez, Garcia-Villatoro E Green C, Lee Y. Little S, Garcia M, Hill L, Brooks M-C, Lawler MS, Keys N, Mohajeri, A, Kamal, K. "Effect of Nox-2 Inhibition on Skeletal Muscle Atrophy and Stress Response Signaling During Mechanical Unloading" American Society for Gravitational and Space Biology & Radiation meeting. Virtual Meeting, 2020.

Mohajeri A, Kamal Khaled, and Lawler JM. "The Evaluation of Nox2 Role in Microgravity–Induced Skeletal Muscle Atrophy," International Journal of Exercise Science: Conference Proceedings: Vol. 2: Iss. 13, Article 76. 2021.

Mohajeri A., Kamal K., & Lawler J. Peptidyl inhibition of Nox2 enhances stress response & mitigates muscle fiber atrophy with simulated microgravity. Experimental Biology, virtual event, April 27–30, 2021.

Kamal K., Mohajeri A., & Lawler J. Stress response proteins & Nox2 signaling in the gastrocnemius muscle of dystrophic mice. Experimental Biology, virtual event), April 27–30, 2021.

Mohajeri A., Kamal K., & Lawler J. The evaluation of Nox2 role in microgravity–induced skeletal muscle atrophy. Texas chapter of ACSM, virtual event, February 25–26, 2021.

Bibliography Type:	Description: (Last Updated: 11/16/2023)
Abstracts for Journals and Proceedings	Mohajeri A, Kamal K, Lawler J. "The evaluation of Nox2 role in microgravity–induced skeletal muscle atrophy." 2021 Texas Chapter of the American College of Sports Medicine meeting, Virtual, February 25-26, 2021. International Journal of Exercise Science: Conference Proceedings. 2021 Feb;2(13):76. Available at: https://digitalcommons.wku.edu/ijesab/vol2/iss13/76 ; accessed 8/25/21. , Feb-2021
Abstracts for Journals and Proceedings	Kamal K, Mohajeri A, Lawler J. "Stress response proteins & Nox2 signaling in the gastrocnemius muscle of dystrophic mice." Experimental Biology, virtual meeting, April 27–30, 2021. FASEB Journal. 2021 Apr; 35(S1). https://doi.org/10.1096/fasebj.2021.35.S1.05252 , Apr-2021
Abstracts for Journals and Proceedings	Mohajeri A, Kamal K, Lawler J. "Peptidyl inhibition of Nox2 enhances stress response & mitigates muscle fiber atrophy with simulated microgravity." Experimental Biology 2021, Virtual, April 27-30, 2021. FASEB Journal. 2021 Apr;35(S1). https://doi.org/10.1096/fasebj.2021.35.S1.05433 , Apr-2021
Abstracts for Journals and Proceedings	Lawler JM, Kamal KY, Mohajeri A. "Probing the Nox2 Pathway and Stress Response Signaling: Identifying Therapeutic Targets in the Gastrocnemius Muscle of Dystrophic Mice." Parent Project Muscular Dystrophy Conference 2021, Virtual, June 23-26, 2021. Abstracts. Parent Project Muscular Dystrophy Conference 2021, Virtual, June 23-26, 2021. , Jun-2021
Articles in Peer-reviewed Journals	Hord JM, Garcia MM, Farris KR, Guzzoni V, Lee Y, Lawler MS, Lawler JM. "Nox2 signaling and muscle fiber remodeling are attenuated by losartan administration during skeletal muscle unloading." Physiol Rep. 2021 Jan;9(1):e14606. https://doi.org/10.14814/phy2.14606 ; PMID: 33400850; PMCID: PMC7785102 , Jan-2021
Articles in Peer-reviewed Journals	Lawler JM, Hord JM, Ryan P, Holly D, Janini Gomes M, Rodriguez D, Guzzoni V, Garcia-Villatoro E, Green C, Lee Y, Little S, Garcia M, Hill L, Brooks MC, Lawler MS, Keys N, Mohajeri A, Kamal KY. "Nox2 inhibition regulates stress response and mitigates skeletal muscle fiber atrophy during simulated microgravity." Int J Mol Sci. 2021 Mar 23;22(6):3252. https://doi.org/10.3390/ijms22063252 ; PMID: 33806917; PMCID: PMC8005132 , Mar-2021