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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.

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Astronaut age, aging heart, exercise: Data from studies that have followed the development of pathology following radiation exposure of the heart and vasculature suggest a process that may mimic some of the effects of aging and Duchenne muscular dystrophy. Specifically, oxidative stress, DNA damage, elevated pro-fibrotic signaling, and accumulation of collagen are shared characteristics of radiation exposure, aging, and Duchenne muscular dystrophy. Oxidative stress and fibrosis lead to increased risk of arrhythmias, cardiovascular disease, and mechanical remodeling that increases stiffness and impairs function.
Effect of Exercise on Nox2-induced Signaling and Fibrosis in the Aging Heart: We have conducted a series of studies that have investigated the ability of exercise training to ameliorate oxidative stress and cardiac remodeling in the astronaut age hearts. NADPH oxidase-2 (Nox2) activity and subunit protein levels were elevated with aging in the heart. Indeed, we found that 12 weeks of exercise training significantly attenuated the age-related increase in Nox2 activity. Exercise training also attenuated age-associated increases in Nox2 subunit localization and protein abundance for gp91phox and p47phox. These data clearly indicate that exercise can ameliorate Nox2 and oxidative stress levels in the astronaut age heart.

Given Nox2 contributes to fibrosis of the heart, and exercise training reduces Nox2, we tested the hypothesis that exercise training reduces connective tissue and collagen I levels in the aging heart. Per our hypothesis, exercise training significantly ameliorated fibrotic tissue in the aging heart. Collagen was markedly elevated with age, and suppressed with daily exercise. Similarly, alpha-smooth muscle actin (alpha-SMA) staining, a marker of myofibroblasts commonly seen in fibrotic tissue, was significantly enhanced by aging. However, exercise training provided a significant inhibitory effect on alpha-SMA. Transforming growth factor-ß (TGF-ß) is a cytokine usually involved in activation of myofibroblasts and collagen production. As predicted, exercise training not only decreased TGF-ß localization in the aging heart, but also significantly reduced TGF-ß abundance when compared with the age-matched sedentary group. Furthermore, smad2/3 phosphorylation (Ser423/425) increased 2.4-fold with aging and was attenuated by regular exercise.

Upregulation of Nox2 in the aging heart has been linked to renin-angiotensin II signaling (RAS). We tested the hypothesis that 12 weeks of endurance treadmill training would significantly reduce abundance of angiotensin II receptor I levels (AT1R). Indeed, while old rats expressed higher AT1R protein levels in the heart, exercise training provided substantial protection against age-induced upregulation of AT1R. These data suggest that exercise-induced protection against fibrosis and remodeling of the aging heart is accomplished by downregulation a pathway involving AT1R, Nox2, and TGF-ß.

The Role of Nox2 in Fibrosis in the mdx Mouse Heart: Similar to aging, Duchenne muscular dystrophy (DMD) causes profound and progressive fibrosis of the heart, impaired function, and the risk of heart failure. Nox2 is upregulated in mdx mice, a model for DMD (Whitehead et al. 2010). We have observed that apocynin, a Nox2 inhibitor and antioxidant, significantly reduces fibrosis in the mdx mouse heart. In addition, apocynin also significantly reduced TGF-ß levels in the dystrophic heart. We also determined that Nox2 effects were linked to oxidative stress by utilizing EUK-134, a mimetic of the antioxidant enzymes superoxide dismutase and catalase. Indeed, EUK-134 reduces muscle damage, inflammation and weakness in the diaphragm muscle of mdx mice (Kim and Lawler 2012).

Effect of Microgravity (Hindlimb Unloading) on Nox2 in the Heart: Oxidative stress is increased with spaceflight and ground analogs for µG in heart and skeletal muscle. We found that Nox2 is upregulated in the heart with the hindlimb unloading µG model in the heart. The specific Nox2 inhibitory peptide gp91ds-tat mitigated oxidative stress in the heart.

Oxidative Stress Is Causal in Age-Related Fibrosis and Apoptosis of the Aging Heart: Our laboratory consistently observed reduction in oxidative stress and cardiac remodeling (e.g., fibrosis, apoptosis) of the aging rat heart (Kwak et al. 2006, 2011, 2015). In addition to downregulation of an AT1R – Nox2 pathway, oxidative stress may also be suppressed with exercise by upregulating antioxidant enzymes and other stress response proteins. Indeed, we found that while aging reduced MnSOD activity in the left ventricle, exercise training increase MnSOD activity in both the old and young hearts. We found that overexpression of MnSOD partially protected against oxidative stress and elevation of collagen I of old mice. Furthermore, the transgenic MnSOD mouse also significantly reduced TGF-ß in the old heart. Overexpression of MnSOD also reduced apoptosis in the aging heart, as indicated by TUNEL+ staining (Kwak 2015). The old transgenic mouse displayed lower TUNEL+ staining vs. old wild-type mice. These data are supportive of the notion that oxidative stress drives apoptosis and remodeling in the aging heart.

Alterations in Redox State in the Heart with Radiation:

Pilot data reveal that redox balance, as assessed by the ratio of reduced to oxidized glutathione (GSH/GSSG) is significant higher with fish oil and pectin in the diet than controls, when exposed to gamma radiation. These data suggest that fish oil and pectin enhance antioxidant protection when tissues are irradiated. Increased antioxidant protection was evident both in the early and delayed responses to radiation.

Fish Oil & Curcumin Intervention in Ground-Based Microgravity: Dietary Fish Oil (FO) supplementation reduces unloading-induced changes in muscle morphology reduced oxidative stress. Similarly, curcumin, a polyphenol that is found in turmeric, inhibits inflammatory signaling and mitigates skeletal muscle atrophy.

Task Progress:

We proposed that a treatment combining 5% fish oil and 1% curcumin (FOC) in the diet would be synergistic in reducing unloading-induced skeletal muscle atrophy because they target independent pathways. Hindlimb unloading (HU) causes muscle atrophy. We hypothesized that FOC would alleviate the translocation (or untethering) of membrane-associated proteins (ex. nNOS) away from the membrane. To test our hypothesis, C57Bl/6 mice were divided in three groups: control group=CON, hindlimb unloading group=HU fed with control diet; fish oil/curcumin+HU group = FOC+HU (n=6) fed specialized diets 10 days prior to HU; continued specialized diets during 7 days of HU period.

Soleus muscle fiber cross sectional area (CSA) and membrane-associated proteins (nNOS, dysferlin, caveolin-3) expression/localization were quantified. We found a marked increase in fiber CSA and soleus mass in the FOC+HU group compared with HU, which suggests that FOC prevents the decrease of CSA during HU. We found no difference in dysferlin localization between groups. We also found that nNOS and caveolin-3 membrane expression were higher in the FOC group comparing with the HU group.

We hypothesized that fish oil, rich in omega-3-fatty acids, combined with polyphenol curcumin protect anabolic (Akt pathway) signaling and heat shock proteins in the rat soleus muscle, concomitant with protection of morphology, is a synergistic countermeasure. FOC mitigated the unloading-induced decrease in CSA and prevented the fiber-type shift normally. FOC also rescued anabolic signaling (Akt phosphorylation, p70S6K phosphorylation) and increased the abundance of HSP70. Therefore, we concluded that the combination of fish oil and curcumin prevents muscle atrophy in concert with the ability to boost heat shock proteins and anabolic signaling in an unloaded state.

HZE Irradiating in the Heart: In a pilot study, C57BL/6 mice were irradiated with a dosage of 0.50 Gy using a 60Co gamma source. Hearts were harvested and frozen in isopentane (-160°C) cooled in liquid nitrogen 8 weeks after radiation exposure. Hematoxylin and eosin (H&E) stains revealed that radiation exposure appeared to increased extracellular matrix space, in a heterogeneous pattern. There were more nuclei visualized in the extracellular matrix space as well. Follow-up experiments with TGF-ß, a cytokine activator of fibroblast, myo-fibroblasts, and fibrosis.

Intervention with Fish Oil, Pectin: TGF-ß positive staining was elevated in the irradiated hearts. These data emphasize the importance of finding effective and safe countermeasures to mitigate radiation-induced damage and fibrosis in the heart of astronauts to address inflight and post-flight cardiovascular risk. We expect Nox2 inhibition to reduce radiation-induced damage. We have been collaborating with Dr. Nancy Turner's laboratory at Texas A&M on two sets of radiation studies. Low LET (linear energy transfer) gamma-ray exposure of mice will be conducted at Texas A&M overseen by Dr. John Ford's laboratory in Nuclear Engineering. Acute 0.5 Gy exposures will be used. Sacrifice of the mice and extraction of the heart and skeletal muscle will occur at 12 hours, 4 weeks, and 8 weeks following radiation treatment. Astronaut age (40-42 weeks) mice were split into controls, non-irradiated with pectin + fish oil, X-ray irradiation, X-ray irradiation with pectin + fish oil, Pectin (6% by weight) and fish oil (15% by weight). We have completed collecting samples and are currently analyzing irradiated hearts. In addition, the impact of HZE (28Si, 48Ti) radiation on cardiac markers of pro-oxidant and pro-fibrotic signaling is currently being examined. We postulate that intervention with fish oil and pectin will abrogate radiation-induced oxidative stress and fibrosis in the heart.

Outcome Markers for Specific Aim 1 include (1) left ventricle damage, (2) oxidative stress, (3) Nox2 subunits (gp91phox, p67phox), (4) pro-fibrotic signaling (TGF-ß, p-smad 2/3, FSP-1), fibrosis (collagen I), and (5) nuclear damage. We expect that pectin and fish oil will reduce oxidative stress and boost stress response proteins (grp94, HSP70) concomitant with protection against pro-inflammatory signaling (TGF-ß, MMP-9, FSP-1, NF-kappaB). These data would demonstrate a reduction in fibrosis linked to antioxidant and anti-inflammatory properties of fish oil combined with pectin under low LET radiation in the mouse heart. Initial results demonstrate that HZE radiation increases oxidative stress, MP-9 levels, TGF-ß, and invasion of inflammatory cells (Macrophages, monocytes). Fish oil + pectin is having a small positive effect.

Collaboration with Aging -- HZE radiation Project with Dr. Melinda Sheffield-Moore: We are commencing a new tissue sharing project with Dr. Melinda Sheffield-Moore. C57/BL6 mice were irradiated at 1 Gy, 0.5 Gy, and 0.25 Gy with HZE. Mice were allowed a latent period of 60 days and 2 years post-radiation. We will be testing hearts from irradiated or control mice for the following outcomes: 1) oxidative stress, 2) Nox2 subunits, 3) pro-inflammatory signaling, 4) invasion of inflammatory cells, 5) profibrotic signaling.

We expect these data to reveal the long-term effects of exposure to HZE radiation, particularly important in understanding the increased risk factors for cardiac fibrosis and cardiovascular disease following long-term spaceflight, especially in deep space.

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Bibliography Type: Description: (Last Updated: 06/05/2025)

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Abstracts for Journals and Proceedings	Ryan P, Lawler MS, Holly, D, Janini Gomes M, Hord J, Lawler JM. "Nox2 Inhibition Prevents Skeletal Muscle Atrophy and nNOS Translocation in Hindlimb Unloaded Rats." 33rd Annual Meeting of the American Society for Gravitational and Space Research, Seattle, WA, October 25-28, 2017. 33rd Annual Meeting of the American Society for Gravitational and Space Research, Seattle, WA, October 25-28, 2017. , Oct-2017