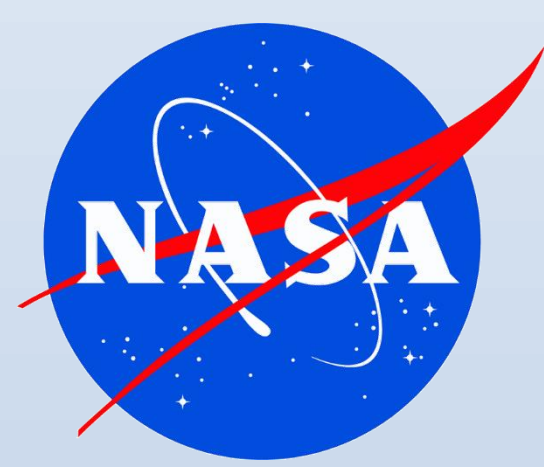


The Integrated Impact of Diet On Human Immune Response, the Gut Microbiota, and Nutritional Status During Adaptation to a Spaceflight Analog



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ABSTRACT

Spaceflight impacts human physiology, including well documented immune system dysregulation. Diet, immune function, and the microbiome are interlinked, but diet is the only one of these factors that we have the ability to easily, and significantly, alter on Earth or during flight. As we understand dietary impacts on physiology more thoroughly, we may then improve the spaceflight diet to improve crew health and potentially reduce flight-associated physiological alterations. It is expected that increasing the consumption of fruits and vegetables and bioactive compounds (e.g., omega-3 fatty acids, lycopene, flavonoids) and therefore enhancing overall nutritional intake from the nominal shelf-stable, fully-processed space food system could serve as a countermeasure to improve human immunological profiles, the taxonomic profile of the gut microbiota, and nutritional status, especially where currently dysregulated during spaceflight. This interdisciplinary study will determine the effect of the current shelf-stable spaceflight diet compared to an "enhanced" shelf-stable spaceflight diet (25% more foods rich in omega-3 fatty acids, lycopene, flavonoids, fruits, and vegetables). The NASA Human Exploration Research Analog (HERA) 2017 missions, consisting of closed chamber confinement, realistic mission simulation, in a high-fidelity mock space vehicle, will serve as a platform to replicate mission stressors and the dysregulated physiology observed in astronauts. Bio sampling of crewmembers will occur at selected intervals, with complete dietary tracking. Outcome measures will include immune markers (e.g., peripheral leukocyte distribution, inflammatory cytokine profiles, T cell function), the taxonomic and metatranscriptomic profile of the gut microbiome, and nutritional status biomarkers and metabolites. Data collection will also include complete dietary tracking. Statistical evaluations will determine physiological and biochemical shifts in relation to nutrient intake and study phase. Beneficial improvements will provide evidence of the impact of diet on crew health and adaptation to this spaceflight analog, and will aid in the design and development of more-efficient targeted dietary interventions.

OBJECTIVE

Implement a ground-control study with HERA analog crew randomized between either the current nominal ISS food system or a spaceflight food system designed to improve dietary quality. Determine the effects of each diet on immune dysregulation, including leukocyte distribution, inflammatory cytokine profiles, T cell function, and other relevant immunological markers, the taxonomic and metatranscriptomic profile of the gut microbiome, and nutritional biomarkers and metabolites at selected intervals, and statistically evaluate to associate shifts across each measure and in relation to nutrient intake.

Spaceflight Food System: Potential Countermeasure for Physiological Changes

CURRENT ISS SPACEFLIGHT DIET

200 options in 8 Standard Menu Categories

- 1. Breakfast
- 2. Rehydratable Meats
- 3. Meat and Fish
- 4. Side Dishes
- 5. Vegetables and Soups
- 6. Fruits and Nuts
- 7. Desserts and Snacks
- 8. Beverages



Freeze-Dried



Low-Moisture



Retort
Thermostabilized or
Irradiated



Powdered
beverages

The food system is one of only a few daily environmental influences that is greatly modifiable for spaceflight and has the potential to promote health

- Limited condiments
- No food refrigeration
- Shelf life of 1-3 years

Immune dysregulation, changes in nutritional status, and changes in the gastrointestinal microbiota have occurred in spaceflight.

Diet and nutritional status is linked to the gastrointestinal microbiota and immune status on Earth.

Increasing consumption of fruits, vegetables, and other foods rich in bioactive components (e.g., omega-3 fatty acids, lycopene, flavonoids) and enhancing overall nutritional intake during spaceflight (or a ground-based analog of spaceflight) is expected to improve human immunological profiles, the taxonomic profile of the gut microbiota, and nutritional status biomarkers and will provide evidence to better inform the impact of diet on crew health and adaptation to spaceflight and to design targeted more-efficient dietary interventions.

METHODS

Implement a study in the Human Exploration Research Analog (HERA) to Determine the impact of an ‘enhanced’ spaceflight diet compared to the current spaceflight diet on immune markers, nutritional status, and the gut microbiota.



The study will occur in 2017 in HERA Campaign 4.

Analog Attributes:

- Astronaut-like crews and work schedules
- Mimics stressful and isolated conditions
- Altered sleeping patterns
- Closed-system spaceflight diet

Mission plan:

- 4 - 45 day missions
- 2 missions current ISS spaceflight diet
- 2 missions enhanced spaceflight diet
- 4 subjects per diet

The HERA missions will provide a ground-control for baseline physiological data that results from each diet.

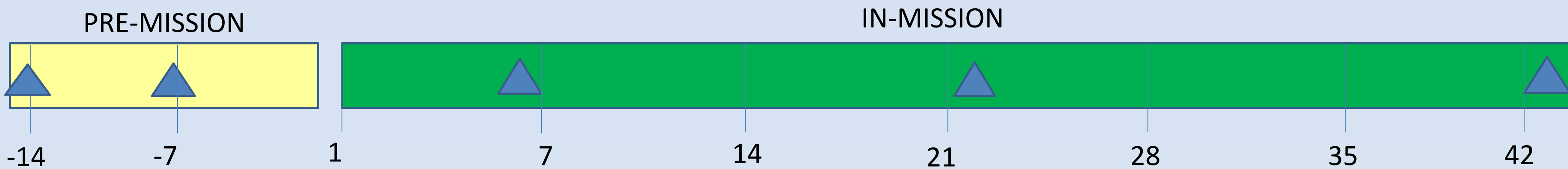
Attributes of the enhanced diet compared to the current ISS diet

Enhanced Diet Will:	Expected Benefits:
Increase Fruit and Vegetable Consumption	Improved nutritional status/bone health Reduced inflammation/Immunomodulation Improved microbiota diversity Increased short chain fatty acid (SCFA) production
Increase fish/omega-3 consumption	Reduced inflammation/Immunomodulation Improved nutritional status/bone health
Increase lycopene consumption	Improved nutritional status/bone health Decreased oxidative damage
Provide 1000-1200 mg Ca/day	Improved nutritional status/bone health
Reduce sodium intake	Improved nutritional status/bone health

	Servings per Week			Average per day	
	Fruit&Veg	Fish	Lycopene	Calcium (mg)	Sodium (mg)
Standard Diet	24	2-3	5	921	2955
Enhanced Diet	48	6	7	1177	2655

Biological Samples and Analysis

Biological Samples will be obtained at 2 pre-mission timepoints and 3 in-mission timepoints (▲) to determine the effect of each spaceflight diet on crew immune markers, nutritional status, and the microbiome throughout the mission.



Biological Sample	Analysis
Blood	Immune: <u>Flow Cytometry</u> – Peripheral leukocyte distribution, T cell function <u>Cytometric Bead Array</u> – Plasma cytokine profiles, mitogen-stimulated cytokine profiles Nutrition: <u>HPLC-MS, HPLC-ESI-MS, GC-MS</u> - Vitamin and mineral status, flavonoids, omega-3 and -6, oxidative markers
Urine	Nutrition: <u>HPLC-MS, HPLC-ESI-MS</u> : Vitamin and mineral status, flavonoids, oxidative markers
Fecal	Microbiome: <u>16S Sequencing</u> : Taxonomic Profiling <u>Illumina Hi-Seq</u> - Metatranscriptomics Nutrition: <u>HPLC/UV-VIS</u> – butyrate, acetate, propionate
Saliva	Immune: <u>Cytometric Bead Array</u> – Salivary cytokine profiles <u>Quantitative PCR</u> – Latent herpes virus
Diet Record	Daily; ISS FIT app
Body Weight	Daily

Statistical associations will be evaluated across each measure and in relation to nutrient intake. The primary aim is to measure effect sizes and variability so that we will have a better understanding of the magnitude of the effect of the enhanced diet, and the power to determine the effects in a spaceflight environment when the proposed diet transitions to spaceflight.

EXPECTED OUTCOMES

This effort will provide pilot data and a ground-control for the impacts of the spaceflight diet on human adaptation to spaceflight. The enhanced diet is expected to:

- Improve nutritional biomarkers and metabolite concentrations (increases in omega-3 fatty acid, flavonoids, and SCFAs in blood, urine, and fecal samples, and decreases in oxidative damage)
- Improve general immune status and mitigate dysregulation, as demonstrated by improvements in T cell function, plasma and secreted cytokine profiles and inflammatory markers that may be associated with target nutrients in the diet.
- Improve overall taxonomic profile of the gut microbiota, such as increments in species diversity, which has been associated with improved gut homeostasis and human health.
- Indicate genes and pathways that participate in the functional response of the gut metagenome to changes in diet habit and environmental factors such as stress. It is expected that among affected genes and pathways will be those that participate in the processing of high-fiber, flavonoid-, lycopene- and omega 3-rich food and in the synthesis of SCFAs and butyrate in the case of samples derived from the enriched-diet cohort.
- Identify genes and pathways whose expression patterns might indicate potential risks to human health, such as downregulation of antibacterial genes or pathways that participate in the production of essential metabolites.

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