Fiscal Year:	FY 2019	**Task Last Updated:**	FY 01/30/2019
PI Name:	Rithidech, Kanokporn Ph.D.		
Project Title:	Effects of Space Flights on the Proteome of Astronauts' Plasma		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Biomedical countermeasures		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	(1) **Immune**:Risk of In Mission Impacts, Adverse Health Events or Long-Term Health Impacts due to Altered Immune Response		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	11794-8691	**Congressional District:**	1
Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2014-15 HERO NNJ14ZSA001N-MIXEDTOPICS. Appendix E: Behavioral Health & Human Health Countermeasures Topics
Start Date:	04/01/2016	**End Date:**	03/31/2020
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
Contact Monitor:	Norsk, Peter	**Contact Phone:**	
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Flight Program:			
Flight Assignment:	Flight definition NOTE: End date changed to 3/31/2020 per NSSC information (Ed., 3/25/19)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Martin, Dwight Ph.D. (State University of New York, Stony Brook)		
Grant/Contract No.:	NNX16AH80G		
Performance Goal No.:			

Performance Goal Text:	
Task Description:	NOTE: This is an integrated project consisting of Dr. Brian Crucian's "Functional Immune Alterations, Latent Herpesvirus Reactivation, Physiological Stress, and Clinical Incidence Onboard the International Space Station" directed research; and Dr. Richard Simpson's "The Impact of an ISS Mission on the Anti-Viral and Functional Properties of NK-cells, T-cells, B-cells and Dendritic Cells," Dr. Kanokporn Rithidech's "Effects of Space Flights on the Proteome of Astronauts' Plasma," and Dr. Honglu Wu's "DNA Damage in the ISS Astronaut's Lymphocytes and Their Association with Stress-Induced Immune Dysfunction" solicited research. Space flight results in exposure of astronauts to several stressors, such as space radiation, microgravity, and physiological stress, that could exacerbate the risks of adverse health effects. To protect astronauts, we must improve our understanding of molecular changes that influence immunological conditions associated with increased astronaut health risks. The in vivo response to the space environment is complex, involving multiple proteins associated with various signal transduction cascades, resulting in different outcomes. Molecular mechanisms responsible for such diverse consequences are poorly understood. It is, therefore, essential to characterize the protein signatures of responses to the space environment in blood plasma samples from astronauts, collected at pre-, in-, and post-flights. Such analyses should help to reveal a particular set of proteins causing adverse immunological changes and to develop methods that help to prevent, or at least to counteract, these effects. In this flight definition project, we will use cutting age proteomic technology to determine protein alterations, qualitatively and quantitatively, in plasma samples collected from astronauts before, during, and after space flights. Our findings will help to provide an understanding of the time course and etiology of immune changes induced by the space environment. Furthermore, since pre- and post-flight samples, in addition to the in-flight samples, will be evaluated in the same astronaut, the direct effects of the space environment can be determined. Hence, our findings will provide high-priority and highly relevant information to NASA. We will further correlate protein expression profiles to the available data on immune dysfunction detected in each astronaut. This approach makes it possible to determine potential predictive biomarkers for space-flight-induced immune dysregulation. Consequently, effective countermeasures against such harmful effects of the space environment can be identified.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Our findings will deliver important information that should advance our understanding of the time course and etiology of immune changes induced by the space environment. Hence, our findings will provide high-priority and highly relevant information to NASA. Importantly, we will correlate protein expression profiles to the available data on immune dysfunction detected in each astronaut. This approach makes it possible to determine potential predictive biomarkers for space-flight-induced immune dysregulation. Such knowledge is important for assessment of health risks and will facilitate the development of appropriate countermeasures that can help astronauts, space travelers, and people on Earth with the impairment of the immune system.
Task Progress:	After the last Task Book report, we obtained plasma samples from four astronauts. Prior to proteomics analysis, astronaut blood plasma was depleted of albumin and IgG using affinity spin columns. The depleted plasma was reduced and alkylated and digested with trypsin. The reaction mixture containing the resultant peptide product from the digestion was desalted by reverse phase chromatography. The desalted peptides were fractionated by a 12 step multi-dimensional chromatography process which delivered the peptides to a Thermo Fisher Orbitrap XL mass spectrometer for analysis by Liquid Chromatography with tandem mass spectrometry (LC-MS-MS). Subsequently, the raw data files were interrogated and searched against a current UniProt human proteome database using the Andromeda search engine within MaxQuant. This produced a list of proteins for each sample which was quantitated by extracted ion chromatogram intensity by the MaxQuant program. The raw data from all the samples were searched simultaneously to reduce the occurrence of homology redundancy. The data from all the samples were compiled into a spreadsheet and the protein quantity was normalized based on the fractional signal strength within each sample. The samples were grouped according to the timeline stages of collection (i.e., pre-, in-, and post-flight). A total of 453 unique and non-redundant proteins were identified at >= 99% confidence. Changes in protein concentrations during the timeline were determined by Student's t-test analysis of comparisons between groups ($p<0.1$ is considered significant). Those proteins displaying statistically significant changes were subjected to interacting network analysis using the Genemania app within the Cytoscape software package. Our data demonstrate that there are 14 proteins with significant changes, i.e., increased or decreased, in expression levels in plasma samples collected in-flight as compared to those collected pre-flight. Four proteins with significant increases were detected in plasma collected in-flight, as compared to the plasma collected pre-flight. These are antithrombin-II (SERPINC1), plasma protease C1 inhibitor (SERPING1), Tropomodulin-3 (TMOD3), thyroxine-binding globulin (SERPINA7). The levels of the remaining 10 proteins were decreased in plasma collected in-flight as compared to those collected pre-flight. Notably, the protein in the SERPIN (serine protease inhibitor) family, i.e., SERPINA7, SERPINC1, and ISERPING1, is the majority of those with increased expression levels. The SERPIN protease inhibitors comprise a large family of molecules involved in inflammation, immune response, blood clotting, hormone transport, and complement activation, dementia, and tumorigenesis. Hence, our findings suggest that dysregulation of these proteins may affect cell/tissue integrity and homeostasis, leading to late occurring health risks. It has been suggested that overexpression of tropomodulin- 3 (TMOD3, actin pointed end-capping protein) leads to decreased endothelial motility. The majority of proteins with decreased levels are those involved in immune response and cytoskeleton systems. Further, there were 16 proteins with significant changes in expression levels in astronauts' plasma collected post-flight, in relation to those collected in-flight. There are eight proteins with significantly increased expression levels, while another set of eight proteins showed significantly decreased levels. The majority of proteins with changes in expression are those involved in immune and cytoskeleton systems. A new set of proteins with significant changes in expression levels was found in astronauts' plasma collected post-flight, e.g., vitronectin, ceruloplasmin, Zyxin, and tubulin beta chain. This set of proteins may be involved in the re-adaptation to the Earth environment. Importantly, our results show that a decreased level of lumican (LUM) and an increased level of antithrombin-III (SERPINC1) persisted in astronauts' plasma collected in- and post-flight. Such a prolonged increase in the level of SERPINC1 may contribute to coagulopathy, the phenomenon observed in individuals exposed to radiation. It is known that LUM is a major keratan sulfate proteoglycan of the cornea responsible for the circumferential growth, corneal transparency, epithelial cell migration, and tissue repair. Hence, LUM has been found to be critical in maintaining corneal clarity. Further, a loss of

	LUM expression has been found to be associated with corneal inflammation. Hence, persistent decreases in the level of LUM after spaceflight would impair the homeostasis of the eyes. Taken together, it is plausible to hypothesize that a reduction in LUM level may be associated with vision impairment that has been observed in many astronauts after spaceflight. Our findings warrant further investigation of the potential role of LUM in vision impairment detected in astronauts. Highlights: The highlights of the findings from our study on the proteome of astronauts' plasma collected at pre-, in-, and post-flights are: • The majority of proteins with altered expression levels detected in plasma samples collected in- or post-flight are those involved in immune and cytoskeleton systems. • A new set of proteins with altered expression levels was detected in plasma collected post-flight as compared to the samples collected in-flight. • An increased level of antithrombin-III (SERPINC1) and a decreased level of Lumican (LUM, an important protein involved in maintaining corneal clarity protein) persisted for a long time post-flight, suggesting the potential role in late-occurring health risks.
Bibliography Type:	Description: (Last Updated: 03/27/2025)