Fiscal Year:	FY 2023	**Task Last Updated:**	FY 04/05/2023
PI Name:	Lawler, John Ph.D.		
Project Title:	Attenuation of Space Radiation-induced Pro-oxidant and Fibrotic Signaling in the Heart by Nutritional and Genetic Interventions: Adventures in Tissue Sharing		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **Cardiovascular**:Risk of Cardiovascular Adaptations Contributing to Adverse Mission Performance and Health Outcomes		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	jml2621@email.tamu.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	979-862-2038
Organization Name:	Texas A&M University		
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PI Web Page:			
City:	College Station	**State:**	TX
Zip Code:	77843	**Congressional District:**	17
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2016-2017 HERO NNJ16ZSA001N-Crew Health (FLAGSHIP, OMNIBUS). Appendix A-Omnibus, Appendix B-Flagship
Start Date:	07/01/2017	**End Date:**	12/31/2023
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:	2	**No. of Master' Degrees:**	
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	2
No. of Bachelor's Candidates:	7	**Monitoring Center:**	NASA JSC
Contact Monitor:	Elgart, Robin	**Contact Phone:**	281-244-0596 (o)/832-221-4576 (m)
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 12/31/2023 per NSSC information (Ed., 4/3/23) NOTE: End date changed to 6/30/2022 per Space Radiation element and NSSC information (Ed., 7/6/21) NOTE: End date changed to 6/30/2021 per NSSC information (Ed., 11/6/20) NOTE: End date changed to 6/30/2020 per NSSC information (Ed., 9/26/19)		
Key Personnel Changes/Previous PI:	July 2021 report: Adding Mariam Atef, PhD student. Adding: Joo Kim, PhD student Adding: Amelia Flug (Aggie Research Scholar) 2021- ; Devon Roeming (Aggie Research Scholar) 2021- ; Corine Harvey (Aggie Research Scholar) 2021- ; Grace Barrow (Aggie Research Scholar) 2021- ; Samhitha Ramanuja (Aggie Research Scholar) 2021- ; Binh Nguyen (Aggie Research Scholar) 2021- . REMOVED due to COVID: Jordyn Johnson, MS student; Myles McFarland (Aggie Research Scholar) 2020; Mollie Linder (Aggie Research Scholar) 2020; Hallie Harris (Aggie Research Scholar) 2020; Aakash Kothari (Aggie Research Scholar) 2020; Francisco Melesio (Aggie Research Scholar) 2020; Sonny Rodriguez (Aggie Research Scholar) 2020; Mia Ngyuen (Aggie Research Scholar) 2020.		

COI Name (Institution):	Ford, John Ph.D. (Texas A&M Engineering Experiment Station) Turner, Nancy Ph.D. (Texas A&M AgriLife Research)
Grant/Contract No.:	80NSSC17K0118
Performance Goal No.:	
Performance Goal Text:	
Task Description:	Spaceflight imposes a unique set of stressors on astronauts as a result of mechanical unloading due to microgravity conditions, while tissues are bombarded by galactic and solar radiation. The cardiovascular system is adversely affected by the disuse and fluid shifts that occur with spaceflight. However, there is a growing concern that cardiovascular disease may be substantially elevated during spaceflight. Indeed, increasing evidence indicates that radiation exposure causes damage and fibrosis in the heart and vasculature. Cellular mechanisms of dysfunction due to disuse and space radiation include increased oxidative stress, pro-inflammatory signaling, and impaired function. Heart, vasculature, and the musculoskeletal system will be exposed to gamma and heavy ion (HZE) radiation. Mitochondria, lysosomes, and nucleic acids are particularly susceptible to HZE and secondary oxidant-induced damage. Previous findings and preliminary data from our laboratory indicate that oxidative stress contributes to apoptosis and fibrosis in aging heart models. However, the contribution by which space radiation (X-Ray, HZE) contributes to secondary oxidative stress and fibrosis in the heart are not well understood. We argue that space radiation induced acceleration of the aging process in heart and skeletal muscle, where susceptibility to fibrosis and apoptosis is high. New studies and Preliminary Data from our laboratory suggest that the renin-angiotensin signaling (RAS) are significant sources of oxidative stress, and thus pro-fibrotic signaling in the heart. Upregulation of RAS in the aging heart upregulates the Nox2 isoform of NADPH oxidase. We have also recently found that Nox2 contributes to oxidative stress and atrophy during ground-based spaceflight of skeletal muscle. Thus secondary and amplified oxidative stress may damage nuclei and stimulate pro-fibrotic signaling, including TGF-ß, smad2/3 phosphorylation, and collagen I accumulation. The current RFA research emphasis in Space Biology Tissue Sharing provides an opportunity to promote sharing of samples with ongoing and archived studies. We will propose a series of studies with X-Ray, HZE, and X-Ray + HZE radiation. Collaboration with Dr. Nancy Turner's laboratory at Texas A&M University will focus on two sets of radiation studies. The first cohort of studies will use X-Ray radiation (0.5 Gy) to induce damage and oxidative stress. Mouse (astronaut age) heart samples will be taken 12 hours, or 4 or 8 weeks after exposure. In the second set of experiments, mice will be exposed to 28Si and 48Ti (0.5 Gy). Mice will be sacrificed and tissues extracted 12 hrs, 4 wks, or 8 wks after radiation exposure. Efficacy of an intervention of fish oil + pectin in reducing cardiac fibrotic signaling will be tested. Fish oil reduces oxidative stress and cardiovascular disease, increases protective heat shock proteins. Our Preliminary Data reveal that fish oil + curcumin also reduces muscle atrophy. Dietary pectin ingestion reduces oxidative stress and apoptosis. Pectin and fish oil have also reduced radiation-induced tissue fibrosis in the kidney and liver, respectively. However, the effects on the irradiated heart are unknown. p53 contributes to apoptosis, cardiac fibrosis, and muscle atrophy. We will also query archived cardiac samples irradiated at the Brookhaven National Laboratory involved in combined X-Ray and 56Fe radiation, where mice with a single p53 allele deletion and wild-types were irradiated.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The cardiovascular system experiences a number of dynamic changes during spaceflight that impair function and predispose it to chronic disease. When space missions travel beyond the protection of the Van Allen belts the hearts and vasculature of astronauts are subject to the profound stressors of both microgravity and radiation from solar and galactic sources. Mechanical unloading of the musculoskeletal system due to microgravity results in severe disuse, eliciting "detraining" of the heart. In addition, a fluid shift toward central blood volume during microgravity results in elevated right atrial pressure and thus elimination of plasma volume via diuresis. Atrial naturietic factor (ANF) and the renin-angiotensin II pathway are involved in increased renal excretion of water. Spaceflight appears to elicit morphological (e.g., collagen fibrosis) and functional changes of the heart that could impede performance, lead to fatigue and orthostatic hypotension upon re-entry to a gravitational environment, and increase the risk of heart and vascular disease. In addition, disuse that occurs with microgravity may predispose the heart to arrhythmias (Moffitt et al. 2013). Radiation enhances apoptosis and loss of myocytes as well as accumulation of collagenous tissue, or "fibrosis." The average age of a typical astronaut has increased to over 50 years of age, and progressive age increases oxidative stress in the heart (Kwak et al. 2006). Spaceflight imposes a unique set of stressors on astronauts as a result of the loss of gravity during spaceflight, while tissues are bombarded by galactic and solar radiation. The cardiovascular system is adversely affected by the disuse and fluid shifts that occur with spaceflight. However, there is a growing concern that cardiovascular disease may be substantially elevated during spaceflight. Indeed, increasing evidence indicates that radiation exposure causes damage and fibrosis in the heart and vasculature. Weightlessness and space radiation during long-duration spaceflight, particularly in outer space between the Earth and the moon or Mars, increases inflammation and oxidative stress in the heart, vasculature, and muscles, joints, and bones. The body is exposed to X-ray and heavy ion (HZE) radiation that damages cell components such as mitochondria, nuclei, and the cell membrane through increase release of oxidants (i.e., oxidative stress). Astronaut age has increased into the 50s, and thus has the risk of damage, cell death, and fibrotic connective tissue, as published by our laboratory and other scientists. However, the contribution by which space radiation (X-Ray, HZE) contributes to secondary oxidative stress and fibrosis in the heart is poorly understood. We argue that space radiation accelerated the aging process in heart and skeletal muscle, increased fibrosis, and contributed to cell death. New publications and pilot data from our laboratories indicate that a potential source of oxidative stress in the heart during radiation is called the renin-angiotensin system (RAS). RAS can trigger the assembly of NADPH oxidase-2 (Nox2), a cluster of proteins that produces oxidative stress. We recently found that Nox-2 is elevated in a ground spaceflight analog in skeletal muscle and heart, and contributed directly to changes in muscle cell size, shape, and infiltration of connective tissue. Antioxidant compounds and nutritional supplement choices that are based upon causal studies may have alleviated changes in the heart, vasculature, and skeletal muscle with spaceflight. For example, fish oil reduces oxidative stress, and thus increases protective heat shock proteins, and reduces cardiovascular disease. For example, a combination of fish oil and curcumin recently prevented muscle fiber atrophy and increased protective stress response proteins in a spaceflight analog. Dietary pectin ingestion reduces oxidative stress and cell death. Pectin and

fish oil have also reduced radiation-induced tissue fibrosis in the kidney and liver, respectively. However, the effects on the irradiated heart are unknown. We propose to determine the effects of a combination of fish oil and pectin on heavy ion-induced radiation in the heart.

The current RFA research emphasis in Space Biology Tissue Sharing provides an opportunity to promote sharing of samples with ongoing and archived studies. We are conducting a series of studies with X-Ray, HZE, and X-Ray + HZE radiation. Collaboration with Dr. Nancy Turner's laboratory at Texas A&M University focuses on two sets of radiation studies. The first cohort of studies will use X-Ray radiation (0.5 Gy) to induce damage and oxidative stress. Mouse (astronaut age) heart samples will be taken 12 hours, or 4 or 8 weeks after exposure. In the second set of experiments, mice will be exposed to 28Si and 48Ti (0.5 Gy). Mice were sacrificed and tissues extracted 12 hrs, 4 wks, or 8 wks after radiation exposure. Effectiveness of fish oil + pectin in reducing heart damage and fibrosis is being tested. Our Preliminary Data reveal that fish oil + curcumin also reduces muscle atrophy. A protein called p53 also contributes to cell death, fibrosis of the heart, and muscle atrophy. We will thus also query archived cardiac samples irradiated at the Brookhaven National Laboratory. We will also query archived cardiac samples irradiated at the Brookhaven National Laboratory involving combined X-Ray and HZE radiation, where mice with a single p53 allele deletion were irradiated.

References

Moffitt JA, Henry MK, Welliver KC, Jepson AJ, Garnett ER. (2013) Hindlimb unloading results in increased predisposition to cardiac arrhythmias and alters left ventricular connexin 43 expression. Am J Physiol Regul Integr Comp Physiol. 304(5):R362-73.

Kwak, H.-B., W. Song,, and J.M. Lawler. (2006) Exercise-training ameliorates age-induced elevation in Bax/Bcl-2 ratio, apoptosis, and remodeling in the aging rat heart. FASEB J.

Task Progress:

TaskBook HRP 2022 bullet points
- We developed a novel Bioreactor to simulate microgravity, radiation exposure, and over/reloading of skeletal muscle cells. We intend to develop co-cultures.

Kamal KY, Othman M, Lawler JM. Proof of Concept: Developing a novel bioreactor for skeletal muscle hypertrophy and atrophy by manipulating uniaxial cyclic strain. ASGSR abstract, 2022, Houston, TX.

- We developed a new RANKL knockdown package with AAV9 gene delivery. RANKL is elevated by microgravity, aging, Duchenne muscular dystrophy in skeletal muscles

Othman, Mariam A, Khaled Y. Kamal, Devon Roeming, Samhitha Ramanuja, John M. Lawler. RANKL protein knockdown applying AAV9/shRNA Systemic Drug Delivery to mitigate spaceflight-induced muscle atrophy, ASGSR Abstract 2022, Houston, TX.

Roeming D, Ramanuja S, Kamal KY, Othman M, Lawler JM. AAV9/shRNA Knockdown of RANKL Protein Expression Utilizing Systemic Drug Delivery: Proof of Concept. Student Research Week, Texas A&M University, 2022.

- We used combination nutritional interventions (fish oil + curcumin to mitigate skeletal muscle oxidative stress, atrophy, fibrosis with microgravity - Also, fish oil + pectin against cardiac fibrosis

Kamal KY, Hord JM, Wu C, Talcott S, Janini Gomes M, Fluckey JF, Ford JF, Nancy D. Turner, Lawler JM. Combination Nutrition Interventions Against Spaceflight Sarcopenia. Human Research Project Meeting. NASA. Galveston, February, 2022.

Lawler JM, Holly H, Ryan P, Janini Gomes M, Brooks M-C, Jennifer Cardona J, Nancy D. Turner ND, Ford JR. Effect of Fish Oil and Pectin on Fibrosis and Inflammation in Mouse Hearts Exposed to HZE Radiation. 2018 Integrative Physiology of Exercise, San Diego, CA, 2019.

- Partial loading and HZE radiation resulted in significant damage and markers of fibrosis in skeletal muscle. This paper has been accepted and is in press.

Wiggs MP, Lee Y, Shimkus KL, O'Reilly CI, Lima F, Macias BR, Shirazi-Fard Y, Greene ES, Hord JM, Braby LA, Carroll CC, Lawler JM, Bloomfield SA, Fluckey JD. Combined effects of heavy ion exposure and simulated lunar gravity on skeletal muscle. Life Sci Space Res. 2023 May;37:39-49.

- Assistant Research Assistant Dr. Khaled Kamal was awarded a SHINE scholarship for radiation research.

NASA SHINE (Space Health Impacts for the NASA Experience) Training Program- Virtual Space Radiation Curriculum (# NNJ22ZSA001L). Dr. Khaled Kamal received this award and participated in the first annual SHINE (Space Health Impacts for the NASA Experience) Space Radiation virtual course.

Bibliography Type: Description: (Last Updated: 11/16/2023)

Abstracts for Journals and Proceedings

Kamal KY, Othman M, Lawler JM. "Proof of Concept: Developing a novel bioreactor for skeletal muscle hypertrophy and atrophy by manipulating uniaxial cyclic strain." 38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022.
Abstracts. 38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022. , Nov-2022

Abstracts for Journals and Proceedings

Othman, Mariam A, Khaled Y. Kamal, Devon Roeming, Samhitha Ramanuja, John M. Lawler. "RANKL protein knockdown applying AAV9/shRNA Systemic Drug Delivery to mitigate spaceflight-induced muscle atrophy." 38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022.
38th Annual Meeting of the American Society for Gravitational and Space Research, Houston, TX, November 9-12, 2022. , Nov-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	Lawler JM, Holly H, Ryan P, Janini Gomes M, Brooks M-C, Cardona J, Turner ND, Ford JR. "Effect of fish oil and pectin on fibrosis and inflammation in mouse hearts exposed to HZE radiation. " Integrative Physiology of Exercise Conference, American College of Sports Medicine (ACSM), San Diego, CA, 2019. Abstracts. Integrative Physiology of Exercise Conference, American College of Sports Medicine (ACSM), San Diego, CA, 2019. , Jun-2019
Articles in Peer-reviewed Journals	Kamal KY, Lawler JM. "Cellular and molecular signaling meet the space environment." Int J Mol Sci. 2023 Mar 22;24(6):5955. https://doi.org/10.3390/ijms24065955 ; PMID: 36983029; PMCID: PMC10058013 , Mar-2023
Articles in Peer-reviewed Journals	Wiggs MP, Lee Y, Shimkus KL, O'Reilly CI, Lima F, Macias BR, Shirazi-Fard Y, Greene ES, Hord JM, Braby LA, Carroll CC, Lawler JM, Bloomfield SA, Fluckey JD. "Combined effects of heavy ion exposure and simulated lunar gravity on skeletal muscle. " Life Sci Space Res. 2023 May;37:39-49. https://doi.org/10.1016/j.lssr.2023.02.003 ; PMID: 37087178 , May-2023
Papers from Meeting 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." Student Research Week, Texas A&M University, College Station, Texas, March 21-24, 2022. Abstracts. Student Research Week, Texas A&M University, College Station, Texas, March 21-24, 2022. , Mar-2022