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Project Title:	Proteomic Profiling of Human Heart Tissue Exposed to Microgravity		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Biomedical countermeasures		
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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		
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Task Description:	Microgravity is a stress experienced during space travel and has been linked to changes in cardiac structure and function in astronauts and cosmonauts. In order to define the mechanisms by which microgravity alters cardiac structure and function, we performed a proteomic investigation of the effects of simulated microgravity on the cardiomyocyte proteome. Using a combination of label-free relative quantitation and dynamic stable isotope labeling by amino acids in cell culture (dynamic SILAC), we compared both the protein expression changes and protein turnover rates between two experimental groups: simulated microgravity and normal gravity. Label-free data revealed that microgravity markedly alters protein expression over time, specifically altering the levels of proteins involved in muscle contraction and structure, translation, metabolism, protein folding and transport, and calcium handling. Dynamic SILAC data demonstrated that protein turnover is diminished in response to simulated microgravity. This observation in combination with the decline in translational proteins suggests that protein translation may mediate the decline in contractile and structural proteins observed in the microgravity group. To validate this finding, we examined three independent measures of protein translation and three surrogates of cell damage and protein degradation. Relative to normal gravity, protein translation was decreased in simulated microgravity, while cell damage and protein degradation was not statistically significant between groups, leading to an overall conclusion that decreased protein translation may mediate microgravity-induced changes to cardiac structure and function.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	In our simulated microgravity experiments we are examining the proteomics changes occurring to cardiac cells and tissues exposed to microgravity compared Earth gravity controls. Therefore, the extensive proteomics data set derived from the Earth gravity controls will be used to inform other studies that perform comparative analysis of cardiac cells exposed to other forms of stress relevant to heart injury and disease. The extensive proteomics data set can also be mined for new targets of heart disease or injury diagnosis or treatments. The data mining may enable new discoveries and products relevant to human heart disease that may be quickly translated into clinical applications. Clinical practice has the potential to be impacted and improved, for example, by the development of assays to assess cardiotoxicity of drugs, new biomarkers to assess disease progression and improvement, and new and better targeted therapeutics for cardiac disease, injury, and failure.
Task Progress:	In order to define the mechanisms by which microgravity alters cardiac structure and function, we performed a proteomic investigation of the effects of simulated microgravity on the cardiomyocyte proteome. Using a combination of label-free relative quantitation and dynamic stable isotope labeling by amino acids in cell culture (dynamic SILAC), we compared both the protein expression changes and protein turnover rates between two experimental groups: simulated microgravity and normal gravity (1x gravity) over time. From our studies the following general concepts were obtained: 1. Protein expression is altered in cardiomyocyte following simulated microgravity. We measured protein-level expression for 848 proteins (6,174 peptides) across all timepoints and gravity conditions. As expected very few proteins are differentially expressed ($p<0.05$) at 12 hours, and the number grows over time, until nearly 100 proteins are differentially expressed. Proteins were classified into three groups: 1) unchanged over time under either condition, 2) equally changed over time under either condition, and 3) differentially changed over time between microgravity and 1x gravity. The significantly altered proteins were then grouped by biological function; seven functional groups were formed: Muscle contraction/structure (26%); Translation (23%); Metabolism (21%); Protein transport and folding (12%); Calcium handling (5%); and Other. 2. Cardiomyocytes under simulated microgravity have diminished protein turnover. Using the ratio of the heavy-isotope label to the total of heavy and light peptide in the dynamic SILAC labeling component of the experiment, our data strongly suggest that amino acid incorporation and thus protein turnover is diminished in the cardiomyocyte subjected to simulated microgravity. No statistical difference exists between measured turnover at 12 hours, however $p<1e-6$ at both 48 hr and 120 hr. This data is highly suggestive that protein turnover is drastically decreased under the microgravity condition, and since access to the translational machinery to precursor amino acid did not seem to be different, the difference points to transcriptional or translational control. 3. Protein translation may be slowed in cardiomyocytes under simulated microgravity. Following the independent quantitative and label-incorporation analyses, two features of the data were connected. The protein expression data revealed that many proteins were downregulated following 120 h of microgravity, specifically, a number of translational proteins were diminished. The decrease in translational proteins suggests that protein translation is slowed, which could be mediating the decline in expression of the other significantly reduced proteins. This revelation was strengthened by the markedly reduced protein turnover observed in the label-incorporation data. Because decreased translation is a characteristic that has been observed in microgravity-induced skeletal muscle atrophy, we decided to perform independent experiments that could validate this potential phenomenon: azidohomoalanine (AHA) incorporation, luciferase activity using adenoviral technology, and total protein-to-total RNA ratio. AHA is a methionine analogue that is incorporated into an individual peptide in a similar fashion as arg or lys. However, AHA was presented to the groups only at 120 h time point and only for 2 h; this design was merely to obtain a snapshot of protein synthesis at the time when the largest separation in protein turnover was observed. AHA incorporation is robust in 0 h controls but is dramatically reduced after 120 h in microgravity relative to 1x gravity. Adenoviral technology was used to deliver the luciferase gene to each group. This technology allows the gene to be transcribed and translated into protein using the cell's inherent machinery. For this experiment, the Adenoviral-Luciferase was delivered to all cells 3 h prior to group separation. The adenovirus was removed upon group separation, and the luciferase protein activity was measured at 120 h. Luciferase activity is a direct correlate to luciferase protein content, since no post-translational modifications are necessary to initiate luciferase enzyme function. Light detection revealed that luciferase protein was significantly diminished 7.5 fold in microgravity compared to 1x gravity. Cell pellets were collected from each group at 120 h, and total protein and total RNA were assessed in order to provide a global examination of protein synthesis relative to the amount of RNA. Data revealed that the total protein-to-total RNA ratio was significantly attenuated 4 fold in microgravity relative to 1x gravity. In all, these independent measures support the SILAC label-incorporation data, indicating that protein turnover in the cardiomyocyte is diminished following 120 h of exposure to simulated microgravity. 4. Indicators of cell damage and protein degradation are not significantly affected in response to simulated microgravity.

	We next examined indicators of cell damage, death, and protein degradation in attempt to determine if microgravity was inducing damage to the cardiomyocyte, thereby altering the proteome, or enhancing protein degradation, thereby slowing protein turnover. Lactate dehydrogenase is a soluble cytosolic enzyme, and its release is an indication of cell membrane permeability or damage. The media was examined from each group at 0, 1, 2, 3, 4, 8, 12, 24, 48, 96, and 120 h. No statistically significant difference was observed between microgravity and 1x gravity at any time period. Caspase-3 is a classical end-effector protease involved in apoptosis. An increase in caspase-3 is a surrogate for enhanced cell death. We surveyed caspase-3 activity at 0, 12, 48, and 120 h, and did not observe a statistically significant difference between microgravity and 1x gravity at any time point. Finally, we assessed global ubiquitination of protein lysates at 0 h and from each group at 120 h. Again, no difference was observed between microgravity and 1x gravity. Together, these data indicate that cardiomyocytes under microgravity are not markedly damaged in response to simulated microgravity. Moreover, because ubiquitination of proteins is a way proteins are tagged for degradation, these data suggest that microgravity is not enhancing protein degradation, which also indicates that protein degradation may not be playing a critical role in the diminished protein turnover induced in microgravity.
Bibliography Type:	Description: (Last Updated: 07/11/2023)
Articles in Peer-reviewed Journals	Feger BJ, Thompson JW, Dubois LG, Kommaddi RP, Foster MW, Mishra R, Shenoy SK, Shibata Y, Kidane YH, Moseley MA, Carnell LS, Bowles DE. "Microgravity induces proteomics changes involved in endoplasmic reticulum stress and mitochondrial protection." Sci Rep. 2016 Sep 27;6:34091. http://dx.doi.org/10.1038/srep34091 ; PubMed PMID: 27670941; PubMed Central PMCID: PMC5037457 , Sep-2016