Fiscal Year:	FY 2015	**Task Last Updated:**	FY 04/19/2016
PI Name:	Kucik, Dennis F. M.D., Ph.D.		
Project Title:	Mechanisms, early events, and dose dependence of radiation-induced atherosclerosis		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
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:	kucik@uab.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	205-934-0062
Organization Name:	University of Alabama at Birmingham		
PI Address 1:	Pathology		
PI Address 2:	1025 18th St S, CBSE room 239		
PI Web Page:			
City:	Birmingham	**State:**	AL
Zip Code:	35205-4831	**Congressional District:**	7
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2009 Space Radiobiology NNJ09ZSA001N
Start Date:	02/01/2011	**End Date:**	07/31/2015
No. of Post Docs:	1	**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:	Simonsen, Lisa	**Contact Phone:**	
Contact Email:	lisa.c.simonsen@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: End date changed to 7/31/2015 per PI and NSSC information (Ed., 8/27/14) NOTE: End date changed to 7/31/2014 per NSSC information (Ed., 4/10/14) NOTE: End date changed to 4/30/2014 per NSSC information (Ed., 1/22/14)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Kabarowski, Janusz Ph.D. (University of Alabama at Birmingham)		
Grant/Contract No.:	NNX11AC61G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Radiation causes vascular inflammation, which is a known risk factor for atherosclerosis. Epidemiological studies have shown that radiation from many sources, including cancer treatments, atomic bombs, and excessive occupational exposure all increase the risk for atherosclerosis. Previous studies, using gamma and/or X-ray radiation, have demonstrated that radiation causes increased white blood cell (WBC) adhesion to the blood vessel wall, an essential early event in atherosclerotic plaque formation. What is not known is whether the cosmic radiation astronauts will be exposed to on missions to the moon and Mars will similarly increase the risk for atherosclerosis. In our last project, we established that X-ray, 56Fe (iron ion), and proton irradiation of blood vessel cells increase adhesiveness of the vessel wall, and that X-rays and 56Fe accelerate development of atherosclerosis in a mouse model (results of proton experiments are pending). The molecular mechanism for this, however, is not yet known. In addition, it remains to be determined how fractionation of doses and irradiation of other tissues affect the dose dependence of both cell adhesion and development of atherosclerosis. With the hypothesis that radiation in general and cosmic radiation in particular directly alter the adhesive properties of vascular endothelium, and resultant vascular inflammation accelerates atherosclerosis, we propose to systematically investigate mechanisms of radiation effects on vascular cells, using both isolated cells and whole mice, to better predict risk and to provide the basis to develop possible future countermeasures. Our specific aims are: Aim 1: Determine whether atherogenic effects of radiation are limited to local effects on vascular endothelium, or if other systems contribute to disease progression and/or modify dose dependence. Aim 2: Determine the molecular mechanism of acute activation of leukocyte-endothelial cell adhesion in response to radiation. Aim 3: Determine how fractionation of doses affects dose-dependence of progression rates, latency periods, and surrogate endpoints.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Therapeutic radiation is a well-established risk factor for cardiovascular disease and stroke. Head and neck cancer patients who undergo radiation treatment are at significantly elevated risk of stroke, even in young patients whose risk would otherwise be near zero. For women with early breast cancer, the benefit of radiotherapy can be nearly offset by the increased risk of mortality from vascular disease. Moreover, new modalities of therapeutic radiation include the use of proton and carbon ion irradiation. Little is known about the adverse effects of these types of radiation, but early results from cell and animal studies suggest that the consequences for cardiovascular disease could be equal to or greater than those for gamma- and X-rays. The risk from accidental exposure is similar. For example, atomic bomb survivors have an increased incidence of coronary artery disease and stroke. Risk for cardiovascular disease after radiation exposure at Chernobyl was increased for those who were exposed to less than 1 Gy. Even radiation technologists in the 1950s (when shielding was less rigorous) had an increased risk of death from cardiovascular disease, demonstrating that repeated exposure at low doses results in significant risk years later. Currently, the principal strategy for reducing risk is avoidance of exposure. Completion of our specific aims will advance the knowledge of the molecular mechanisms of radiation-induced atherosclerosis, enabling better prediction of cardiovascular risk from exposure, facilitating early detection through the use of surrogate biomarkers, and pointing the way toward potential countermeasures to mitigate the cardiovascular consequences of radiation exposure, both in space and on Earth.
	Summary of Research for the entire reporting period (in terms of CPR questions) The overall goal of this project was to determine the effect of radiation, especially cosmic radiation, on atherosclerosis, the disease that results in heart attacks and strokes. This could help to estimate the risk for astronauts on deep-space missions, such as a trip to Mars. This project consisted of two arms. The cell component, using cultured human aortic endothelial cells (HAECs) was designed to elucidate the mechanism by which absorption of radiation, either photons or ions, causes pro-atherogenic changes in the vascular endothelium. The animal component, which used development of atherosclerotic plaques as an endpoint, was to determine the eventual consequences of these changes for development of cardiovascular disease, and to work toward identification of early endpoints that predict later consequences. ApoE -/- mice were used in the animal component of this project because they already had a well-characterized response to x-ray radiation, especially with respect to atherosclerosis. Previous studies had established that this mouse model reproduces the pathology seen in humans after radiation exposure. Moreover, these mice mimic an adult human population in that they unavoidably develop some degree of asymptomatic atherosclerotic disease spontaneously with age. In this project, we made progress on three CPR questions, as detailed below. Degen-1, “How can tissue specific risk models be developed for the major degenerative tissue risks, including heart, circulatory, endocrine, digestive, lens and other tissue systems in order to estimate GCR (galactic cosmic radiation) and SPE (solar particle event) risks for degenerative diseases?” The tissue of interest for this project is the vascular system, especially the major vessels of the chest and neck, which are the major sites of clinically significant atherosclerosis. To address Degen-1, we established that 56Fe (i.e., iron ions, a particularly damaging component of cosmic radiation) targeted to the aorta and carotid arteries has similar effects as x-rays, but at a lower dose. That is, while 8-14 Gy x-rays have been shown to exacerbate atherosclerosis in this mouse model, 56Fe has significant effects at as little as 2 Gy. The fact that 56Fe can produce effects at lower doses is not surprising, since particle radiation can be particularly damaging. In radiobiology, the relative biological effect (RBE) is defined as the ratio of absorbed dose of a radiation in question to the absorbed dose of a reference radiation (usually gamma- or x-rays) required to produce the same biological effect in a particular tissue. Based on this definition, the RBE of approximately 4-7 for 56Fe ion-induced atherosclerosis (signifying that iron ion causes similar adverse cardiovascular effects at a 4-7 fold lower dose than x-rays) is consistent with those associated with other effects. It is known from human epidemiologic data that x-ray or gamma-ray doses as low as 1 Gy can increase the risk of

	cardiovascular disease, suggesting that humans are 8-14 times more sensitive to the pro-atherogenic effects of high-energy photon radiation as apo-E -/- mice. Assuming an RBE of 4-7 for humans as well as mice, the prediction would be that 56Fe as low as 0.14-0.25 Gy might significantly increase the risk for astronauts. Further work will be needed to confirm that this extrapolation from mice to humans is valid. Although 56Fe targeted to the aortic arch accelerated development of atherosclerosis, the dose dependence of 56Fe-induced atherosclerosis varied by site. While 5 Gy was required for a significant effect in the aortic arch, 2 Gy was sufficient in the aortic root (where the aorta emerges from the heart) and carotid arteries. These differences might be due to pro-atherogenic contributions of local factors, such as shear stress (the force felt by the inside wall of the vessel from blood flowing by). This advances our understanding of the how radiation induces atherosclerosis by demonstrating that, while direct radiation effects on the vessel are clearly a component of the 56Fe effect on atherosclerosis, the risk can be modified by other local factors.
Task Progress:	The above experiments were done by irradiating only the heart and major blood vessels of mice. Since the entire astronaut will be irradiated during spaceflight, we then asked the question of whether radiation-induced atherosclerosis is primarily dependent on local effects on the vessels themselves or whether significant contributions, either positive or negative, from other organ systems might be important when the whole body is exposed. At least a component of the pro-atherogenic effect of 56Fe was a direct effect on the vessel itself, because radiation did not increase atherosclerosis in un-irradiated areas of the aorta. However, potential effects on the immune system may either exacerbate or ameliorate the local radiation effects. Therefore, we compared whole-body irradiation to that targeted to the chest and neck. Our data indicate that, as with direct, local factors, the influence of systemic factors also depends on the site and perhaps on the particular effect measured. Thickening of the carotid artery wall, a hallmark of atherosclerosis, was largely unaffected by 56Fe irradiation of the rest of the body. Plaque formation in the aortic arch, however, seemed to be slightly blunted in whole-body 56Fe irradiated mice vs. those with radiation targeted to the major vessels. This was a minor effect however, because significant exacerbation of plaques was seen at 2 Gy in either case. We conclude that the major effect of radiation is on the vessels themselves, but that systemic effects from irradiation of other tissues may modify the damage to a small extent. Degen-2, “What are the mechanisms of degenerative tissues risks in the heart, circulatory, endocrine, digestive, lens, and other tissue systems? What surrogate endpoints do they suggest?” The cell biology arm of this study (Aim #2) is specifically designed to elucidate the mechanism of radiation-induced atherosclerosis. It is known that gamma- and x-radiation increase the adhesiveness of vascular endothelium, a key, early step in the pathogenesis of atherosclerosis. We established that 56Fe increases adhesiveness as well. It is hypothesized that this increased endothelial adhesiveness initiates endovascular inflammation in atherosclerosis-prone arteries, and that this inflammatory response can become self-perpetuating. Although a common mechanism for making cells stick to each other is to increase the number of adhesion molecules on the surface of the cell, we showed that x-ray-induced endothelial adhesiveness can occur even without up-regulation of adhesion molecules. We further showed that the mechanism by which this occurs is dependent upon chemokine signaling. We determined that the integrin alpha4beta1 (also known as VLA-4), an important adhesion molecule needed for atherosclerosis to develop, is one of the adhesion molecules activated by radiation. This was not a direct effect, but was mediated by production of signaling molecules, known as chemokines, by cells in the blood vessels. Experiments at Brookhaven National Laboratory (where particle accelerators can mimic components of cosmic radiation) then confimed that, despite the major differences in how x-rays and heavy ions interact with tissue, 56Fe also increases endothelial adhesiveness through a chemokine-dependent mechanism. Moreover, the 56Fe radiation-induced adhesiveness also depends on signaling to the integrin alpha4beta1. Degen-3, “What are the progression rates and latency periods for degenerative risks, and how do progression rates depend on age, gender, radiation type, or other physiological or environmental factors?” Degen-3 is addressed by Aims #1 and #3. Since Aim #1 involves experiments with both x-rays and 56Fe, progression rates for these two radiation types can be compared. We showed earlier that x-rays and 56Fe have similar pro-atherogenic effects, increasing adhesiveness of human aortic endothelial cells. The dose of 56Fe required to do this was much lower than that required for x-rays, however. We also demonstrated a linear energy transfer (LET) dependence of radiation-induced atherosclerotic changes in the major blood vessels. Thus, to estimate risk for astronauts on deep space missions it might be better to consider not only the total dose of heavy ion likely to be absorbed, but to take into account the mix of ions and their energies as well.

Bibliography Type:	Description: (Last Updated: 04/12/2018)
Abstracts for Journals and Proceedings	Kucik DF, Chanda D, Gupta K, Kabarowski JH. "Whole-body irradiation of wild type mice results in chemokine-dependent increased adhesiveness of aortic endothelium, an early pro-atherosclerotic change." Presented at the 20th IAA Humans in Space Symposium, Prague, Czech Republic, June 29-July 3, 2015. Published in meeting proceedings, 20th IAA Humans in Space Symposium, Prague, Czech Republic, June 29-July 3, 2015. , Jul-2015
Abstracts for Journals and Proceedings	Babitz SK, Dunaway CW, Steele C, Kucik DF. "Radiation results in IL-8 mediated intercellular signaling that increases adhesion between monocytic cells and aortic endothelium." Presented at the Committee on Space Research (COSPAR) 2014 40th Scientific Assembly, Moscow, Russia, August 2-10, 2014. Published in meeting proceedings, Committee on Space Research (COSPAR) 2014 40th Scientific Assembly, Moscow, Russia, August 2-10, 2014. , Aug-2014
Abstracts for Journals and Proceedings	Babitz SK, Dunaway CW, Steele C, Kucik DF. "Involvement of IL-8 in radiation-induced, atherosclerosis-related adhesiveness changes of arterial endothelium." Presented at the 60th Annual Meeting of the Radiation Research Society, Las Vegas, Nevada, September 21-24, 2014. Published in meeting proceedings, 60th Annual Meeting of the Radiation Research Society, Las Vegas, Nevada, September 21-24, 2014. , Sep-2014

Abstracts for Journals and Proceedings	Babitz SK, Dunaway CW, Steele C, Kucik DF. "IL-8 mediated intercellular signaling following 56Fe irradiation results in increased aortic endothelial adhesiveness." Presented at the 30th Annual Meeting of the American Society for Gravitational and Space Research, Pasadena, CA, October 22-26, 2014. 30th Annual Meeting of the American Society for Gravitational and Space Research, Pasadena, CA, October 22-26, 2014. , Oct-2014
Abstracts for Journals and Proceedings	Chanda D, Gupta K, Kabarowski JH, Kucik DF. "56Fe irradiation of wild type C57BL/6 mice results in increased adhesiveness of aortic endothelium." 2015 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 13-15, 2015. 2015 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 13-15, 2015. , Jan-2015
Abstracts for Journals and Proceedings	Kucik DF, Chanda D, Gupta K, Kabarowski JH. "Space-relevant dose of 56Fe radiation results in a pro-atherogenic change in the aortic endothelium of wild type C57BL/6 mice." Presented at the Space Radiation and Heavy Ions in Therapy Symposium 2015, Osaka, Japan, May 22-24, 2015. Space Radiation and Heavy Ions in Therapy Symposium 2015, Osaka, Japan, May 22-24, 2015. , May-2015
Articles in Peer-reviewed Journals	White RC, Yu T, Gupta K, Babitz SK, Black LL, Kabarowski JH, Kucik DF. "Early changes in vascular reactivity in response to 56Fe irradiation in ApoE -/- mice." Acta Astronautica. 2015 Mar-Apr;108:40-5. http://dx.doi.org/10.1016/j.actaastro.2014.10.010 , Apr-2015