Transcriptomics analysis reveals potential mechanisms underlying mitochondrial dysfunction and T cell exhaustion in astronauts' blood cells in space

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Background

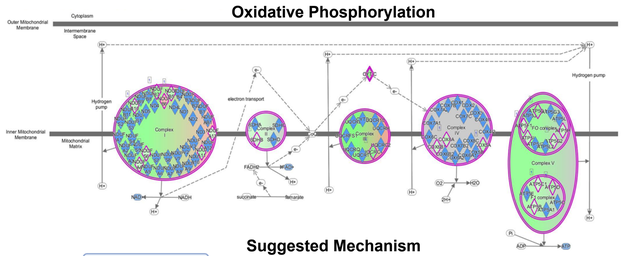
- Astronauts are exposed to the microgravity environment during spaceflight, disrupting both cellular and homeostatic functions (1,2).
- Microgravity can induce transcriptional changes that modify cell structure and function (3).
- Mitochondrial and immune dysfunction have been a documented consequence of spaceflight for over 30 years, but the upstream mechanisms have remained unknown (4,5).
- Telomere elongation in T cells has also been reported during spaceflight, but the molecular mechanisms that underly this phenomenon were previously unknown (6).

Objectives

- Analyze the most comprehensive transcript-omics dataset from astronauts, utilizing Ingenuity Pathway Analysis (IPA) tool.
- Uncover the molecular mechanisms behind spaceflight induced mitochondrial dysfunction and its downstream implication which include immune dysfunction and telomere elongation
- Data was collected from 11 ISS crew-members in space in comparison to the pre-flight ground controls

Mitochondrial Dysfunction

- Microgravity induces an upregulation of GPCRS that provide a pathway for calcium ions to enter intracellularly.
- Alongside upregulated GPCRs, transcriptional changes within the cytoskeleton and G protein interacting molecules promote an increased influx of calcium.
- Upregulation of calcium transporters within the mitochondria were seen in our dataset.
- Signaling pathways in the TCA Cycle, oxidative phosphorylation and Cristae Formation were downregulated in our dataset, coinciding with mitochondrial calcium overload.



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Immune Dysfunction

- While T cell activation initially occurs in spaceflight the downstream immune function is inhibited.
- We propose that microgravity induced mitochondrial dysfunction is an intrinsic trigger that metabolically reprograms T cells into an exhausted like state.
- Our data shows the upregulation of key genetic markers for T cell exhaustion.

T cell exhaustion like phenotype

- **Inhibitory Receptors:** (↑) CTLA-4, TIGIT, CD274
- **Transcription Factors:** (↑) EOMES, BCL6, BACH2, BLIMP1, NFATC1, NFATC2
- **GPCRs:** ADORA2A, GPR65, FFAR

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Take my breath away—mitochondrial dysfunction drives CD8⁺ T cell exhaustion

Felix Clemens Richter ✉, Marija Saliutina, Ahmed N. Hegazy & Andreas Bergthaler

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Simulated microgravity inhibits the genetic expression of interleukin-2 and its receptor in mitogen-activated T lymphocytes

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Affiliations + expand

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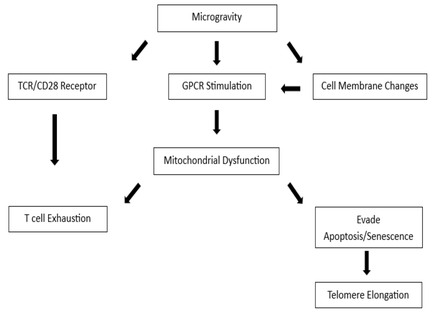
Diseases or Functions Annotation	Activation z-score	p-value
Endocytosis	-3.2	1.2E-19
Endocytosis by eukaryotic cells	-3.0	8.1E-16
Engulfment of cells	-3.0	1.8E-16
Infection of cells	-2.8	1.1E-16
Cell movement of antigen presenting cells	-2.7	1.1E-10
Engulfment of myeloid cells	-2.6	1.2E-12
Infection by RNA virus	-2.6	4.9E-30
Phagocytosis of myeloid cells	-2.6	6.5E-13
Phagocytosis of blood cells	-2.5	2.2E-14
Cell movement of macrophages	-2.5	3.5E-09
Phagocytosis of antigen presenting cells	-2.4	2.5E-08
Viral Infection	-2.4	7.9E-37
Internalization of cells	-2.4	1.1E-13
Cell-cell contact	-2.3	5.6E-07
Infiltration by T lymphocytes	-2.3	2.5E-09
Phagocytosis by macrophages	-2.3	4.2E-08
Phagocytosis	-2.3	1.9E-15
Engulfment of blood cells	-2.2	3.2E-14
Phagocytosis of phagocytes	-2.2	2.6E-09
T cell migration	-2.2	5.3E-18
Cellular infiltration by macrophages	-2.2	1.4E-07
Systemic autoimmune syndrome	-2.2	2.2E-25
Development of progenitor cells	-2.2	5.3E-15
Leukocyte migration	-2.2	7.8E-24
Cell movement of blood cells	-2.1	6.1E-24
Immune response of myeloid cells	-2.0	2.2E-14
Migration of endothelial cells	-2.0	1.9E-09

Impact of Spaceflight on PBMC's- Downregulated functions

- IPA predicted diseases and functions
- Endocytosis, phagocytosis, engulfment, and cell migration are negatively impacted by spaceflight
- These downregulated functions coincide with T- cell exhaustion, demonstrating that this cell state is not just seen at a genetic level but also manifests into physiological immune effector dysfunction.

Summary

- Microgravity induces the upregulation of GPCRS and signaling cascades that increase intracellular calcium concentrations.
- Calcium overload induces mitochondrial dysfunction which leads to adaptational responses
- In conjunction to constant GPCR stimulation, microgravity induces a suboptimal signal from TCR and CD28 receptors
- All together this leads to T cell exhaustion



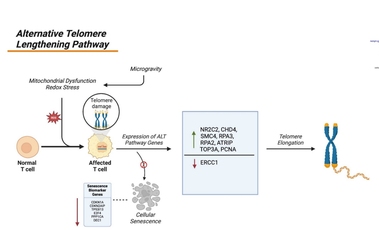
G Protein Coupled Receptors (GPCR) Dysregulation

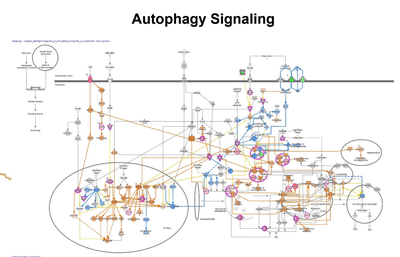
- G protein Coupled Receptors (GPCRs) are the largest class of membrane receptors and function by converting extracellular signals into biochemical responses that can activate or suppress cell function (7,8).
- Activation of GPCRs can occur through a ligand dependent and independent manner, activating a signaling cascade that's both receptor and cell specific (9,10).
- Our data shows the upregulation of 18 ligand dependent GPCRs and that have been linked to increasing cytosolic calcium and promoting calcium mobilization.
- Adhesion GPCRs were dysregulated in our dataset.

	Upregulated GPCRs that increase cytosolic Ca2+		Adhesion GPCRs
HCAR2	hydroxycarboxylic acid receptor 2	ADGRG3	adhesion G protein-coupled receptor G3
CXCR2	C-X-C motif chemokine receptor 2	ADGRE3	adhesion G protein-coupled receptor E3
HCAR3	hydroxycarboxylic acid receptor 3	ADGRA2	adhesion G protein-coupled receptor A2
FPR2	formyl peptide receptor 2	ADGRE1	adhesion G protein-coupled receptor E1
FFAR2	free fatty acid receptor 2		**Downregulated GPCRS that increase cytosolic Ca2+**
C5AR2	complement C5a receptor 2	S1PR2	sphingosine-1-phosphate receptor 2
CCR3	C-C motif chemokine receptor 3	NTSR1	neurotensin receptor 1
ADORA2A	adenosine A2a receptor	P2RY2	purinergic receptor P2Y2
CCR1	C-C motif chemokine receptor 1	GPRC5C	G protein-coupled receptor class C group 5 member C
PTAFR	platelet activating factor receptor	PTGIR	prostaglandin I2 receptor
FPR1	formyl peptide receptor 1	VIPR1	vasoactive intestinal peptide receptor 1
P2RY13	purinergic receptor P2Y13	P2RY1	purinergic receptor P2Y1
GPR15	G protein-coupled receptor 15	PTGDR2	prostaglandin D2 receptor 2
GPR174	G protein-coupled receptor 174		**Downregulated GPCRS that decrease cytosolic Ca2+**
GPR65	G protein-coupled receptor 65	GPR35	G protein-coupled receptor 35
GPR18	G protein-coupled receptor 18	GABBR1	gamma-aminobutyric acid type B receptor subunit 1
P2RY10	P2Y receptor family member 10		**Upregulated GPCRs that decrease cytosolic Ca2+**
S1PR5	sphingosine-1-phosphate receptor 5	SSTR3	somatostatin receptor 3

Telomere Elongation During Spaceflight

- Mitochondrial dysfunction induced by microgravity leads to increased redox stress within the cell.
- Upregulation of cell survival mechanisms such as autophagy allow T cells to evade apoptosis but unless the cells can evade redox induced telomere attrition, they will become senescent.
- The Alternative Lengthening of Telomere Pathway is an adaptive response that lengthens telomeres, allowing the cells to evade senescence
- Apoptotic signaling pathways were also downregulated, promoting cell survival.





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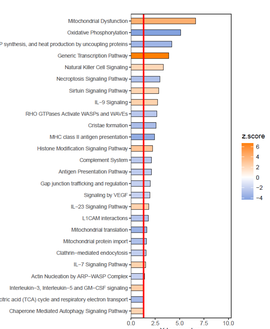
Dysregulated Pathways (Ingenuity Pathway Analysis)



- Mitochondria/Cellular respiration
- Actin Cytoskeleton
- Immune Response
- Cell death/survival

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