Fiscal Year:	FY 2023	**Task Last Updated:**	FY 11/04/2022
PI Name:	Delp, Michael Ph.D.		
Project Title:	Effects of Spaceflight on Ocular Oxidative Stress and the Blood-Retinal Barrier		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	SPACE BIOLOGY--Cellular and molecular biology		
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) Developmental Biology (2) Neurobiology		
Space Biology Special Category:	(1) Translational (Countermeasure) Potential		
PI Email:	mdelp@fsu.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	352-214-1195
Organization Name:	Florida State University		
PI Address 1:	College of Human Sciences		
PI Address 2:	242 Sandels Building, 120 Convocation Way		
PI Web Page:			
City:	Tallahassee	**State:**	FL
Zip Code:	32306-0001	**Congressional District:**	2
Comments:	Previous affiliations were University of Florida (mid-2007-June 2014), West Virginia University (mid-2005 to mid-2007), and Texas A&M University (1995 to mid-2005).		
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2014 Space Biology Flight NNH14ZTT001N
Start Date:	02/01/2015	**End Date:**	06/04/2022
No. of Post Docs:	1	**No. of PhD Degrees:**	1
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:	Loftus, David	**Contact Phone:**	650-604-1011
Contact Email:	david.j.loftus@nasa.gov		
Flight Program:	ISS		
Flight Assignment:	Tissue Sharing NOTE: Extended to 6/4/2022 per F. Hernandez/ARC (Ed., 7/27/21) NOTE: Extended to 6/4/2021 per F. Hernandez/ARC and NSSC information (Ed., 6/12/19) NOTE: Extended to 7/30/2019 per F. Hernandez/ARC and NSSC information (Ed., 2/14/19) NOTE: Extended to 1/31/2019 per NSSC information (Ed., 3/12/18) NOTE: Extended to 1/31/2018 per F. Hernandez/ARC (Ed., 2/12/17)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Pecaut, Michael Ph.D. (Loma Linda University) Mao, Xiao Wen M.D. (Loma Linda University)		
Grant/Contract No.:	NNX15AE86G		
Performance Goal No.:			

Performance Goal Text:	
Task Description:	Approximately 29% of astronauts on short-term (~2 wk) space shuttle flights and 60% on long-duration (~6 mo) missions to the International Space Station (ISS) are reported to have experienced some impairment in distant or near visual acuity. These visual disturbances have been hypothesized to be related to increases in intracranial pressure (ICP) and intraocular pressure. Modeling studies have shown that a compromise in the integrity of the vascular blood-brain barrier (BBB) would serve to elevate ICP. While much attention has been directed toward the role of the cerebral vasculature in elevating ICP, little work has been done to examine conditions of the vasculature in the eye and the potential role of microgravity in altering the blood-retinal barrier (BRB), which maintains a similar function in the eye for regulating intraocular pressure as the BBB in the cranium. One condition known to compromise the BRB is oxidative stress. For example, in diabetic retinopathy, the leading cause of blindness in Western society, elevations in oxidative stress compromise the BRB and increase vascular permeability in the eye. The proposed studies through the ISS Rodent Tissue Sharing Opportunity will provide new and important information regarding the effects of spaceflight on oxidative stress in the eye and its potential deleterious effects on the BRB.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Through the collection of 300 post-flight questionnaires, it has recently been reported that approximately 29% of astronauts flying short-duration missions and 60% of astronauts on long-duration missions experience an impairment of distance and near visual acuity. Furthermore, some of these changes remain degraded for years after flight. It is hard to imagine a more severe, prevalent, and potentially intractable condition threatening human space exploration than the loss of visual acuity. In 2010, NASA Space Life Sciences at Johnson Space Center in Houston held a Visual Impairment Intracranial Pressure (VIIP) Summit of leading clinicians and scientists with expertise in ophthalmology and cerebral fluid dynamics, and it was hypothesized that the visual impairment experienced by astronauts was the result of a microgravity-induced cephalad fluid shifts and corresponding increases in ICP and intraocular pressure. The proposed studies will provide new and important information regarding the effects of spaceflight on oxidative stress in the eye, its potential deleterious effects on the blood-retinal barrier and, consequently, factors that may function to increase intraocular pressure. In addition, understanding the relation between oxidative stress in the eye and disruption of the blood-retinal barrier may provide new insight into other conditions that affect visual acuity, including diabetic retinopathy, the leading cause of blindness in Western society, where elevations in oxidative stress compromise the blood-retinal barrier and increase vascular permeability in the eye.
Task Progress:	The goal of Study 1 was to address Specific Aims #1 and #2, i.e., to investigate the effects of spaceflight on oxidative stress and apoptosis in retinal endothelial cells and to identify spaceflight-induced changes in protein expression profiles in mouse ocular tissue. Additionally, we sought to determine whether the application of 1g artificial gravity (AG) during spaceflight could mitigate any detrimental effects of microgravity on the retina. We hypothesized that spaceflight would induce elevations in oxidative stress and apoptosis in retinal endothelial cells, as well as alter ocular proteins associated with apoptosis, cell repair, inflammation, and metabolic function. We further hypothesized that the application of 1g AG would mitigate these changes. Twelve male 9-week old C57BL/6 male mice, obtained from a United States (US) breeding colony, were launched July 18, 2016, at NASA Kennedy Space Center (KSC) on a SpaceX-9 rocket for the 35-day Mouse Habitat Unit-1 (MHU-1) mission to the International Space Station (ISS). The animals were housed in the mouse Habitat Cage Unit (HCU) located in the Japan Aerospace Exploration Agency (JAXA) "Kibo" facility on the ISS. The 12 flight mice were subdivided into two groups. The first group of flight mice (n=6) were exposed to ambient microgravity conditions (µg group), while the second group of flight mice (n=6) were exposed to continuous artificial Earth gravity (µg + 1g group) while they were in the HCU. AG was achieved through the use of a short-arm centrifuge for the duration of their stay on the ISS. The flight mice were then returned live to Earth and splashed down in the Pacific Ocean on August 26, 2016. It took approximately 40 hours for the mice to be recovered in the Pacific Ocean, brought to shore and transported to the testing and processing laboratory located in San Diego, California on August 28, 2016. The spaceflight mice were then sacrificed and their eyes were removed and prepared for analysis. Ground control mouse studies were completed in Japan after the return of the flight mice. Control mice (Habitat Controls, n=6; Vivarium controls, n=6) were acquired on August 31, 2016 from a breeding colony in Japan and shipped to the JAXA animal facility in Tsukuba, Japan. HC mice were acclimated to the water lixit system and the same special food bar diet as the spaceflown mice were fed. They were first housed in the Transportation Cage Unit (TCU) to simulate launch and flight to the ISS, and then placed in the HCUs to simulate the housing conditions experienced by µg mice on the ISS. They were again placed in the TCU to simulate the return to Earth flight. The control mouse dissections took place on November 3, 2016. Control mouse eye tissue was then shipped to the US for analysis. All mice received the same ad libitum access to food and water. Study 1 Conclusions: 1) The data demonstrated that spaceflight alone induced apoptosis in retinal vascular endothelial cells, which suggests disruption in the integrity of the blood-retinal barrier. 2) The number of apoptotic cells in the retina was reduced 24% during spaceflight with continuous artificial 1g while the animals were housed on the ISS. 3) Proteomic analysis showed that many proteins were significantly altered after spaceflight compared to that in habitat control mice; these proteins are involved in cell death, cell repair, inflammation, carbohydrate metabolism, and apoptosis. 4) Continuous artificial 1g showed lower organismal death and greater cellular organization and function signaling compared to the spaceflight alone group. The purpose of Study 2 was to more directly address Specific Aim #2, i.e., to characterize the effects of spaceflight on the retinal vasculature and possible alterations in blood-retinal barrier (BRB) integrity, and to identify spaceflight-induced proteomic significance and biomarkers in mouse ocular tissue. The data demonstrate that spaceflight induces apoptosis in the retinal vascular endothelial cells and photoreceptors, as well as evokes alterations in vascular levels of aquaporin-4 (AQP-4), platelet endothelial cell adhesion molecule (PECAM-1) and zonula occludens-1 (ZO-1) proteins related to BRB integrity. Study 2 Conclusions: 1. The results of this study demonstrate that exposure to a spaceflight environment is associated with increased retinal endothelial cell and photoreceptor cell death. 2. The changes in retinal microvasculature BRB integrity (i.e., vascular levels of AQP-4, PECAM-1, and ZO-1), indicate decrements in barrier function in the eye. 3. Protein expression profiles and pathway analysis provide evidence that spaceflight induces changes in cellular organization, cell cycle, mitochondrial function, circadian clock, and oxidative stress in the retina. 4. Collectively, these observations are consistent with, and extend, previous findings in rodents exposed to a weightless environment and

suggest that spaceflight involves a complex combination of stressors that leads to alterations and impairment of ocular structure and function.

The purpose of Study 3 was to address Specific Aim #1, i.e., to characterize the effects of spaceflight on the retinal oxidative stress and extracellular matrix remodeling in the eye. Specifically, this study characterized the physical response of the retina, the degradation of photoreceptors, and the presence of oxidative stress markers. The findings also indicate that spaceflight induces a distinct gene expression signature in the retina of mice. This signature is enriched for genes related to visual perception, the phototransduction pathway, and numerous retina and photoreceptor phenotype categories. Several genes with significant differential expression in the spaceflight condition are also differentially expressed in the disease retinitis pigmentosa.

Study 3 Conclusions: The results suggest that the differential expression induced by spaceflight may be pathological. Additionally, we suspect the changes observed during spaceflight are influenced by alternative splicing and chromatin reorganization.

The purpose of Study 4 was to determine the effects of spaceflight on possible alterations in DNA methylome and transcriptome of the retina, and to determine whether the primary impacted genes belong to physiologically relevant cellular processes and pathways. These include processes and pathways associated with oxidative stress, inflammation, mitochondrial function, tissue remodeling, fibrosis, and angiogenesis. This study represents a more sophisticated experimental approach than that originally proposed in the specific aims and, consequently, provides more information regarding the broad effects of spaceflight on the retina.

Female C57 BL/6J mice that were 16 weeks old were used in this study. Both spaceflight and ground control animals were housed in NASA's animal enclosure modules (AEM), with control mice being exposed to the same environment conditions (12-hour light cycle, temperature and humidity) as those flown on the ISS. Control animals were kept inside an environmental simulator (ISSES) at the Space Life Science Laboratory (SLSL) at Kennedy Space Center, and the spaceflight animals were transported to the ISS by SpaceX4 on September 21, 2014. All animals were fed with a special NASA food bar diet and their health was checked daily. The spaceflight mice were sacrificed and frozen in orbit after 37 days of flight, while ground control mice were simultaneously sacrificed and frozen under identical conditions. After the frozen carcasses were returned to KSC, the ocular tissues were removed from both groups.

Study 4 Conclusions: Approximately one in three astronauts flying on long-duration space missions experience visual impairment and morphologic changes to their eyes that include choroidal and retinal folds, optic disc edema, focal areas of retinal ischemia (i.e., cotton wool spots), globe flattening, and hyperopic shifts. This collection of ocular disorders has been termed Spaceflight Associated Neuro-ocular Syndrome (SANS). A variety of potential mechanisms have also been proposed to account for the unusual physiologic and pathologic neuro-ophthalmic findings in astronauts, including elevations in intracranial pressure (ICP) from cephalad fluid shifts, altered autoregulation of cerebral perfusion, impaired cerebrospinal fluid drainage from the brain and orbital optic nerve sheath through venous, glymphatic, and lymphatic drainage systems, and disruption of blood-brain, blood-retinal, and blood-optic nerve barrier function. This seemingly multifaceted pathological process, which varies from astronaut to astronaut, indicates a complex origin for these neuro-ophthalmic findings associated with SANS. The integrated DNA methylome and RNA transcriptome analysis demonstrates that spaceflight had profound effects on extracellular matrix (ECM) / cell junction and cell proliferation/apoptosis signaling in the retina. Although these data do not address all the possible mechanisms involved in the etiology of SANS, they provide crucial insight into the potential adverse consequences of spaceflight on the retina that could be functionally important for maintaining proper visual acuity among astronauts.

Bibliography Type:	Description: (Last Updated: 06/21/2023)
Articles in Peer-reviewed Journals	Mao XW, Nishiyama NC, Byrum SD, Stanbouly S, Jones T, Drew A, Sridharan V, Boerma M, Tackett AJ, Zawieja D, Willey JS, Delp M, Pecaut MJ. "Characterization of mouse ocular response to a 35-day spaceflight mission: Evidence of blood-retinal barrier disruption and ocular adaptations." Sci Rep. 2019 Jun 3;9(1):8215. https://doi.org/10.1038/s41598-019-44696-0 ; PMID: 31160660 PMCID: PMC6547757 , Jun-2019
Articles in Peer-reviewed Journals	Overbey EG, da Silveira WA, Stanbouly S, Nishiyama NC, Roque-Torres GD, Pecaut MJ, Zawieja DC, Wang C, Willey JS, Delp MD, Hardiman G, Mao XW. "Spaceflight influences gene expression, photoreceptor integrity, and oxidative stress-related damage in the murine retina." Sci Rep. 2019 Sep 16;9(1):13304. https://doi.org/10.1038/s41598-019-49453-x ; PMID: 31527661; PMCID: PMC6746706 , Sep-2019
Articles in Peer-reviewed Journals	Chen Z, Stanbouly S, Nishiyama NC, Chen X, Delp MD, Qiu H, Mao XW, Wang C. "Spaceflight decelerates the epigenetic clock orchestrated with a global alteration in DNA methylome and transcriptome in the mouse retina" Precis Clin Med. 2021 Jun;4(2):93-108. https://doi.org/10.1093/pcmedi/pbab012 ; PMID: 34179686; PMCID: PMC8220224 , Jun-2021
Articles in Peer-reviewed Journals	Willey JS, Britten RA, Blaber E, Tahimic CGT, Chancellor J, Mortreux M, Sanford LD, Kubik AJ, Delp MD, Mao XW. "The individual and combined effects of spaceflight radiation and microgravity on biologic systems and functional outcomes." J Environ Sci Health C Toxicol Carcinog. 2021 Apr 27;39(2):129-79. https://doi.org/10.1080/26896583.2021.1885283 ; PMID: 33902391 , Apr-2021