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| **Fiscal Year:** | FY 2023 | **Task Last Updated:** | FY 06/14/2023 |
| **PI Name:** | Gonzalez-Juarbe, Norberto | | |
| **Project Title:** | Understanding the Impact of Hypobaric Hypoxia and Confinement Stress on Intestinal Immunity and Host-Microbiome Interactions | | |
| **Division Name:** | Human Research | | |
| **Program/Discipline:** | | | |
| **Program/Discipline--Element/Subdiscipline:** | | | |
| **Joint Agency Name:** | | **TechPort:** | No |
| **Human Research Program Elements:** | (1) **HFBP**:Human Factors & Behavioral Performance (IRP Rev H) | | |
| **Human Research Program Risks:** | (1) **BMed**:Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders<br>(2) **Hypoxia**:Risk of Reduced Crew Health and Performance Due to Hypoxia [inactive] | | |
| **Space Biology Element:** | None | | |
| **Space Biology Cross-Element Discipline:** | None | | |
| **Space Biology Special Category:** | None | | |
| **PI Email:** | NGonzale@jcvi.org | **Fax:** | FY |
| **PI Organization Type:** | NON-PROFIT | **Phone:** | 787-566-1271 |
| **Organization Name:** | J Craig Venter Institute, Inc. | | |
| **PI Address 1:** | Infectious Diseases and Genomic Medicine Group | | |
| **PI Address 2:** | 9605 Medical Center Dr, Suite 150 | | |
| **PI Web Page:** | | | |
| **City:** | Rockville | **State:** | MD |
| **Zip Code:** | 20850-6380 | **Congressional District:** | 6 |
| **Comments:** | | | |
| **Project Type:** | GROUND | **Solicitation / Funding Source:** | 2019 HERO 80JSC018N0001-HHCHFBP: Human Health Countermeasures, Human Factors, Behavioral Performance. Appendix D |
| **Start Date:** | 05/10/2021 | **End Date:** | 12/10/2023 |
| **No. of Post Docs:** | 1 | **No. of PhD Degrees:** | 1 |
| **No. of PhD Candidates:** | | **No. of Master' Degrees:** | 1 |
| **No. of Master's Candidates:** | | **No. of Bachelor's Degrees:** | 1 |
| **No. of Bachelor's Candidates:** | | **Monitoring Center:** | NASA JSC |
| **Contact Monitor:** | Whitmire, Alexandra | **Contact Phone:** | |
| **Contact Email:** | alexandra.m.whitmire@nasa.gov | | |
| **Flight Program:** | | | |
| **Flight Assignment:** | NOTE: End date changed to 12/10/2023 per NSSC information (Ed., 7/6/23).<br>NOTE: End date changed to 05/10/2023 per NSSC information (Ed., 6/6/22). | | |
| **Key Personnel Changes/Previous PI:** | Original PI: Dr. Hernan Lorenzi, Co-investigators: Dr. Norberto Gonzalez-Juarbe and Dr. Yanbao Yu. After Dr. Lorenzi's departure, Dr. Manolito Torralba took over the project, however he also left the institute during the pandemic. Dr. Norberto Gonzalez-Juarbe took over the project after Dr. Torralba left. The COVID-19 pandemic delayed the start of the project and in addition caused multiple personnel changes at the institute. | | |
| **COI Name (Institution):** | | | |
| **Grant/Contract No.:** | 80NSSC21K1116 | | |
| **Performance Goal No.:** | | | |
| **Performance Goal Text:** | | | |

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| **Task Description:** | Background:<br>It is expected that future crewed space missions involving extravehicular activities (EVA) will require novel EVA architectures including a slightly hypobaric hypoxic cabin atmosphere (8.2 psia, 34% O2). Humans are well-adapted to live at Earth altitudes with similar O2 partial pressures. However, it is not clear if the combined effect of hypobaric hypoxia (HH) with other space-associated stressors such as microgravity, altered circadian rhythm or confinement, will have a synergistic detrimental effect on crew health. Several studies suggest that some hypoxic conditions may affect the host immune response and gut microbiota (1-4). Also, healthy individuals exposed for ~1-year to the HH environment of the Antarctic Concordia station show immune sensitization (5, and Life Sciences Data Archive (LSDA) experiment: Confinement and Hypobaric Hypoxia on Immunity in the Antarctic Concordia Environment (CHOICE)). The Antarctic Neumayer and Concordia stations represent a high-fidelity spaceflight ground analog, reflecting some conditions of long-duration space missions, such as extreme isolation and altered circadian rhythm. Neumayer is a coastal base located at the sea level. Concordia resides 1,000 km inland at an altitude of 3,232 m and therefore, has a HH environment. Herein, we propose to investigate the combined impact of long-term HH and isolation on the human microbiome and immune system homeostasis in the intestinal tract by using an existing collection of stool specimens derived from 34 healthy individuals that spent ~1-year at either the Concordia or Neumayer stations.<br><br>Hypothesis:<br><br>We hypothesize that the combined effect of HH and confined environment stressors will induce changes to the human intestinal immune response and gut microbiota in the context of microbial diversity, activity, composition, and protein post-translational modifications (PTMs, such as acetylation and oxidation), which tend to be associated with impaired host metabolism, immune response and aggravated cellular damage (1, 2).<br><br>Aims:<br><br>Aim 1. Characterization of gut microbiota and immune profiles of Concordia and Neumayer crewmembers. 16S rRNA sequencing and mass spectrometry based meta-proteomic approaches will be employed to investigate both the microbial composition and host immune responsive proteins.<br><br>Aim 2. Global profiling of gut protein PTMs. Enrichment (e.g., antibody-based) and quantitative (e.g., chemical labeling) strategies will be utilized to examine protein acetylation and oxidation, which will be correlated to host metabolism and oxidative damage.<br><br>Methods:<br><br>Stool specimens were collected from healthy individuals before, during, and after a ~1-year stay at the Neumayer or Concordia stations and kept frozen for further analysis at the J. Craig Venter Institute. We will apply the well-established protocols in our laboratories to extract genomic DNA and proteins from stool samples for taxonomic profiling and proteomic analyses.<br><br>Deliverables:<br><br>A detailed qualitative and quantitative analysis of the impact of Neumayer and Concordia extreme conditions and HH on human gut microbiome and host immunity, interpretation of identified microbial PTMs, and assessment of potential risks to human health.<br><br>Significance:<br><br>The crosstalk between the intestinal microbiome and immune system is essential to human health. Understanding the response of intestinal microbiota and immunity to extreme stress conditions at the taxonomic, metaproteome and PTM levels will offer novel insights into the immune system-microbiome interactions during HH and isolation conditions, and may set the bases for potential therapeutic targets for spaceflight-induced immune and microbial dysregulation.<br><br>References:<br><br>1. Zhang X, Ning Z, Mayne J, Deeke SA, Walker K, Farnsworth CL, Stokes MP, Mack D, Stintzi A, Figeys D. Deep characterization of the protein lysine acetylation in human gut microbiome and its alterations in patients with Crohn's disease. Systems Biology. bioRxiv; 2019. p. 337<br><br>2. Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem. 1997 Aug 15;272(33):20313–20316. PMID: 9252331 |
| **Rationale for HRP Directed Research:** | |
| **Research Impact/Earth Benefits:** | Our studies inform about alterations in metabolic activity of the gut microbiome and, potentially, of the gut epithelium due to confinement and hypoxia leads to modulation of inflammation. In addition, proteomic analysis of microbial and host proteins in the gut will allow us to assess the impact of HH and confinement on host-microbiome interactions and how they correlate to inflammatory profiles. To our best knowledge, there is very little information about how the combination of HH and confinement stressors affect the human microbiome composition and metabolic activity and the intestinal immune response. Therefore, the product of this study is information that will help to define the likelihood and consequences of the risk of reduced crew health due to long-term HH exposure and will dramatically decrease the degree of uncertainty in this risk. |
| | Task-1: Processing of stool samples and generation of 16S taxonomic profiles: DNA will be extracted from stool samples with Qiagen DNeasy Powersoil Kit including negative and positive controls. For each sample, we will amplify by PCR 6 regions spanning the bacterial 16S rRNA gene V1-V9 variable using barcoded primers and the Swift Amplicon 16S Panel Kit (Swift Biosciences). PCR amplicons will be sequenced on an Illumina machine. Sequencing reads will be processed using the Swift Biosciences 16S workflow to remove primers and chimeric sequences, cluster reads into exact Amplicon Sequence Variants (ASVs) and perform further taxonomic classification of ASVs by comparison with the Silva 16S rRNA databases.<br>Task-1 Progress: Processing of stool samples and generation of 16S taxonomic profiles: Our group has extracted DNA from 428 samples that cover the effects of long-term hypobaric hypoxia (HH) and confinement on the human gut |

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| **Task Progress:** | microbiome from stool samples that were collected from subjects spending one year in confinement in Antarctica at either the coastal base Neumayer or at the high-altitude Concordia base.<br><br>Task-2: Analysis of 16S taxonomic profiles and stressors associated with the Concordia and Neumayer stations: To assess the impact of confinement with/without HH on the gut microbiota, we will compare the microbial composition of samples from Concordia and Neumayer bases using pre-mission time points. Briefly, we will measure changes in microbiome's Shannon alpha diversity (based on the number of species present and their abundance) and Richness (total number of species in the sample) using the diversity and rarefy functions from the R package Vegan. Also, we will assess changes in overall taxonomic profiles using Bray-Curtis beta diversity with the Vegan function vegdist, and changes of specific taxa with the R package DESeq2.<br><br>Task-2 Progress: Analysis of 16S taxonomic profiles and stressors associated with the Concordia and Neumayer stations. Current analysis of the 16S data shows that the microbial composition of the crewmembers of Concordia and Neumayer is altered depending on the time of the year and is different when comparing each station against the other. In addition, we have compared the microbiome similarities and differences between the two bases.<br><br>Task-3: Extraction of stool proteins and global metaproteomics analysis. Stool samples will be processed following a SDS and ultrasonication-based lysis protocol. Protein extracts will be subjected to Suspension Trapping digestion followed by nanoflow liquid chromatography (nanoLC) and tandem mass spectrometry (MS/MS) analysis. For quantitation and functional enrichment analyses, we will use the MaxQuant-Perseus software suite, which enables large-scale label-free proteome-level quantitation. The raw MS data will be searched against the National Institutes of Health (NIH) Human Microbiome Reference Genome DB.<br><br>Task-3 Progress: Extraction of stool proteins and global metaproteomics analysis. Our group has processed over 100 pooled samples for global proteomic analysis. All samples are in the queue at the mass spec facility.<br><br>Task-4: Integration of proteomics, 16S and inflammation data. All sequencing and proteomic data will be analyzed and then integrated with cytokine and chemokine inflammation data (to be provided by collaborator Alexander Chouker). Data integration will allow baseline immune and bacterial signatures to predict how Antarctic conditions affect the host microbiome interactions in Concordia and Neumayer crewmembers.<br><br>Task-4 Progress: Integration of proteomics, 16S and inflammation data. Pending upon completion of proteomic analysis. |
| **Bibliography Type:** | Description: (Last Updated: ) |