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PROCEEDINGS OF THE 2023 ISGP MEETING

Antwerp, Belgium, 2 - 7 July 2023

ISGP Annual Meeting, 2023

Antwerp, Belgium

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July 2–7, 2023, Antwerp, Belgium

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Welcome to the ISGP Annual Meeting, 2023

ISGP, the International Society of Gravitational Physiology holds this annual meeting that allows the presentation of original experimental research and reviews of current topics. The broad scientific spectrum of ISGP emphasizes gravity, life, and physiology as its anchors. We were very happy to organize our 42nd annual meeting in 2023, in Antwerp, Belgium.

LIST OF ORGANIZERS

Floris Wuyts, Antwerp
Catho Schoenmaekers, Antwerp
Steven Jillings, Antwerp
Chloe De Laet, Antwerp
Alain Maillet, ISGP
Martina Heer, ISGP
Alexander Choukér, ISGP
Marc-Antoine Custaud, ISGP

Preface

42nd ISGP meeting report - Antwerp, Belgium

During the first week of July 2023, we hosted the 42nd ISGP conference in the auditoria of the University of Antwerp. While usually numerous students fill the space in and around the classrooms, it was now lively filled with 170 researchers from 19 different countries throughout the world for gathering, talking, discussing and presenting the space science that drives our professional lives. Highlights during this first "in-person" ISGP meeting since 2019 were the great science presented during the plenary and the Young Investigator Sessions in the morning, including the space agencies panel discussion by representatives of ESA, NASA, CSA, DLR, ASI, BELSPO and CNES, the parallel sessions in the afternoon, and poster sessions. The welcome reception in cafe Horta, the "Careers and Beers" event to inspire young professionals to pursue their career in spaceflight research and the gala dinner in the magnificent gothic "Handelsbeurs" provided a superb informal platform to meet each other and continue scientific discussions after each day's scientific program. They also provided space and time for merely enjoying yourself and each other's company with food, drinks, including a silent disco with a great DJ. When the scientific meeting ended, attendees had the opportunity to enjoy some Belgian culture (next to e.g. the beer they had already discovered), including a visit to the Royal Museum for Fine Arts, a city tour in Antwerp, or a daytrip to Bruges. All these events were glued and mixed with interesting conversations during the coffee breaks, lunches as well as during the poster sessions, and of course the lively discussions following the presentations using a "flying microphone" (the blue cube). As you can see, for me the entire event from beginning till the end was the main highlight. And I think it is fair to say that it was perceived as such by many attendants. This event would never have been so successful without the impeccable organization by my team from LEIA drs. Catho Schoenmaekers, dr. Steven Jillings, Chloe De Laet, and Pinja Kaariainen, assisted by Tessa Debi Ide from the Physics department of the University of Antwerp. They surely exceeded my hope to put Antwerp on the map within the ISGP community. We were supported in this task by the sponsors (ESA, Redwire, OIP Space Instruments, ELG RA, the City of Antwerp, the University of Antwerp and BELSPO). But the greatest success was made by all researchers gathering together in Antwerp during the 42nd ISGP meeting.

Thank you all so very much for making this happen.
Floris Wuyts

About ISGP

The broad scientific spectrum of the **International Society of Gravitational Physiology emphasizes gravity, life and physiology as its anchors.** Gravitational physiology is considered to include the effects of the magnitude and direction of the gravitational force environment on cells, integrated physiological systems and behavior/performance of humans, animals and plants.

Benefits of Membership

- Discover student and post-doctoral opportunities
- Connect with experts in the field
- Get news on upcoming and recent space events
- Find out about new gravitational physiology publications
- Be notified first about upcoming meetings

Join today! www.isgp-space.org

In proud collaboration with frontiers


YOUNG ISGP
INTERNATIONAL SOCIETY FOR GRAVITATIONAL PHYSIOLOGY ISGP 1991
Are you a student, postdoc, or early-career scientist with an interest in gravitational physiology?
Join the
ISGP Young Investigators!
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our Research Groups and Career Opportunities
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in annual meetings, award sessions and seminars
WANT TO KNOW MORE?
VISIT WWW.ISGP-SPACE.ORG/STUDENTS/
CONTACT YOUNG.ISGP@GMAIL.COM
JOIN US AT THE 43RD ISGP MEETING IN DUBAI (26-31 MAY 2024) AND ATTEND THE DEDICATED EVENTS FOR YOUNG INVESTIGATORS!

Charles A. Fuller

2023 Nello Pace Award

The Nello Pace Award, established in 1997 through a partnership between the Galileo Foundation and the International Society for Gravitational Physiology, seeks to recognize individuals who have made extraordinary contributions to both research and service in the field of Gravitational Physiology. It is with great pleasure that we announce the recipient of the 2023 Nello Pace Award as Professor Charles A. (Chuck) Fuller.

Dr. Fuller's journey in the realm of science has been nothing short of extraordinary. His passion for unraveling the mysteries of gravity has guided him throughout his illustrious career, and it is safe to say, "He's all about gravity." In 1984, he took the helm of the Chronic Acceleration Research Unit (CARU) Lab at UC Davis, a momentous step that would pave the way for groundbreaking research. Dr. Fuller's pioneering work has centered on the effects of gravitational forces, with a particular focus on primates. His dedication and commitment have seen him conduct research on centrifuges of varying sizes, some as colossal as 20 meters. Through these endeavors, he has provided invaluable insights into the intricate interplay between our biological systems and gravity.

However, Dr. Fuller's contributions extend beyond the laboratory. He is not only a distinguished scientist but also a gifted mentor, nurturing and shaping the careers of many successful scientists. His generosity in sharing knowledge and guiding the next generation of researchers is truly commendable.

From 1995 to the present day, he has held the role of President at the Galileo Foundation, a non-profit organization dedicated to fostering the exchange of scientific knowledge among researchers concerned with the effects of Earth gravity on living systems, as well as its augmentation or reduction. The foundation supports these scientific exchanges and their publication and promotes collaboration with organizations sharing an interest in expanding human knowledge regarding the impact of gravity on living organisms.

As we honor Dr. Charles A. Fuller, we pay tribute to his unwavering commitment to advancing our understanding of gravity's impact on the human body. His work has far-reaching implications, from space exploration to healthcare here on Earth.

His dedication, passion, and pioneering spirit have left an indelible mark not only within the International Society for Gravitational Physiology but also on the world of science. His accomplishments have revealed new frontiers of understanding, and his mentorship has served as an inspiration for countless individuals, motivating them to reach for the highest achievements.

We extend our warmest congratulations and deepest gratitude to Dr. Fuller for his exceptional contributions. His legacy will serve as a wellspring of inspiration for future generations of scientists, guiding them toward new discoveries and advancements.


MOHAMMED BIN RASHID SPACE CENTRE
DUBAI HEALTH
ISGP
1991
INTERNATIONAL SOCIETY FOR GRAVITATIONAL PHYSIOLOGY
The International Society for Gravitational Physiology is inviting you to the
43rd Annual ISGP Meeting in Dubai
May 26th to 31st, 2024
As the proud host for ISGP 2024, the Mohammed Bin Rashid Space Centre (MBRSC) stands at the forefront of the United Arab Emirates' space exploration and innovation endeavours. Dedicated to advancing the knowledge and application of space sciences in the region and fostering a culture of scientific excellence and technological innovation, MBRSC aims to facilitate an international platform for dialogue, collaboration, and discovery in gravitational physiology.
Unlock the opportunity to share your ground-breaking research with peers and pioneers in the field of gravitational physiology. We invite researchers, scientists, professors, scholars and students to submit abstracts and contribute to the advancement of our scientific community. Submit your abstract now and shape the future of space studies.
Discover the ISGP Young Investigator Award at our annual meetings, aimed at students and early career researchers. Showcase your work, gain recognition, and connect with a global community.
Explore Dubai's exquisite landmarks at ISGP 2024 with tours of the cutting-edge innovations at MBRSC, and iconic Dubai tourist spots. Experience the city's vibrant heritage and futuristic skyline, culminating in an exclusive gala amidst Dubai's architectural wonders.
Early registration fees
Regular Registration 500€
Young Investigators and students 250€
Accompanying Person 150€
Opening registrations
01st March
08th March
Abstract submission deadline
Abstracts selected and pre-program available
29th March
12th April
Early bird registration deadline
26th to 31st May
ISGP Meeting in Dubai
Visit our website
www.isgp-space.org

INTERNATIONAL SOCIETY FOR GRAVITATIONAL PHYSIOLOGY

Hosted by Prof. Floris Wuyts

42nd Annual ISGP Meeting in Antwerp
July 2 – 7, 2023

A word from the sponsors

A word from the sponsors	
	European Space Agency (ESA) The European Space Agency (ESA) is Europe's gateway to space. Its mission is to shape the development of Europe's space capability and ensure that investment in space continues to deliver benefits to the citizens of Europe and the world. ESA is an international organisation with 22 Member States. ESA is strongly invested in implementing space and exploration science across a variety of ground and space platforms within ESA's SciSpacE team. For more information visit https://www.esa.int/.
	Redwire Redwire is a global leader in mission critical space solutions and high reliability components for the next generation space economy, with valuable intellectual property for solar power generation, in-space 3D printing and manufacturing, avionics, critical components, sensors, digital engineering and space-based biotechnology. We combine decades of flight heritage with an agile and innovative culture. Our "Heritage plus Innovation" strategy enables us to combine proven performance with new, innovative capabilities to provide our customers with the building blocks for the present and future of space infrastructure. For more information visit *https://redwirespace.com/*.
	OIP Space Instruments OIP specializes in design, development and production of high-end electro-optical systems for the Defence and Space markets. The OIP Space Instruments department has delivered electro-optical equipment for space since the 90s. The application fields include Earth & planetary observation, atmospheric analysis, space situational awareness, navigation & landing, and scientific instrumentation. For more information visit https://oipspace.be/.
	European Low Gravity Research Association (ELGRA) The European Low Gravity Research Association (ELGRA) is a non-profit international society devoted to the promotion of scientific research under various gravity conditions in Europe. The organization, established in 1979, provides a networking platform for all scientists interested in life and physical sciences and technology in space or on ground. ELGRA aims at representing and strengthening the scientific community of gravity-related research and helping young scientists and engineers get involved in low- and hyper-gravity research through educational programs. It has both a professional and student body (SELGRA) and is composed of academics, representatives of space agencies, industry, students and more. For more information visit *https://www.elgra.org/*.
	Belgian Science Policy Office (BELSPO) BELSPO, the Federal Science Policy, is one of the main actors of scientific research in Belgium. It brings together many prestigious research programs and manages ten federal scientific institutes. With 2,300 employees, BELSPO brings together a wide range of expertise in astrophysics, meteorology, history, climatology, anthropology, molecular biology... BELSPO's "Space Research and Applications" department manages the Belgian contributions to the international space programs from ESA and other intergovernmental organisations (EUMETSAT, ECMWF, ESO...) as well as those developed in the framework of bilateral agreements. It is also in charge of the follow-up of the space activities and programmes of the European Union. The department also manages specific national R&D programmes mainly in the domain of Earth Observation. For more information visit https://www.belspo.be/.

Program Overview

Plenary sessions | Student sessions | Parallel sessions | Poster sessions | Social & Others

	Monday 3	Tuesday 4	Wednesday 5	Thursday 6	Friday 7
	Registration	Registration	Registration	Registration	
09:00	Opening word	Plenary 2: Discussion pannel "Exercise and Countermeasures"	Plenary 3: Keynote lectures "Hibernation and hypometabolic state "		Social Day
09:30	Plenary 1: Keynote lectures "Current Concepts"			Plenary 4: Keynote lectures "Neuroscience"	
10:00					
10:30					
11:00	Coffee break	Coffee break	Coffee break		
11:30	Young Investigators Session	Young Investigators Session	Young Investigators Session		
12:00				Closing ceremony	
12:30	Lunch Break	Lunch Break	Lunch Break	Lunch Break	
13:00	Lunch & poster	Lunch & poster	Lunch & poster		
13:30				ISGP General assembly	
14:00	Spaceflight analogues and countermeasures / Reproduction and biology	Space Agency Session	Metabolism and Immunology / Neuroscience		
14:30					
15:00				Royal Museum of Fine Arts (KMSKA) visit	
15:30					
16:00	Coffee break	Coffee break	Coffee break		
16:30	Cardiovascular and respiratory system / Bone and musculoskeletal system	Spaceflight Applications / Neuroscience	Cardiovascular system / Bone and musculoskeletal system		
17:00					
17:30					
18:00		ELGRA General Assembly			
	Welcome Reception (20:00 - 22:00)	Careers & Beers (18:30 - 20:00)	Gala Dinner and Young Investigators Award Ceremony (19:00 - 01:00)		

July 03, 2023 - Monday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
8:00 – 9:00	*Registration*			
9:00 – 9:15	**Opening word** *Speakers* Marc-Antoine Custaud – President of ISGP Floris Wuyts – Host of the 42nd Annual ISGP Meeting Representative Belgian Science Policy (BELSPO)			
9:15 – 11:00	**Plenary session "Current Concept"** *Moderators* Brandon Macias and Danielle Greaves *Speakers* **9:15** Michael Stenger – *"NASA physiological research for human space exploration"* **10:00** Erik Hougland – *"Human research program plans on Artemis research"* **10:45** Frank De Winne – *"ESA's Terrae Novae and Master Plan"*			
11:00 – 11:30	*Coffee Break*			
11:30 – 12:30	**Young Investigators Session** *Moderators* Anna-Maria Liphardt, Catho Schoenmaekers, Rodrigo Coutinho De Almeida *Speakers* **11:30** Katherine Warthen – *"Quantification of intracranial tissue, fluids, and ocular structural changes due to long-duration spaceflight"* **11:45** Silvana Miranda – *"Gravity's Effect on T-Cell Activation: Unraveling the Mechanisms of Immune System Dysregulation in Space"* **12:00** Constance Badali – *"SpaceBike – Preliminary data on cycling neuromechanics in weightlessness"* **12:15** Laurent Opsomer – *"Movement direction drift in astronauts performing point-to-point arm movements without visual feedback"*			

July 03, 2023 - Monday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
12:30 – 13:00	*Lunch Break*			
13:00 – 14:00		**Lunch & Posters** Tatiana Kostrominova – *"P2YI and P2Y2 receptors differ in their role in the regulation of signaling pathways during unloading-induced rat soleus muscle atrophy"* Bing Han – *"Modulation of macrophage pryptosis by acute or chronic stress - an in vitro approach"* Sharon van Rijthoven – *"Ageing and altered gravity: a cellular perspective"* Debora Angeloi – *"Microgravity and space radiation inhibit autophagy in human capillary endothelial cells"* Natassi Navasiolava – *"Chronic increase in renin-angiotensin-aldosterone activity at steady state of microgravity: why and by which mechanisms?"* Roman Zhedyaev – *"Direct comparison of tilt test and lower body negative pressure effects on human hemodynamics and baroreflex regulation after dry immersion"* Artur Fedianin – *"The effect of various spinal cord stimulation and support afferentation on the condition of the muscles of the rat's lower leg during simulated gravitational unloading"* Daniela Santucci – *"Neurobehavioural evaluation in mice exposed to acute 2G hypergravity in normothermic or synthetic torpor conditions"* Ann-Sofie Schreurs – *"Candidate countermeasure to mitigate adverse effects of spaceflight on musculoskeletal, cardiovascular and central nervous systems tissues"* Kevin Tabury – *"A blood vessel microfluidic chip to study vascular remodeling in space"* Stijn Meel – *"Influence of prolonged exposure to microgravity on perception of verticality"* Victoria Sampson – *""Space dentistry" - An invention or a modern necessity?"* Margot Winters – *"Optimizing Scientific Output: Assessing the Benefits and Risks of Analog Astronaut Missions for Human Physiology Research"*		

July 03, 2023 - Monday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
14:00 – 15:45			**Spaceflight analogues and countermeasures** Moderators Angelique Van Ombergen and Philip Carvil Speakers **14:00** Alamelu Sundaresan – *“Development and Characterization of 3D culture of bone tissue as a physiological test bed for pharmaceutical formulations”* **14:15** Ilya Rukavishnikov – *“Comparative Results Of 21-Day Dry Immersion And 21-Day Head Down Tilt Bed Rest”* **14:30** Bjorn Baselet – *“Combined biological effects of ionizing radiation, psychological stress and microgravity in space: the hind limb unloading mouse model”* **14:45** Vivekanand Vimal – *“Vibrotactile Feedback as a Countermeasure to Spatial Disorientation”* **15:00** Carole-Anne Vollette – *“A wearable-based system to reduce space motion sickness by multi-sensory pre-habituation”* **15:15** Jonathan Scott – *“Effects of body size and countermeasure exercise on estimates of life support resources during all-female crewed exploration missions”* **15:30** Jack van Loon – *“Reduce Cost by Permanent Artificial Gravity”*	**Biology** Moderators Sharmila Bhattacharya and Sarah Baatout Speakers **14:00** Elena Gorbacheva – *“Hormonal Status Of Woman And Structural Characteristics Of The Ovaries And Uterus After A 5-Day "Dry" Immersion”* **14:15** Irina V. Ogneva – *“Cell Stiffness And Protein Content In Drosophila Melanogaster' Oocytes After Space Flight”* **14:30** Dagmara Stasiowska – *“Impact of honey bee (Apis mellifera) queen exposure to hypergravity on colony development - case study”* **14:45** Simon Wuest – *“Analyzing Calcium Signaling by CaMPARI2 during Parabolic Flights”* **15:00** Christian Liemersdorf – *“Human Neural Network Activity Reacts to Gravity Changes”* **15:15** Jess Bunchek – *“VEG-04: How Lighting Affects Crop Production, Quality, And Acceptability of Mizuna Mustard Grown on the International Space Station”* **15:30** Jennifer-Vernice Pauly – *“Veggie on ICE: The Effects of Plant Production on Human Behavioral Health in Long-Duration Antarctic Overwintering Missions”*
15:45 – 16:15	*Coffee Break*			

July 03, 2023 - Monday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
16:15 – 18:00			**Cardiovascular and respiratory system** _Moderators_ Jérémy Rabineau and Ana Diaz-Artilles _Speakers_ **16:15** Dag Linnarsson – *“Lung diffusing capacity for nitric oxide in space: microgravity gas density interactions”* **16:30** Paniz Balali – *“Adaptive Changes in Heart Rate Variability and Cardiac Function during Long-Term Spaceflight: Insights from Wearable Devices”* **16:45** Jason Fisher – *“Cardiovascular And Cutaneous Blood Flow Responses To Artificial Gravity And Temperature: A Pilot Study”* **17:00** Damien Lanéellé – *“Cerebral Blood flow distribution according to cerebral arterial pattern variations during hemodynamic stress induced by simulated hypovolemia or hypercapia: a triplex ultrasound and magnetic resonance imaging study”* **17:15** Danielle Greaves – *“Robotic Ultrasound on ISS Operated from Canadian University's Mission Control Center - Results from Vascular Echo Experiment”* **17:30** Philippe Arbeille – *“Spaceflight and Dry Immersion Increases the Reflectivity of the Arterial Wall to Ultrasound Radio Frequency Waves”* **17:45** Damian Bailey – *“Gravitational transitions increase blood-brain barrier permeability in humans; focus on the redox-regulation of cerebral hyper-fusion”*	**Bone and musculoskeletal system** _Moderators_ Anna-Maria Liphardt and Stefan Schneider _Speakers_ **16:15** Ivan Ponomarev – *“Effect of 5-day dry immersion on viscoelastic and contractile properties of the lower extremities muscles in women”* **16:30** Alexandra Ryabova – *“Effects of 5-day dry immersion on characteristics of shin muscles motor evoked potentials in women”* **16:45** Maren Dreiner – *“Sex-specific cartilage biomarker response to 5 days of dry immersion”* **17:00** Alessandra Bosutti – *“Impact of 60-bed rest and human artificial gravity on serum oxidative stress biomarkers and skeletal muscle protein oxidative modifications”* **17:15** Peter Fernandez – *“Insights and Conundrums Involving Spaceflight and Bone - An 18-Month Perspective”* **17:30** Anna-Maria Liphardt – *“Effect of microgravity during long-duration spaceflight on transverse relaxation times of the femorotibial cartilage (MRI T2) - the ESA Cartilage Health study”*
20:00 – 22:00	***Welcome reception at Horta***			

July 04, 2023 - Tuesday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
8:00 – 9:00	*Registration*			
9:00 – 11:00	**Plenary session "Discussion panel exercise and countermeasures"** *Moderators* Anna-Maria Liphardt and Rodrigo Fernandez-Gonzalo *Discussion panel* **9:05** Meghan Everett – *"Enabling the Human to Explore the Moon and Beyond"* **9:20** Rodrigo Fernandez-Gonzalo – *"Is the "optimal exercise" for future exploratory missions optimal for everyone? The individual variability issue"* **9:35** Martina Heer – *"Is the pantry on the way to Mars big enough? The tangle relationship between exercise load and energy requirements"* **9:50** Jonathan Scott – *"One giant leap for Mankind: Why plyometric exercise could be the future of countermeasure exercise in space"* **10:05** Stefan Schneider – *"Exercise recommendations? A hedonistic brain perspective!"*			
11:00 – 11:30	*Coffee Break*			
11:30 – 12:30	**Young Investigators Session** *Moderators* Rodrigo Fernandez-Gonzalo, Steven Jillings, Inês Antunes *Speakers* **11:30** Roxy Fournier – *"Increased cerebrovascular tone following long duration spaceflight is associated with increased pulsatility and reduced conductance"* **11:45** Emil Rehnberg – *"Compartimentalized chip for improved formation of human cardiac spheroids using simulated microgravity"* **12:00** Timo Frett – *"Comparison between maximal rowing ergometry in artificial gravity and terrestrial conditions"* **12:15** Carmen Possnig – *"Influence of gravitational gradients on cerebral and ocular blood flow"*			
12:30 – 13:00	*Lunch Break*			

July 04, 2023 - Tuesday				
	Plenary room (M.01)	**Poster hall**		
13:00 – 14:00		**Lunch & Posters** Patrick Lau – *"FLUMIAS - high-resolution live-cell fluorescence microscopy and identification of gravity-dependent thresholds on-board the ISS"* Fuminori Kawano – *"Histone chaperone SPT16 triggers histone turnover in mouse skeletal muscle"* Junya Shimizu – *"Role of exercise-induced H3K27me3 for the gene response to exercise in mouse skeletal muscle"* Simon Vandergooten – *"Directional accuracy of point-to-point arm movements in different body orientations with respect to gravity"* Katharina Biere – *"Isolation and lack of stimuli in Antarctica temporarily increase the alertness of the innate immunity"* Vivek Mann – *"Blood vessels and microgravity"* Ryo Masuzawa – *"Examination of histone variant induction as a novel countermeasure for long term stay in space using mouse model"* J.J.W.A. van Loon – *"Effect of 30 day-3G-exposure on C57BL6J mice: neurobehavioural and biomolecular effects on ocular and brain tissues"* Nelly Abu Sheli – *"Practical applications of electrical stimulation space model with "Russian Currents" in neurological elderly patients"* Margot Winters – *"Assessing the Resilience of Lab-On-Chip Devices with OoC-STRAT: A study on High-Radiation Effects Using Stratospheric Balloons"*		
14:00 – 15:30	**Space Agency Session** *Moderators* Sarah Baatout and Catho Schoenmaekers Speakers: Angelique Van Ombergen (ESA), Markus Braun (DLR), Didier Chaput (CNES), Sara Piccirillo (ASI) Valerie Gil (CSA), Sharmila Battacharya (NASA BPS), Jancy McPhee (NASA HRP)			

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<tr><td>15:30 – 16:00</td><td colspan="4">Coffee Break</td></tr>
<tr><td>16:00 – 17:45</td><td></td><td></td><td>Spaceflight applications

<u>Moderators</u>
Angelique Van Ombergen and Jonathan Scott

<u>Speakers</u>
16:00 Inês Antunes – “ESA's SciSpacE team”

16:15 Masahiro Terada – “The implementation of the educational programs for space medicine in Japan”

16:30 Andrei Sapera – “New commercial applications for space physiology and human spaceflight”

16:45 Ryan T. Scott – “Open Science for Life in Space: Data Sharing, Standards, and Informatics for Reuse and Knowledge Discovery”

17:00 Anna Catharina Carstens – “Fluorescent live cell imaging on a centrifuge in space: FLUMIAS”

17:15 Marina Cara Tuschen – “µIMMUNE - Development of automated microfluidic immune monitoring for spaceflight, a first report”</td><td>Neuroscience

<u>Moderators</u>
Steven Jillings and Kunihiko Tanaka

<u>Speakers</u>
16:00 Nikita Shishkin – “Sensory Organization of Postural Control After Long Term Space Flight”

16:15 Kunihiko Tanaka – “Magnetic Vestibular Stimulation Amplifies Posture and Arterial Pressure Control”

16:30 Maria Bekreneva – “Effect of 5-day Dry Immersion On Vertical Stability And Voluntary Walking in Woman”

16:45 Olga Kuldavletova – “Comparison of postflight mission critical tests between astronauts and bilateral vestibular patients”

17:00 Philippe Lefèvre – “A haptic illusion created by gravity”

17:15 Michele Tagliabue – “The Role of Gravity in Eye-Hand Coordination: Effects of Short-Term and Long-Term Head-Gravity Misalignment”

17:30 Deborah Cecilia Navarro Morales – “Duration judgement in astronauts and patients with idiopathic bilateral vestibular loss”</td></tr>
<tr><td>18:00 – 18:30</td><td colspan="4">ELGRA General Assembly</td></tr>
<tr><td>18:30 – 20:00</td><td colspan="4">Careers & Beers</td></tr>
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July 05, 2023 - Wednesday				
	Plenary room (M.01)	**Poster hall**	**Parallel room (M0.4)**	**Parallel room (M0.5)**
8:00 – 9:00	*Registration*			
9:00 – 11:00	**Plenary session "Hybernation and hypometabolic state"** *Moderators* Alexander Choukér and Jürgen Bereiter-Hahn *Speakers* **9:00** Dominique Moser – *"Is torpor slumbering in all of us? A comparative cross-species analysis"* **9:40** Matteo Cerri – *"Hypometabolism in hypergravity: metabolic and behavioral effects"* **10:20** Fabrice Bertile – *"Hibernation as a source of innovation for space biology and biomedicine: a focus on brown bears"*			
11:00 – 11:30	*Coffee Break*			
11:30 – 12:30	**Young Investigators Session** *Moderators* Anna-Maria Liphardt, Kimia Seyedmadani, Rodrigo Coutinho De Almeida *Speakers* **11:30** Peter Fernandez – *"Preliminary insights into bone adaptation and differences in energy metabolism between males and females under (DI) conditions"* **11:45** Florian Pfeiffer – *"Inspired by Space: E-Nose technology provides diagnostic tool for SARS-CoV-2 infection by breath analysis"* **12:00** Federico D'Amico – *"Immune cell activation state in 5-day Dry Immersion: a comparative study between a female and male cohort"* **12:15** Angela Kubik – *"Defining CDKN1a/p21-induced changes to the functional and regenerative capabilities of bone-marrow derived stem cells in models of aging, loading, and spaceflight"*			
12:30 – 13:00	*Lunch Break*			

July 05, 2023 – Wednesday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
13:00 – 14:00		**Lunch & Posters** Katharina Block – *"Project NEMUCO - The neuromuscular junction-like structure in vitro to study muscle and nerve cell-cell communication in space"* Kyung-Ju Shin – *"Microgravity induces oxidative stress and mitochondrial dysfunction which is mittigated by TPP-niacin in retinal epithelial cells"* Darshan Chandramowli – *"Targeted proteomic analysis of S. cerevisiae in microgravity conditions in response to antifungal stress"* Ivan Vasilev – *"Study of vascular hemodynamics in healthy men undergoing 21-day antiorthostatic hypokinesia"* Hendrik Bouwman – *"Effects of sustained hypergravity on growth and reproduction in earthworms"* Laurence Stevens – *"Chronic recording of EMG activity by telemetry in mice skeletal hindlimb muscles during long-term inactivity"* Danielle Greaves – *"Cervical intervertebral distance measured using 3D ultrasound after dry immersion and 6 month spaceflight"* Adriana Salatino – *"Motor awareness in microgravity: action self-monitoring during parabolic flights"* Sarah Schunk – *"Astrocyte reactivity can be modulated by altered gravity"* Alina Saveko – *"Human postural responses to artificial gravity training"* Judith Nottage – *"Alterations in gravity influences conscious experiences"* Pinja Kääriäinen – *"The influence of spaceflight experience on structural brain changes"* Dirk Neefs – *"Dental care in space as an interdisciplinary procedure"* Thomas Angeli – *"Implementation of Blood Flow Restricted Exercising for Strength Increase in Quadriceps Muscle on the Multifunctional Dynamometer for Application in Space"*		

July 05, 2023 - Wednesday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
14:00 – 15:45			**Metabolism and immunology** Moderators Bjorn Basalet and Alexander Choukér Speakers **14:00** Mathieu Horeau – *"Simulated microgravity differentially affects the iron metabolism in male and female rats"* **14:15** Frédéric Derbré – *"Iron metabolism regulation in women and men exposed to simulated microgravity: results from the randomized trial AGBRESA"* **14:30** Martina Heer – *"Glucose tolerance onboard the International Space Station: First results from an Oral Glucose Tolerance Test"* **14:45** Dominique Moser – *"Preconditioning with mild hypergravity mitigates simulated microgravity-induced T cell dysfunctions"* **15:00** Judith-Irina Buchheim – *"Role of individual immune profiles for wound healing under chronic stress during long-term space flight"*	**Neuroscience** Moderators Angelique Van Ombergen and Katherine Warthen Speakers **14:00** Ge Tang – *"Establishing a link between brain and eye in long-duration spaceflight"* **14:15** Kimia Seyedmadani – *"Superior Baseline Oculomotor Performance Viewing with the Dominant Eye"* **14:30** Jean Pauly – *"Psychological and cognitive adaptation during a 3-weeks confinement in a space-like environment"* **14:45** Gabriel De La Torre – *"Neurocognitive assessment in microgravity: Review under a clinical perspective"* **15:00** Elisa Raffaella Ferrè – *"Cognition in Zero-G: How Altered Gravity Influences Human Brain and Behaviour"* **15:15** Steven Jillings – *"Brain structural and functional changes after long duration spaceflight"* **15:30** Jennifer-Vernice Pauly – *"Brains on ICE - Hippocampal Changes in Response to Prolonged Isolation and Confinement"*
15:45 – 16:15	*Coffee Break*			

July 05, 2023 - Wednesday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
16:15 – 18:00			**Cardiovascular system** _Moderators_ Roxy Fournier and Damian Bailey _Speakers_ **16:15** Adrien Robin – *"Venous filling and emptying properties during 5-day strict dry immersion microgravity simulation"* **16:30** Ana Diaz Artiles – *"Quantification Of The Internal Jugular Vein Characteristics During Fluid Shift Induced By Lower Body Negative Pressure"* **16:45** Richard Hughson – *"Is fainting after 14-day head-down bed rest in 55-65 year-old men and women a chicken and egg question?"* **17:00** Jérémy Rabineau – *"Correlation of changes in aortic stiffness with other parameters of cardiovascular health after 60-day head-down bed rest"* **17:15** Jacques-Olivier Fortrat – *"Increased Cardiovascular Self-Organized Critically After Prolonged Head-Down Bed Rest"* **17:30** Stefan Möstl – *"Autonomic cardiovascular control following 30 days strict head down tilt bedrest - Results from the SANS CM studies"* **17:45** Danniella Hurt – *"Cephalad fluid shifts associated with neuroprotective alterations in cerebral perfusion and haemostasis independent of systemic oxygenation status"*	**Bone and musculoskeletal system** _Moderators_ Rodrigo Fernandez Gonzalo and Laurence Vico _Speakers_ **16:15** Britt Schoenrock – *"Muscle stiffness in astronauts during long duration missions onboard the International Space Station (MYOTONES)"* **16:30** Elena Fomina – *"The weight load simulation mode every other day is quite effective for preserving muscular strength on long space missions"* **16:45** Philip Carvil – *"Effects of a proposed microgravity countermeasure, the MK VI SkinSuit, upon markers of lumbar geometry and kinematics following unloading"* **17:00** Margot Issertine – *"The NEBULA Project: effect of preflight endurance and resistance training as a countermeasure against microgravity-induced musculoskeletal deconditioning"* **17:15** Mona Nasser – *"Jaw movement in microgravity"* **17:30** Elie-Tino Godonou – *"Changes in urinary concentration of the cartilage degradation marker Coll2-1NO2 in response to bed rest immobilization and countermeasures"*
19:00 – 1:00	***Gala dinner at Handelsbeurs and announcement of Young Investigator Awards (YIA)***			

July 06, 2023 - Thursday				
	Plenary room (M.01)	Poster hall	Parallel room (M0.4)	Parallel room (M0.5)
8:45 – 9:45	Registration			
9:45 – 12:20	**Plenary session "Neuroscience"** *Moderators* Floris Wuyts and Elisa Raffaella Ferrè *Speakers* **9:45** Floris Wuyts – *"How does the brain react to space"* **10:20** Brandon Macias – *"Spaceflight Associated Neuro-ocular Syndrome: The Gravity of the Mission"* *------ 15 min Break ------* **11:10** Jay Buckey – *"A re-evaluation of the acute adaptation to weightlessness and what it means for SANS"* **11:45** Elena Tomilovskaya – *"Effects of Dry Immersion on the sensory-motor system"*			
12:20 – 12:30	*Closing Ceremony*			
12:30 – 13:30	*Lunch Break*			
13:30 – 15:30	***ISGP General Assembly***			
15:30 – 18:00	***Royal Museum of Fine Arts (KMSKA) visit***			

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SpaceBike – Preliminary data on cycling neuromechanics in weightlessness

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Citation
Badalì, C., Wollseiffen, P., Schneider, S. SpaceBike – Preliminary data on cycling neuromechanics in weightlessness.

Introduction

Regarding the plans of international space agencies for Lunar and Martian explorations, it is important to understand the effects of microgravity on human motor control. After long stays in space, astronauts report impairments in their visual, motor and vestibular systems that are known to be based on well-established neural communication sequences, called motor engrams. Motor engrams are structural changes in the brain caused by frequent repetitions of the same perceptual and processing content and describe a neuronal network through which information can be retrieved at a short notice and in a highly efficient manner. In a similar way to running, walking and jumping, the pedalling movement in cycling are also based on motor engrams. It has already been shown, that the detection of motor engrams during cycling is possible by recording and localizing brain cortical activity with an electroencephalogram (EEG). Schneider et al. combined this with electromyography (EMG) and observed comparatively stable oscillations over different intensities and with a strong correlation at higher intensities (Schneider et al., 2013). Despite motor engrams are really stable, they might be influenced by external parameters. These could be an increased intracranial pressure (ICP), which is experienced by astronauts in weightlessness due to a fluid redistribution. The use of a highly stable, automated movement pattern like pedalling allows us to identify the effect of an increased ICP on existing neuronal activation patterns. It is already known, that neural communication changes under reduced gravity. In vivo and in vitro studies show a slight depolarisation of nerve cells in weightlessness, caused by an increased pressure, which irritates neural communication (Hanke et al., 1993; Kohn & Ritzmann, 2018). It is expected, that the accuracy of the neuronal activation pattern will show higher variability in microgravity due to an increased ICP compared to normal gravity. If neuronal innervation patterns are affected, this will confirm the results of previous studies in weightlessness (Kohn & Ritzmann, 2018). It can further help to develop appropriate countermeasures as well as re-adaptation processes after long term space missions and enable suitable neurorehabilitation programs for neurological rehabilitation.

Material and Methods

Data from two individual subjects were recorded during two parabolic flight campaigns. Parabolic flights take place from Merignac Airport in Bordeaux, France and are carried under the lead of the German Aerospace Center (DLR) or the European Space Agency (ESA). During these flights, participants have to pedal at an individually pre-defined crank rate for 24 consecutive parabolas in weightlessness and at 1G. The load is increased by 1 W/kg body weight after every eighth parabola, so that the participants reach their individual maximum after the 16th parabola. The remaining six parabolas are used as back-up in case of failures. As each phase of weightlessness lasts 22s, participants start pedalling 2s before entering weightlessness, so that the individual crank rate is reached beforehand and the total 22s remain for data acquisition. The resulting average of all repetitions within the same intensity allows to determine a clear motor engram. EEG and EMG are collected simultaneously and are synchronised by an additional analogue signal at the start of each pedal cycle. When the right crank passes the top dead centre, a reed switch attached to the bike frame is closed momentarily and an electrical pulse is generated using a custom-made electronic box to both recording software.

EMG Activity

EMG activity is continuously recorded with wireless surface EMG electrodes from 14 muscles which are activated by the central nervous system (CNS) and known to be responsible for producing the pedalling movement (MacIntosh et al., 2000; Raasch & Zajac, 1999; Ryan & Gregor, 1992). The electrodes are located according to the recommendations by the Surface EMG for Non-invasive Assessment of Muscles project (seniam.org). The signals are analysed with self-created scripts in Python3 (Python Software Foundation, https://www.python.org/ (VanRossum & Drake, 2010)) in Jupyter Notebooks. After applying the processing steps of smoothing, filtering and rectifying, muscle activity is time normalized to one pedal cycle and averaged over all valid trials and muscles (Rouffet & Hautier, 2008; Rouffet et al., 2009).

EEG Activity

Oscillation of the cortical innervation in the motor cortex is assessed by using a head-size adapted 64-channel EEG cap (actiCap-64Ch, Brain Products GmbH, Munich, Germany) in the classic 10-20 arrangement. Each electrode is referenced to a reference electrode FCz. For optimal signal transmission, all electrodes are filled with Electro-Gel™ (Electro-Cap International, USA). Analogue data are recorded with a sampling frequency of 1000 Hz, amplified and converted into digital signals via Brain Vision amplifier and RecView software (Brain Products GmbH, Munich, Germany). The EEG signal is analysed offline using Brain Vision Analyzer 2.2 (Brain Products, Munich, Germany). By using the integrated sLORETA module (Bai et al., 2007; Grech et al., 2008; Pascual-Marqui, 2002) cortical current density is calculated from the averaged EEG activity of a pedalling cycle for Montreal Neurophysiological Institutes (MNI) coordinates (x/y/z) 0/0/60, 0/-10/60, 0/-20/60, 0/-30/60, 0/-40/60 and 0/-50/60 with a sphere of 10mm on the MNI-Average-305-T1 head model.

Results

Data presented here are preliminary results from two participants during parabolic flights. Cortical activity of the motor cortex shows an oscillation which increases during higher cycling intensities throughout both gravity conditions. Additionally, a more pronounced oscillation is observed in weightlessness compared to 1G. The summed muscle activity of 14 muscles shows an oscillation pattern, which is more pronounced in higher cycling intensities. Especially in weightlessness a peak-to-peak match of current cortical density and muscle activity could be seen (s. Figure 1 and Figure 2).

Discussion

The results achieved in this project could help to better understand the effects of weightlessness on neuromuscular performance. Both participants show a similar behaviour of muscle and cortical activity during all cycling intensities and in both gravity conditions. At the moment it could be

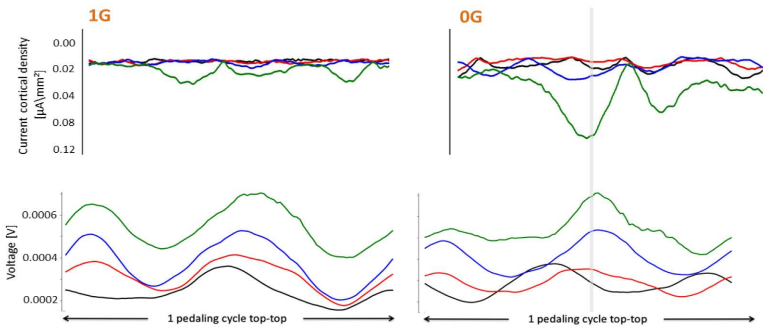

FIGURE 1

Data from one subject averaged over all valid trials. The upper graphs show the oscillation of the cortical density in the motor cortex for different intensities in 1G and weightlessness. The lower graphs show the oscillation of muscle activity averaged over all muscles. Both signals show higher amplitudes in higher intensities and a peak-to-peak match in weightlessness between EEG and EMG. All graphs are normalized to one pedal cycle. Black = 2W/kg body weight, red = 3W/kg body weight, blue= 4W/kg body weight, green = 5W/kg body weight.

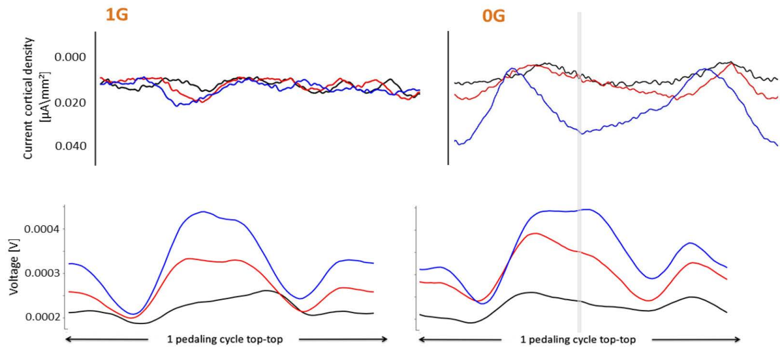

FIGURE 2

Data from one subject averaged over all valid trials. The upper graphs show the oscillation of the cortical density in the motor cortex for different intensities in 1G and weightlessness. The lower graphs show the oscillation of muscle activity averaged over all muscles. Both signals show higher amplitudes in higher intensities and a peak-to-peak match in weightlessness between EEG and EMG. All graphs are normalized to one pedal cycle. Black = 1W/kg body weight, red = 2W/kg body weight, blue= 3W/kg body weight.

hypothesized, that the increased activity in weightlessness is shown either because cycling in weightlessness is totally unfamiliar and requires a different movement pattern to keep up with the desired performance or it is caused by physiological changes in cell properties.

A physiological model, developed by Kohn and Ritzmann in 2018, could explain changes in neural communication in weightlessness (Kohn & Ritzmann, 2018). Due to the increase in ICP in microgravity, the membrane viscosity is affected and becomes more permeable for ions. This in turn leads to a reduction of the open state probability of ion channels which results in a slightly depolarized resting membrane potential of neuronal cells up to 3 mV. Ultimately, this leads to a lower threshold for triggering an action potential and could therefore explain the more pronounced oscillation in weightlessness.

It seems at the moment, that the frequently discussed spaceflight associated neuro-ocular syndrome (SANS) manifests itself also in well-established neuronal processes such as vision or vestibular system, but not in cognitive performance (Lee et al., 2018; Marshall-Goebel et al., 2019). Originally, SANS was associated with visual impairment, however, other neurological problems such as motor control are currently also connected with the term SANS. At this stage, it is still unclear whether these neurological problems occur because they are not needed in microgravity or whether it is ultimately caused by a structural change (neuroplasticity) of the central nervous system (van Ombergen, Demertzi, et al., 2017; van Ombergen et al., 2018; van Ombergen, Laureys, et al., 2017). A reliable way to further investigate SANS is to assess the influence of microgravity on neural communication, neural drive and/or neural excitability in well-established motor engrams.

Conclusion

A detailed understanding of neural de- and re-adaptation is further important for post-flight rehabilitation for astronauts returning from long-term space flights. In addition, studying the influence of weightlessness on firmly anchored movement patterns can also help to understand other neurological problems linked with spaceflight associated syndromes and to develop countermeasures if necessary. The project is embedded in life science research in space, however it can be linked to terrestrial applications, as a deeper understanding of astronaut (re)adaptation processes will support neurorehabilitation strategies for patients with neurological diseases such as a stroke.

Acknowledgments

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Role of individual immune profiles for wound healing under chronic stress during long-term space flight

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Citation
Buchheim, J., Biere, K., Matzel, S., Hoerl, M., Feuerecker, M., Moser, D., Monici, M., Choukér, A. Role of individual immune profiles for wound healing under chronic stress during long-term space flight.

Introduction

Wound healing is a multistep process that requires the coordinated interplay of different cell types and the efficient recruitment of immune cells to the site of the damage. Long-term stress (allostatic load) can interfere with wound repair and can result in infections or extensive scar tissue. In the health sector, non-healing wounds are an enormous economic burden and are estimated to cause about $50 billion per year in the US alone. Among the patient groups at risk are people that suffer from high stress. Astronauts are subjected to high workload and other chronic stressors such as microgravity, isolation, sleep disruption and immune dysregulation placing them at risk for wound healing problems (Monici et al. 2022, Puhl et al. 2022).

Material and Methods

Study Design and Funding

In the frame of the ESA funded MAP project Wound Healing In Space: problems and PErspectives for tissue Regeneration and engineering (WHISPER) (Contract Number 4000130928/20/NL/PG/pt), data derived from the IMMUNO2 study (funded by ESA:ELIPS 3 and 4 and SciSpaceE programs and the German Space Agency (DLR) on behalf of the Federal Ministry of Economics and Technology/Energy (50WB0919, 50WB1319 and 50WB1622)) was analyzed to evaluate and focus on stress load and wound healing problems associated with individual immune alterations in seven Russian cosmonauts staying at least five months aboard the International Space Station (ISS). The studies were carried out according to the ethical code of the World Medical Association (Declaration of Helsinki). Ethical approval was obtained from the institutional review board of the Ludwig-Maximilians-University (LMU) Munich, Germany, and the medical board of the European Space agency (ESA) and the Russian Space Agency (Roscosmos).

Wound Healing Survey and Stress Assessment

Perception of wound healing problems was measured by completion of the NASA wound healing survey at least once pre-, in, and post-flight for each subject. We evaluated allostatic load employing questionnaires such as the current stress test (CST), profile of mood states (POMS), post-traumaticsymptom scale-10 (PTSS-10) and a numeric analogue scale for lower back pain. Additionally, the physical stress response was monitored in saliva, blood and hair samples, respectively.

Immune Function and RNAseq Analysis
The functional immune status was assessed by immune cell counts as well as the cytokine response 48 hours after *in vitro* stimulation of whole blood with Lipopolysaccharide (LPS). Furthermore, transcriptome profiling by next generation sequencing (NGS) was performed on whole blood samples of four subjects at pre- and post-flight time-points. After total RNA isolation, quality controls, random primed cDNA synthesis and RNAseq using next generation sequencing (NGS) was performed at Eurofins® (Konstanz, Germany) on an Illumina HiSeq2500 platform. FASTQ files of RNA sequencing files were imported into the Array Studio software v10.0.1.118 (QIAGEN, USA) and aligned to the gene model Ensembl.v92 and to the reference library Human B38 using the proprietary OmicSoft Aligner OSA. Differential gene expression of each timepoint versus preflight was assessed using DESeq2. Differentially expressed genes were sent to IPA (http://www.ingenuity.com, QIAGEN, USA) for biological analysis.

Statistical Analysis
Statistical analyses of RNAseq data was performed by Array Studio, OmicsSoft and IPA (all QIAGEN, USA).

All other data were processed using SigmaPlot 13.0 (Systat Software, Germany) and SPSS 24 (IBM, USA). Data was tested for normal distribution using the Kolmogorov-Smirnov Test. Box-Cox transformation of the data was performed only when residuals of the tested variable were found to be non-normally distributed. The data was then analyzed using a linear mixed effects (LME) model, where the time-points measured were regarded as fixed and subjects as random effects. A value of $P < 0.05$ was considered as statistically significant.

Results

Preliminary analysis of data revealed 85% of participants estimated wound healing to be altered and 67% of those estimated the process to be delayed. 86% of the subjects believed that applying ointment on wounds would support healing. Perception of stress by cosmonauts seemed generally low suggesting that resilience and coping strategies in this cohort appear to work. Lower back pain was reported by few individuals after return from space, mostly on day R+7. Levels of the stress hormone cortisol, driven by the hypothalamic-pituitary-adrenal (HPA) axis evidenced a preserved circadian rhythm. Analysis of leukocyte subsets confirmed some of the data already seen in a previous long-term spaceflight study (Buchheim et al. 2019) but showed a lasting significant increase in monocytes on R+1 and R+7 after return.

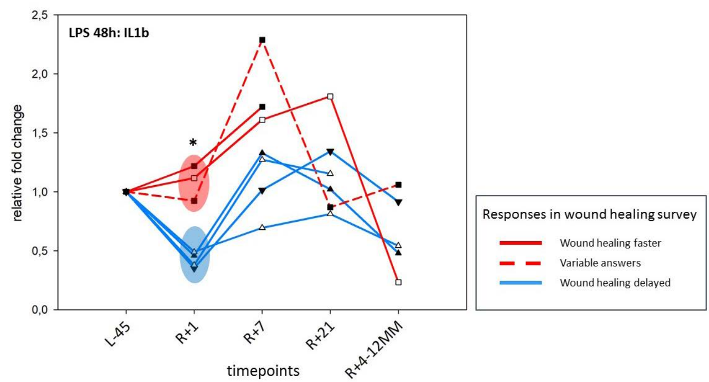

FIGURE 1

Results of the wound healing survey correlated with functional stimulation assays. The group stating that wound healing progressed faster (red colour) showed higher levesl of IL1b than the other crew members reporting a slower wound healing (blue colour). n=7,*P<0.05 red vs. blue (LME).

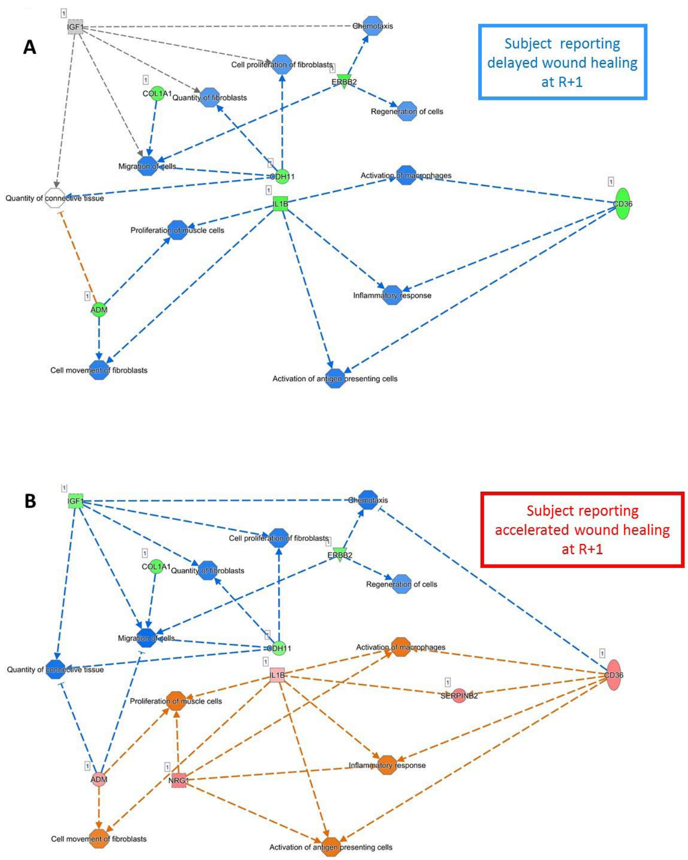

FIGURE 2

Differences in RNAseq trancriptome profiles between subjects of the two groups.
Subject reporting delayed wound healing **(A)** showed more functions critical for wound healing to be inactivated (blue) and more genes to be downregulated (green colour) compared to a subject reporting accelerated wound healing **(B)**.

Stimulation assays revealed individually different trajectories of cytokine amounts after stimulation with lipopolysaccharide (LPS). Interestingly, two subgroups mirrored the results from the wound healing survey. Individuals that perceived wound healing not to be impaired on at least one of the time-points also showed preserved interleukin 1b (IL1b) levels, a key cytokine for wound healing, after stimulation with LPS on R+1 after return. By contrast, the other subjects that reported impaired wound healing showed a lower IL1b concentration (Figure 1). RNAseq data of cosmonauts again confirmed the individual genetic disposition or response to the spaceflight environment. In a pre- to post comparison, two subjects showed a very distinct RNA expression pattern compared to each other (Figure 2). Interestingly the "antagonistic" patterns in these two subjects reflected again the results from the individual stimulation assays and the reported wound healing. A detailed analysis of altered genes showed that one subject with impaired wound healing and lower IL1b levels showed also lower IL1b RNA expression and an inactivation of several cell functions critical for wound healing such as *movement of fibroblasts, activation of macrophages* and *antigen presenting cells* as well as *inflammatory response* (Figure 2A). A subject from the other group showed higher RNA expression levels of IL1b (Figure 2B) and an activated state of the latter functions. Both subjects, however, evidenced inactivation of other critical functions such as *chemotaxis* suggesting immune dysfunction present also in the high IL1b group.

Discussion

Immune dysfunction and recurrent viral reactivation during long-term spaceflight are known problems (Crucian and Sams 2009, Agha et al. 2020). As such, it is not surprising that most cosmonauts reported wound healing impairment. Nevertheless, the observation of a visual acceleration of the observed closure of a wound in a minority suggests individual risk profiles. Our experiments showed that the stimulated IL1b protein release, a key cytokine in the wound healing process was reduced which could also be shown for the baseline RNA expression. This distinct profile was inversed in a subject reporting accelerated wound healing. It should be investigated further, ideally together with a detailed photo documentation of inflight wounds, if the subject's personal observation can be correlated with objective imagery. IL1b might be an interesting target for further studies with the aim to unravel the regulation of the wound healing process under spaceflight conditions.

Conclusion

Our data show that individual immune responses to the very specific spaceflight environment render some participants more prone to wound healing problems than others despite similar training and preparation for the mission. Individual data analysis can help to develop personalized prevention strategies for future interplanetary missions.

Acknowledgements

We thank all the helping hands, operators, scientists and administrators at DLR and all space agencies who fund and support the study on different levels. We express our sincere gratitude to the ISS crews who supported this project and participated with great professionalism.

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A re-evaluation of the acute effects of weightlessness and what it means for SANS

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Citation
Buckey, J.C., Lan, M. A re-evaluation of the acute effects of weightlessness and what it means for SANS.

Introduction
Headward fluid shifts have long been described as the primary effect of weightlessness on the human body. Without the force of gravity pulling blood towards the legs, blood redistributes towards the upper body. On Earth, fluid shifts occur when a standing individual moves to the supine or head down tilt positions. Moving from upright to supine increases central venous pressure (CVP), while heart rate decreases and stroke volume increases. A fluid shift in microgravity might be expected to produce similar effects. The fluid shifts that occur with postural changes in Earth and the fluid shift in weightlessness, however, may differ in fundamental ways.

Discussion
Although experience from head-down tilt studies show increases in CVP above supine levels, in 1993 when CVP was measured invasively on a Space Shuttle mission, it decreased below supine levels. One possible explanation presented for this was decreased intrathoracic pressure. Decreased

intrathoracic pressure would create a transmural pressure gradient at the right atrium leading to distension of the atria and reduction of central venous pressure. Noninvasive measures of intrathoracic pressure using an esophageal balloon in parabolic flight seemed to support this [1]. This study, however, failed to account for postural inaccuracies in the supine esophageal manometry measures caused by mediastinal weight. Comparing upright esophageal pressures on Earth to esophageal pressures in space showed no major changes. This weakens the theory that reduced intrathoracic pressure caused CVP to drop in weightlessness [2].

Another way to explain the CVP reduction below supine levels in weightlessness is increased vascular compliance in the circulatory system. Loss of gravity not only eliminates hydrostatic gradients in the blood, it also removes the extravascular pressures exerted by the weight of tissues. This would alter transmural pressures throughout the body in a way that would promote distension of veins and arteries, rendering them more compliant overall. With compliance increased, the venous and arterial blood could be contained at lower pressures (Figure 1). If venous blood is contained at lower pressures in 0G, this would explain reduced CVP in spaceflight. This is supported by results from hypergravity using Gx centrifugation, which show that hypergravity increases CVP [3].

Results from numerical modeling experiments that incorporate the effects of tissue weight align well with experimental results and observations made in spaceflight. A cranio-vascular model, previously described by Lan et al., models the effects of tissue weight in gravity as a column of water with height defined by anthropometric measures of neck circumference, chest circumference and waist circumference [4]. Tissue weights at these locations affect the numerical model's calculation of vascular compliance in that area. Body position relative to the gravitational field is also defined in the model. Using this model, the hemodynamic response to the head down tilt position is predicted accurately, with increased central venous pressure, increased jugular venous flow, and increased intracranial pressure. Importantly, the acute hemodynamic response of the body to weightlessness can also be

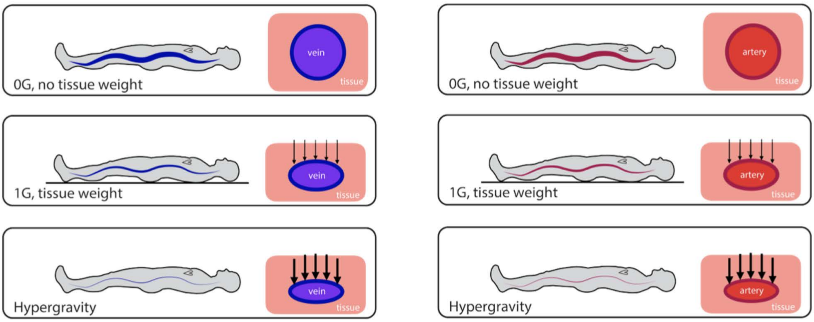

FIGURE 1
Venous and arterial compliance in different gravity conditions.

TABLE 1: Hemodynamic changes observed in head down tilt studies and weightlessness juxtaposed against simulated results from the cardiovascular model from Lan et al

Modeling agreement				
	Supine to HDT change		Supine to 0g change	
	Literature	Simulated	Literature	Simulated
ICP	↑	↑	↓	↓
CVP	↑	↑	↓	↓
IJV Flow Rate	↑	↑	↓	↓

replicated correctly and shows reduced central venous pressure, reduced jugular venous flow, and reduced intracranial pressure relative to the ground based supine position (Table 1). This mismatch between the hemodynamic effects of head down tilt and real 0G implies that head down tilt is not a good analog for replicating the cardiovascular effects of spaceflight (although prolonged head down tilt or supine bed rest is still likely a good analog for spaceflight induced orthostatic intolerance, muscle atrophy, and bone loss).

Overall, these data suggest that the adaptation of weightlessness is not simply due to a fluid shift. The primary event is the loss of hydrostatic gradients in fluid-filled columns (like blood vessels) and the loss of tissue compressive forces. This leads not just to fluid shifts, but other effects as well, such as a reduction in venous pressures. Also, these effects may be affected by body size. The hydrostatic pressure in a column of fluid depends on its length, and compressive forces in tissue depend on the size of the tissue. So, body size as measured by various anthropometric measurements, is important for the pressure changes that will occur when entering weightlessness. The idea that body size or body weight affects the magnitude of weightless affects is supported by a retrospective analysis of astronaut data collected in the Longitudinal Surveillance of Astronaut Health (LSAH) database. Using regression analysis, preflight body weight was found to be a significant predictor for the development of significant optic disc edema and/or choroidal folds, two signature signs of Spaceflight associated neuro-ocular syndrome (SANS)—a condition that affects crew on long duration spaceflight missions. [5]

Spaceflight associated neuro-ocular syndrome (SANS) has been hypothesized to be caused by elevated intracranial pressures, similar to the terrestrial condition idiopathic intracranial hypertension (IIH). But, both invasive and non-invasive measures of intracranial pressure (ICP) show that ICP does not increase above supine levels in weightlessness [6, 7]. Rather ICP is at a pressure level below supine but greater than upright. This is in contrast to IIH patients who typically have pathologically elevated ICP well above supine levels. Other differences between SANS patients and IIH patients are that astronauts with SANS do not report headaches, tinnitus, or other symptoms typical of elevated ICP and IIH. This suggests a different mechanism for the development of SANS. Hypotheses for how SANS develop should consider the unique effects of weightlessness on hydrostatic gradients and how that might affect pressures in the fluid compartments of the eye and of the head [8]. The hyperopic shift and globe flattening that occur with SANS need to be explained without presuming elevated ICP from fluid shifts. When hydrostatic gradients within the eye and head are removed, it's possible that the net transmural pressure at the posterior of the eye creates a force that tends towards flattening.

Conclusion

Imaging the adaptation to weightlessness as a response to a headward fluid shift, similar to what occurs during head-down tilt bedrest, has framed the thinking about SANS and other weightlessness-unique conditions, such as jugular venous thrombosis risk [9]. The fluid shift, however, is only one part of the adaptation to weightlessness, which is caused primarily by the removal of hydrostatic gradients and body weight. The pressure changes that occur in fluid columns and tissue in weightlessness will depend upon body size, so anthropometric measures are important when studying the effects of weightlessness.

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Targeted proteomic analysis of *S. cerevisiae* in microgravity conditions in response to antifungal stress

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Chandramowli, D., Devreese, B., Willaert, R. Targeted proteomic analysis of *S. cerevisiae* in microgravity conditions in response to antifungal stress.

Introduction

The search for life in extraterrestrial environments has always been one of the most appealing aspects of astrobiology. However, one common hurdle facing astronauts during long-term space missions in this regard is the drop in their immune responses. This is, in part, due to microgravity, which has been shown to have an effect on human cells after prolonged exposure. Further, since astronauts do not have the luxury of the atmosphere to protect against radiation, they receive does of ionising radiation several times what they would normally be exposed to on Earth (Blaber et al., 2010). These stress conditions result in increased susceptibility to bacterial and fungal infections.

Conversely, microbes present in the astronauts' environments also subject to these adverse conditions adapt in such a way that they show increased antimicrobial resistance, thus making even simple diseases potentially problematic (Klaus and Howard, 2006). The constant co-evolution between microbes and humans in these conditions limits effective treatment options, thereby necessitating the timely development of suitable therapy. In order to do so, it becomes important to understand what cellular processes are affected in pathogens upon exposure to antibiotic/antifungal agents.

Here, we examine a list of proteins from the model organism *Saccharomyces cerevisiae* that are known to be involved in a range of functions (cell death, cell division and cell cycle regulation, response to antifungals, etc.) to see how the levels are altered in response to different antifungals in conditions of simulated microgravity (sim-μg). This is accomplished using targeted proteomics, viz. the mass spectrometry method known as multiple reaction monitoring (MRM).

Materials and Methods

1. **Experimental design:** For ground control (1g) experiments, overnight cultures of *S. cerevisiae* strain BY4741 (mating type a) were back-diluted in 1 litre of yeast extract-peptone-dextrose (YPD) medium at 30°C and 200 rpm. These cultures were allowed to grow for 1 generation, following which a sub-minimal inhibitory concentration (sub-MIC) of an antifungal was added to the appropriate culture vessel – the antifungals used were amphotericin B (concentration-dependent), fluconazole (fungistatic), and anidulafungin (fungicidal). The cultures were then allowed to grow for a further 3 generations. Cells were harvested, and intracellular proteins were isolated and prepared for analysis using multiple reaction monitoring (MRM). For simulated microgravity (sim-μg) experiments, the same experimental workflow was followed, but cells were grown in a 1 litre rotating wall vessel (RWV) maintained at 5 rpm to simulate the effects of microgravity.

2. **Multiple reaction monitoring (MRM):** Proteins were reduced, alkylated, and digested using trypsin to yield peptide mixtures. Peptides were purified using solid phase extraction (SPE) and resuspended in a solution of Hi3 PhosB standard (Waters). The LC-MS/MS apparatus consisted of a Waters NanoAcquity M-Class UPLC console and an IonKey source connected to a Waters Xevo TQ-S triple quadrupole mass spectrometer. The IonKey source contained a 150 µm x 100 mm, 1.8 µm HSS T3 iKey separation device to separate peptides entering the mass spectrometer. Briefly, 5 µl of each sample (0.5 µg of peptides) was injected into the loop and trapped for 3 minutes with a flow rate of 10 µl/min on a 300 µm x 50 mm, 5 µm, 100 Å Acquity UPLC M-Class Symmetry C18 trap column (Waters). The peptides were then separated on the iKey using a 15-minute gradient of 3-50% acetonitrile (ACN) with a flow rate of 2 µl/min. The mobile phase consisted of 0.1% formic acid in UPLC-grade water (solvent A) and 0.1% formic acid in ACN (solvent B). The triple quadrupole was operated in positive ion mode, and the machine was set to MRM mode to detect peptides at a set cone voltage (35 V) and variable collision energies and dwell times for each precursor ion. The data was then imported into the Skyline software for visualisation and analysis.

3. **Analysis of MRM data:** The normalised areas under the curve (AUCs) were calculated using the Skyline software. This was done by subtracting the background area from the area under the target peptide chromatograms, following which the area was normalised to the area of a peptide from the spike-in control bovine serum albumin (BSA). The relative abundance of each target peptide was then determined by comparing the ratio of the peptide in each condition relative to an untreated control across both conditions of gravity. Graphs were plotted comparing the levels of each target peptide across conditions of antifungal stress and gravity. Paired t-tests were used to assess statistical significance, and this was carried out using the GraphPad Prism software.

Results and Discussion

Based on the results of our MRM experiments, we observed marginal differences in protein abundance between conditions of gravity for the majority of proteins tested (results not shown). This means that most proteins are unaffected in conditions of sim-µg (and by extension, the corresponding metabolic functions in which they are involved). However, significant differences were observed between certain proteins (figure 1) across conditions of gravity. These include proteins involved in antifungal response (*PDR5*) and regulation of cell cycle (*NUC1*). Such proteins could be interesting candidates for measuring the diagnostic efficacy of antifungals.

It should also be noted that multiple combinations of antifungals and gravity were tested simultaneously (144 conditions across gravity and antifungals), and some significant differences were observed in some conditions that were not apparent in others (results not shown). This is to be expected, since the targets of each antifungal are generally specific to one or a group

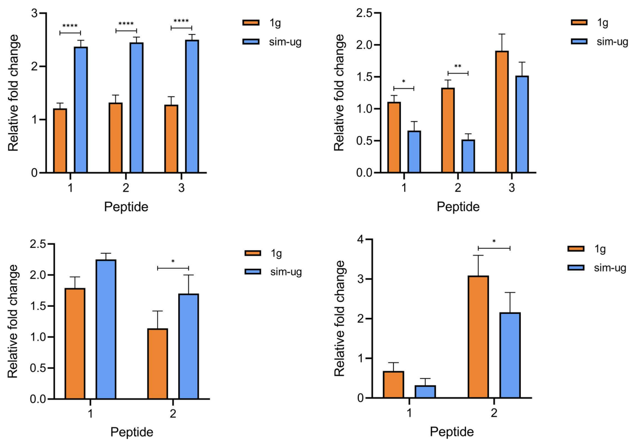

FIGURE 1

Relative protein abundances in response to antifungal and microgravity stresses. Each graph (mean ± S.D.) represents the level of a specific protein in response to a specific antifungal in conditions of 1g and sim-ug. Both have been normalised to an un-treated control for the respective condition of gravity. A t-test was used to assess statistical significance ($p < 0.05$).

Top-left – *PDR5* in response to amphotericin B; top-right – *EFG1* in response to amphotericin B; bottom-left – *RAD55* in response to fluconazole; bottom-right – *NUC1* in response to anidulafungin.

of related functions while others remain unaffected. In future iterations of these experiments, it would therefore be more meaningful to only draw conclusions based on proteins known to be affected by each antifungal, as this would allow for a better understanding of which cellular processes are most affected by the treatment.

Future Perspectives

So far, we have managed to develop MRM methods to quantify the levels of 18 proteins in *S. cerevisiae*. In the future, we hope to expand on this list to include proteins involved in other metabolic processes (such as cell death). Further, we intend to investigate these proteins using a different medium to assess whether our results are medium-specific. Lastly, we also wish to investigate the effect of radiation on the levels of these proteins, since this is also a significant stress encountered during space flights.

Acknowledgments

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Immune cell activation state in 5-day Dry Immersion: A comparative study between a female and a male cohort

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D´Amico, F., Choukér, A., Moser, D. Immune cell activation state in 5-day Dry Immersion: A comparative study between a female and a male cohort.

Introduction

Immune system dysregulations can occur both during and after spaceflight, and pose a serious threat to long-duration manned missions (Crucian et al., 2008). Spaceflight-related immune impairment can result in reactivation of latent viruses such as HSV (Mehta SK et al., 2014), increase in allergic reactions or newfound allergies, worsening or slatentization of autoimmune diseases, and more (Crucian et al., 2018). An array of factors can affect the immune system in such conditions, including microgravity, physical and psychological stress, physical activity, radiation, alteration of circadian rhytms and nutrition (Crucian et al., 2018), although the extent and details of such alterations is still subject of ongoing investigations. Given the peculiar logistic of conducting investigations in a spaceflight setting, ground analogues are often adopted as a surrogate to simulate one or more of spaceflight-associated conditions like isolation and weightlessness. Most used models for microgravity include head-out water immersion (WI), Dry Immersion (DI), horizontal bed rest (HBR) and head-down tilt bedrest (HDBR). WI was first proposed to investigate rapid-onset microgravity effects (Beckman et al., 1961) until DI was developed for long-duration studies (Shulzhenko et al., 1976). In DI, subjects are immersed in a water bath covered by a folded waterproof fabric, allowing the participant freedom of movement. This model provides hypokinesia and a greater degree of mechanical unloading when compared to other analogues, such as HDBR.

While the effects of DI on muscoloskeletal, cardiovascular and sensori-motor systems have been thoroughly investigated (Tomilovskaya et al., 2019), few studies have assessed the immune system in this model (Ponomarev et al., 2013). Moreover, until very recently (Tomilovskaya et al, 2021), DI studies have been performed only on male subjects. As of December 2022, roughly 12% of all-time astronauts have been female, a percentage quickly destined to increase – thus, the need for a systematic approach to sex difference in spaceflight-related health effects becomes clear, as well as for comparative studies in ground models.

Material and Methods

In the ESA-sponsored VIVALDI 1 & 2 studies performed at the MEDES Space Clinic (Toulouse, France), a team of experts investigated the effects of dry immersion on many physiological systems for 5 days in females and males. 20 healthy females (regular menstrual cycle, no oestroprogestative contraception) and 20 healthy males aged between 20 and 40 were separately enrolled in each study. In this consortial effort our team has evaluated innate and adaptive immune cell activation states, analysing through flow cytometry for monocytes, granulocytes and lymphocytes expressing HLA-DR, TLR2, TLR4, CD4, CD8, CD11b, CD14, CD16, CD28, CD40, CD62L, CD66b, CD69 and CD86 receptors. Peripheral blood was drawn in heparinized monovettes at multiple timepoints during the study: one day prior to protocol (BDC), at third (DI-3) and fifth (DI-5) day of DI and one day after DI (R+1). Collected blood was immediately conserved with

TransFix™ (Cytomark, Buckingham, UK) and stored at 4°C. Whole blood was incubated with antibody mixes for 20 minutes and red blood cells were lysed with FACS Lysis solution (BD Biosciences, San Diego, USA). After washing with PBS, event acquisition was performed on a Guava easyCyte© (Merck, Darmstadt, Germany) flow cytometer. GuavaSoft™ software was used for acquisition and data analysis. Statistical analysis was performed with SigmaPlot™ 13.0; data were compared by repeated measures ANOVA for variability within groups and by Mann-Whitney U test for comparability between the two groups. Statistical significance was set at $p \leq 0.05$.

Results

Activation markers on monocytes (CD14^{+}) were not significantly affected by DI in both sexes, although males showed a higher pre-existing activation, as demonstrated by higher level of activation markers such as CD40, TLR2 (Figure 1) and CD69. Monocytes subsets were, instead, significantly affected

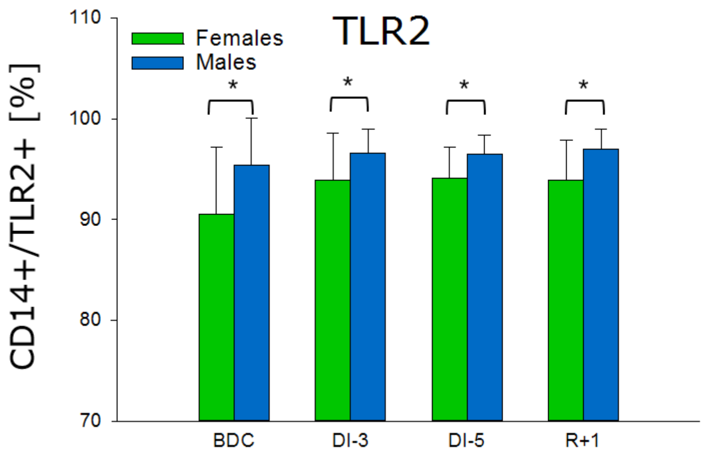

FIGURE 1

Comparison of TLR2 expression on monocytes (CD14^{+}) between males and females at four timepoints (BDC-1 = baseline, D-3 = DI day 3, D-5 = DI day 5, R+1 = first day after DI). Bars represent mean + SD. *$p \leq 0{,}05$.

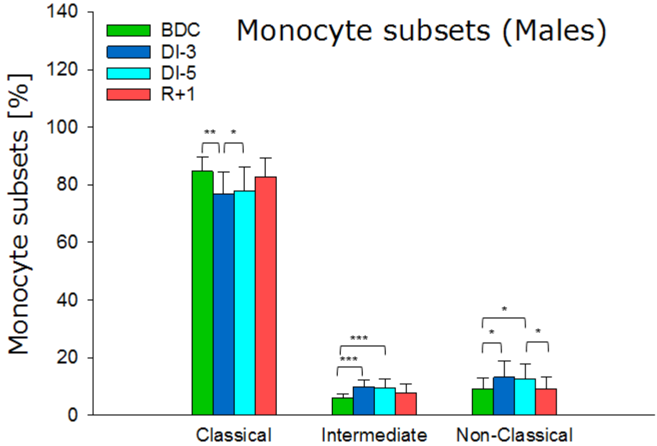

FIGURE 2
Comparison of monocyte subsets over the four timepoints (BDC-1 = baseline, D-3 = DI day 3, D-5 = DI day 5, R+1 = first day after DI). Bars represent mean + SD. *p≤0,05; **p≤0,01; ***p≤0,001.

by DI in males (Figure 2): classical monocytes ($CD14^{++}$, $CD16^{-}$) decreased with a corresponding increase of intermediate ($CD14^{+}$, $CD16^{+}$) and non-classical ($CD14^{dim}$, $CD16^{+}$) monocytes, whereas in females no such trend was observed.

Granulocyte ($CD16^{+}$) activation markers were, as well, not significantly affected by DI, with males showing again higher surface marker levels compared to females, particularly with CD66b and CD62L (Figure 3) at all timepoints.

DI did not significantly affect T-cell ($CD3^{+}$) activation state in both sexes. A significant difference in activation markers was present between sexes, where males displayed a lower CD28 expression (Figure 4) and a higher CD69 (Figure 5) expression than females on both $CD4^{+}$ and $CD8^{+}$ T cells.

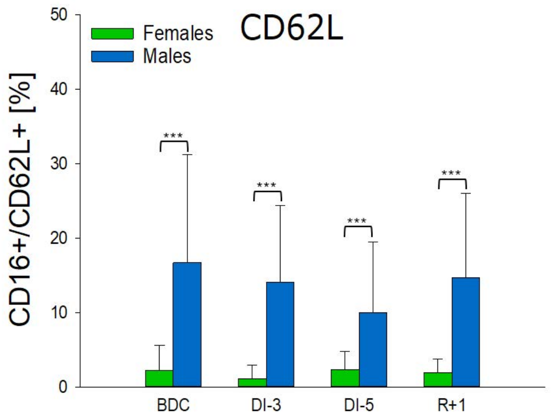

FIGURE 3
Comparison of CD62L expression on granulocytes (CD16+) between males and females at four timepoints (BDC-1 = baseline, D-3 = DI day 3, D-5 = DI day 5, R+1 = first day after DI). Bars represent mean + SD. ***p≤0,001.

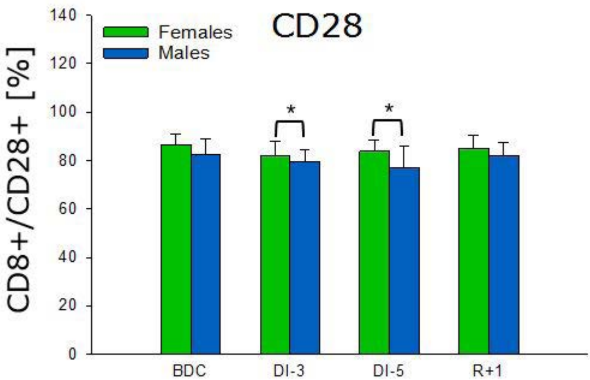

FIGURE 4
Comparison of CD28 expression on CD8+ T cells between males and females at four timepoints (BDC-1 = baseline, D-3 = DI day 3, D-5 = DI day 5, R+1 = first day after DI). Bars represent mean + SD. *p≤0,05.

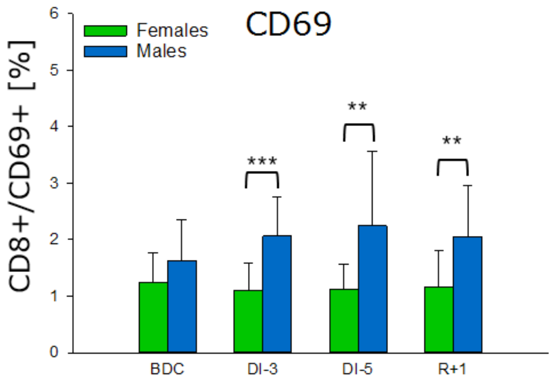

FIGURE 5
Comparison of CD69 expression on CD8+ T cells between males and females at four timepoints (BDC-1 = baseline, D-3 = DI day 3, D-5 = DI day 5, R+1 = first day after DI). Bars represent mean + SD. **p≤0,01; ***p≤0,001.

Discussion

This study is, to our knowledge, among the firsts to include an all-female cohort in a DI protocol, and the first to compare immunity markers between males and females in this setting. These preliminary data, which will be further complemented by humoral factors - such as cytokine panels and assessment of circulating soluble markers - show that DI does not significantly affect immune cell activation state, while highlighting pre-existing differences between sexes. Interestingly, some differences were evident already at BDC and persisted throughout DI, such as for TLR2 on monocytes; others reached stastical significance only during the DI, such as CD28 on T cells; moreover, some differences appeared during and persisted after DI, as shown by CD69 on T cells. Curiously, both CD62L and CD66b levels were persistently higher in male subjects. CD62L is an adhesion molecule (a selectin) presented by many white blood cell types including granulocytes. It's been known for a long time that, upon activation, CD62L is rapidly shedded (Griffin et al., 1990), and higher granulocyte surface levels in males would suggest a lower activation of these cells. This seems conflicting with the higher CD66b and CD11b levels in the same cohort, which point to higher activation state. Therefore, our finding has to be integrated with circulating levels of CD62L for a comprehensive overview of adhesion readyness in these cells.

Increase in non-classical and intermediate (a mid-step between classical and non-classical conversion) monocytes has been associated with injury, inflammation, infection and worse outcomes in several pathologies, including cardiovascular and autoimmune diseases, although a causal relationship has to be estabilished yet (Kratofil et al., 2017). Here we suggest that this shift, observed only in male subjects, could represent a „priming" of the innate immune system to the stress of DI, although this claim has to be investigated further.

Taken together, these differences point to a higher activation state of white blood cells in males compared to female subjects. Although literature on sexual dimorphism of white blood cell surface markers is scarce, plenty of studies have investigated the effects of sexual hormones on the immune system as a whole. On a superficial level, our findings may conflict with the general hypothesis that females possess stronger innate and adaptive immune responses (Jaillon et al., 2019); further investigation in this cohort is required to support this statement, particularly regarding cytokine levels.

Although biological sex plays a considerable role in infections (Goble et al., 1973), cancer (Kim et al., 2018) and autoimmune diseases (Whitacre et al., 2001), few studies tried to assess sex-based differences in immunity during spaceflight, partly due to reduced sample sizes (Kennedy et al., 2014). Given the known differences in immune responses and the higher likelihood of mixed crews in the near future, it is important to assess if ground-based models are adequate to investigate particular aspects of immunity. A deeper immune function analyses of these VIVALDI cohorts in samples available to us will verify these findings and help integrating these observations into other organ changes assessed by the VIVALDI study group. The results of such an integrative view will pave future studies and mission designs, respectively.

Conclusion

A 5-day Dry Immersion protocol does not seem to affect the majority of immune cell activation states as assessed by phenotyping through flow cytometry, with the notable exception of monocyte subsets in males. In male subjects, the shift from classical to intermediate and non-classical subsets could suggest an adaptive response to the Dry Immersion condition. Moreover, males appear to show higher immune cell activation states than females, independently of Dry Immersion.

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What is the optimal exercise for future exploration missions: A panel discussion

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Introduction

With the transition from the International Space Station (ISS) to a lunar gateway or interplanetary exploration missions, exercise countermeasures will need to be redefined in light of the new realities (Liphardt et al., 2023).

Exercise equipment will have to change and adapt to the new environments (e.g., lunar habitat or exploration vehicle) and flight durations, likely resulting in smaller and less numerous equipment than currently exists on the ISS. To preserve tissue health and body mass while being physically active, adequate energy supply is essential, but upload mass will change dramatically during exploration missions and part of the supply will likely be self-grown food. Therefore, the energy cost of exercise must be considered when planning exercise regimens for space crew. Finally, the individual response to exercise countermeasures and the preferences for types of exercises and protocols vary widely. Given the above, the research community may need to move beyond the “one-fits-all” approach to truly personalized spaceflight exercise countermeasures. In a plenary session during the 42nd ISGP Annual Meeting in Antwerp, Belgium, in July 2023, we discussed with five experts their views on the optimal exercise for exploration missions and specifically aimed to take different perspectives on this complex question.

Enabling the Human to Explore the Moon and Beyond

The exercise countermeasure system on the International Space Station (ISS) has enabled astronauts to perform microgravity Extravehicular Activities and return to the Earth in a relatively healthy state with return to most normal daily activities within weeks on landing. Although physical performance is sufficiently protected for ISS missions, as space exploration extends beyond low Earth orbit, the physical and psychological demands on the astronaut will be higher and the current level of protection may not be sufficient for all astronauts and mission scenarios. Additionally, the capabilities of the vehicle to support countermeasures systems for human health and performance will likely be limited compared to the ISS. With the potential for increased human requirements and decreased vehicle capabilities it is critical to develop countermeasures systems that are feasible and reliable for use in new spaceflight vehicles and protect the astronauts to an acceptable level. This may require refinement of current countermeasures, novel countermeasures, or adjunct interventions.

One Giant Leap for Mankind

The current ISS countermeasure exercise programme consists of 10-12 sessions / week of aerobic and resistance exercise, resulting in approximately 75 minutes of prescribed (30-min aerobic, 45-min resistance) exercise per day. This high volume, multi-modal programme has successfully reduced the magnitude of spaceflight-induced adaptation of the cardiovascular and musculoskeletal systems, but individual effectiveness appears to vary markedly. It also comes at a relatively high cost is terms of time and metabolic expenditure. Plyometric exercise, which typically includes hops and jumps, has been shown in long-term bed rest studies to be an effective method of managing physiological adaptation to prolonged gravitational unloading while requiring only a few minutes of exercise each day on a single countermeasure exercise device. This combination provides the potential for a form of countermeasure exercise that is both effective and efficient, as well as simplifying the design and development of future countermeasure exercise systems.

Is the Pantry on the Way to mars big Enough? The Tangle Relationship Between Exercise Load and Energy Requirements

Body mass loss due to insufficient energy intake remains a concern during ISS missions. Additionally, space travelers lose muscle mass and strength, which prompts them to increase their exercise sessions in microgravity. Recent publications demonstrate that, even in spaceflight, energy expenditure mainly increases due to exercise loads (Bourdier et al., 2022). To prevent the use of endogenous energy stores, such as fat and muscle mass, more food must be provided. However, providing more food means additional upload mass and/or a greater need for production and storage space. Therefore, finding the optimal balance between the type and duration of exercise and minimizing energy expenditure is crucial for exploratory missions.

Is the "Optimal Exercise" for Future Exploratory Missions Optimal for Everyone? The Individual Variability Issue

Interindividual variability in physiological responses to an intervention (e.g., exercise) has attracted research attention in recent decades with the goal of identifying 'responders' and 'non-responders," finding the mechanisms that influence individual response, and advancing "personalized medicine" (Mann et al., 2014;Hecksteden et al., 2015;Ross et al., 2019). This is also true for space physiology. However, there are many limitations that make the study of individual variability in space physiology a difficult endeavor. To improve the study of individual variability in space physiology, it is first necessary to define how much of the variability is due to random and technical sources. This will facilitate the identification of the actual biological individual response to an intervention. In addition, there are several issues that researchers need to consider before they begin analyzing the data, i.e., during study design, which will facilitate experiments on individual variability. For example, including a well-defined and relevant control group or using a cross-over design, running multiple tests per time point for a given outcome, and running all samples simultaneously (this is very relevant for molecular outcomes, especially omics data) will help researchers identify biological variability. In addition, using robust and validated approaches to study individual variability should be a priority (Atkinson and Batterham, 2015;Hopkins, 2015). In this way, researchers can determine individual variability in response to an intervention and even examine the moderators that influence the observed individual response. Following these recommendations, we found significant individual variability in calf and thigh muscle area after bed rest (Fernandez-Gonzalo et al. 2021). We also reported that baseline values were a moderator of the individual response and that it is possible to build mathematical models to predict individual response to changes in calf muscle area induced by bed rest (Fernandez-Gonzalo et al., 2021).

F* Exercise Recommendations: A Hedonistic Brain Perspective!**

With an increasing understanding of physiological mechanisms and adaptational processes in recent years, there is a significant increase in exercise recommendations. All these recommendations often target one specific area: cardiovascular, musculoskeletal and sensorimotor systems and are probably of relevance when aiming for the last 5% of performance. But we need to be aware of the fact that we are training astronauts, not athletes. In a health setting, on earth as well as in space, the best exercise is the one, that is done. And this is a question of personal drive and motivation. From a holistic and sustainable health perspective it is strongly advised to develop an internal awareness of the mode of action of exercise rather than follow blindly external recommendations.

Conclusion

We still have a lot of work to do to define the optimal excise strategy that offsets the harmful effects of space travel on the human body. For example, training loads must be controlled and fine-tuned to reduce damage to important tissues (e.g., cartilage) and risk of injury, while providing stimuli strong enough to produce positive effects (e.g., preservation of skeletal muscle and bone mass). All of this must be achieved with exercise modes that are enjoyable and fun for the individual crew member, and ensuring that adequate nutritional intake is consumed. Another important aspect of developing optimal exercise countermeasures is determining the degree of deconditioning that space crew members can endure without jeopardizing mission success or long-term health outcomes upon return to Earth. Finally, a better understanding of the different individual responses to specific exercise routines is needed, followed by true individualization of exercise countermeasures to maximize the benefits for each crew member. Although the challenges discussed here are colossal, the space physiology research community appears ready to tackle this major undertaking to facilitate long-term human space exploration on the Moon, Mars, and beyond.

Acknowledgments

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Self-organized criticality assessed on Holter heart rate variability during spaceflight simulation by dry immersion

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Citation
Fortrat, J., Robin, A., Navasiolava, N., Custaud, M-A. Self-organized criticality assessed on Holter heart rate variability during spaceflight simulation by dry immersion.

Introduction
Self-organized criticality (SOC) is a universal theory that explains the behavior of dynamical systems, including the cardiovascular system (Bak, 1996; Fortrat & Gharib, 2016; Muñoz, 2018). The term "criticality" refers to the occurrence of large, spontaneous, sudden dynamical events known as catastrophes.

The time distribution of these catastrophes follows power laws, such as the Gutenberg-Richter and Zipf's laws. The demonstration of self-organized criticality is particularly evident in cardiovascular time series obtained during the gravitational stress of the standing position (Fortrat & Ravé, 2020). This position poses a challenge to brain perfusion and increases the risk of vasovagal syncope, which are cardiovascular catastrophes (Fortrat & Gharib, 2016). Spaceflight or its simulation through prolonged head-down bed-rest or dry immersion poses challenges to cardiovascular function, leading to cardiovascular deconditioning and increased orthostatic intolerance upon returning to the standing position (Robin et al., 2020). This raises the question of whether simulated spaceflight influences the self-organization of cardiovascular dynamics. In this study, we hypothesized that long-term dry immersion alters Zipf's law of heart rate variability in Holter recordings.

Material and Methods

A long-term spaceflight simulation was conducted using a five-day dry immersion on nine healthy men. Detailed information about the experiment has been previously reported (Robin et al., 2020). Holter recordings were taken three days prior to the immersion and on day 1, 3, and 5 of the immersion (referred to as B3, I1, I3, and I5, respectively). For each Holter recording, two hours of continuous heart rate data were collected during both daytime and nighttime, totaling at least 5000 heartbeats for each period. The heart rate time series were manually filtered by a trained operator (JOF). Episodes of bradycardia were identified and counted based on their length in terms of the number of beats, following a previously described method, in order to construct the Zipf's plots (Fortrat, 2020). The slope of long and short bradycardias was determined according to the initial description of the bradycardia Zipf's law. A comparison of the immersion periods was performed using Friedman's tests.

Results

The slope of the Zipf's distribution for long bradycardias was consistently higher during the day compared to the night, whereas the slope for short bradycardias was consistently lower during the day compared to the night.

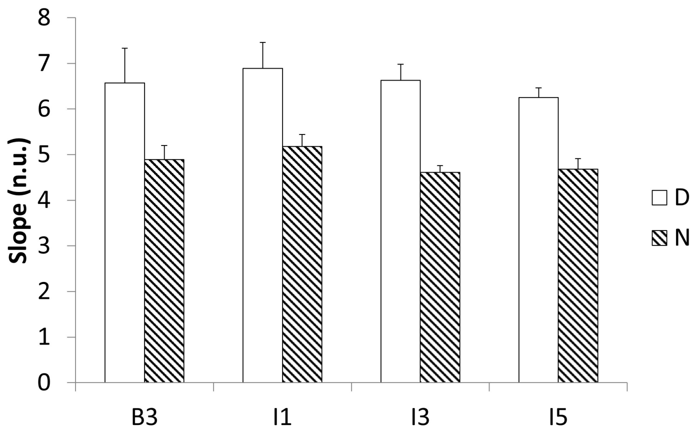

FIGURE 1
Slope of Zipf's Distribution of Spontaneous Long Bradycardias during 5-Day Dry Immersion. The figure displays the slope of Zipf's distribution of spontaneous long bradycardias throughout a 5-day dry immersion period. The labels B3, I1, I3, and I5 correspond to measurements taken 3 days before the immersion and on days 1, 3, and 5 of the immersion, respectively. The data presented in the figure were collected during both daytime (D) and nighttime (N). Notably, no significant changes in the slope of the distribution were observed during the study.

There was no significant change in this slope observed during the dry immersion period (Figure 1). Despite consistently obtaining high regression coefficients, the overall shape of the Zipf's distribution appears to be curvilinear instead of the expected linear shape (Figure 2).

Discussion

Despite previous reports of the gravitational influence on cardiovascular self-organized criticality, a long-term dry immersion did not have an impact on Zipf's law of heart rate variability in Holter recordings. Studying self-organized criticality during a long-term spaceflight simulation is based on the intriguing hypothesis of a connection between the gravitational

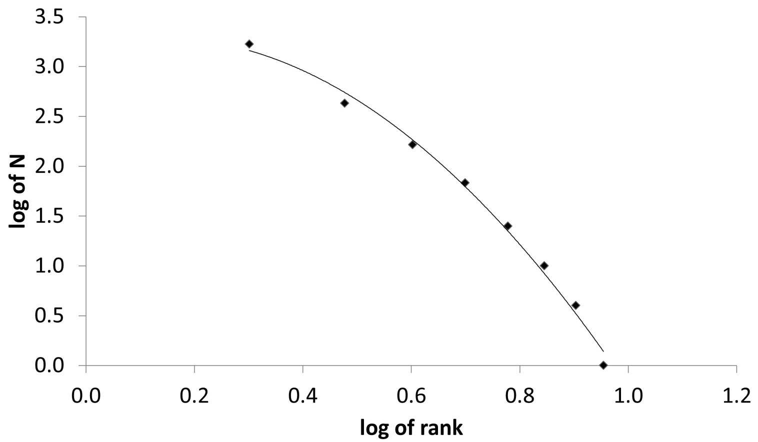

FIGURE 2
Zipf's Distribution of Spontaneous Bradycardias Based on Duration in a Healthy Subject before Dry Immersion. Data shown represent daytime period recordings obtained using a Holter monitor. Surprisingly, the observed distribution exhibited a curvilinear pattern, deviating from the expected linear trend.

challenge of the standing position and criticality, as evidenced by vasovagal syncope. However, spaceflight simulation leads to an increase in orthostatic intolerance. Conducting such a study is challenging because spaceflight simulation eliminates the standing position, in which self-organized criticality is more readily observed. To overcome this limitation, the length of the data analyzed in this experiment was significantly increased compared to previous studies, aiming to mitigate this drawback. Through the use of Holter, the data length was expanded by a factor of 10 in order to capture more spontaneous bradycardia events. Unfortunately, this experiment failed to demonstrate any influence of simulated spaceflight on cardiovascular self-organized criticality. The difficulty in studying cardiovascular self-organized criticality during simulated spaceflight may stem from the absence of the standing position in the dataset. Obtaining a Holter recording immediately after the spaceflight simulation that includes periods of standing positions might provide more informative results compared to one obtained at the end of the immersion period.

The extended duration of data analysis provided an opportunity to accurately describe the distribution of bradycardia episodes. However, this distribution appears to be curvilinear, deviating from the expected Zipf's distribution. The presence of a curvilinear distribution challenges the self-organized criticality of the time series. Previous experiments that have explored HRV power laws to investigate self-organized criticality were conducted on motionless subjects, not utilizing Holter recordings. We have previously demonstrated that the data collection process itself can influence heart rate variability analysis, even when considering mathematical complexity (Fortrat et al., 1999). The question remains whether Holter recordings may obscure the self-organized criticality of cardiovascular dynamics.

Conclusions

Further studies should aim to elucidate the impact of Holter settings on self-organization of heart rate variability, particularly when compared to laboratory settings. Moreover, these studies should incorporate recordings that encompass the standing position during the recovery phase immediately following the conclusion of spaceflight simulation.

Acknowledgments

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P2Y1 and P2Y2 receptors differ in their role in the regulation of signaling pathways during unloading-induced rat soleus muscle atrophy

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Kostrominova, T., Zaripova, K., Belova, S., Shenkman, B., Nemirovskaya, T. P2Y1 and P2Y2 receptors differ in their role in the regulation of signaling pathways during unloading-induced rat soleus muscle atrophy.

Introduction

Hypokinesia leads to skeletal muscle atrophy due to the disruption of the balance between protein synthesis and protein degradation (Fluck and Hoppeler, 2003). One of the mechanisms regulating alterations of signaling pathways could be an accumulation of ATP during the first three days of unloading (Zaripova et al., 2021). Extracellular ATP is one of the major mediators of autocrine/paracrine signaling (Lazarowski et al., 2003). P2Y1 and P2Y2 receptors are sensors of the extracellular ATP concentration (Casas et al., 2014). P2Y2 receptors increase skeletal muscle atrophy and the activation of fibroblasts/fibrosis in damaged muscle (Chen et al., 2021).

At present, there are no well-described functional differences between P2Y1 and P2Y2 receptors. The current study tested a hypothesis that ATP-dependent signaling via P2Y1 and P2Y2 receptors is involved in the activation of unloading-induced signaling cascades. To test this hypothesis two purinergic receptor inhibitors were used during three days of soleus muscle unloading: P2Y1 receptor inhibitor MRS2179 and P2Y2 receptor inhibitor AR-C 118925XX.

Material and Methods
All animal experiments for this study were approved (protocol 585; 05/31/2021). Thirty-two male Wistar rats were randomly assigned to one of the four groups (8 animals per group): control rats with placebo (C), 3 days of unloading/hindlimb suspension with placebo (HS), 3 days of unloading with P2Y1 receptors inhibitor MRS2179 (intraperitoneal injection of 25 mg/kg of body weight; HSM), and 3 days of unloading with P2Y2 receptors inhibitor AR-C 118925XX (intraperitoneal injection of 10 mg/kg of body weight; HSA). The procedures of muscle dissection, Western blotting, QRT-PCR, and statistical analysis were performed as previously described (Belova et al., 2022).

Results
After three days of unloading soleus muscle mass was significantly decreased in HS and HSM groups when compared with the control rats (C: 73,0 $\pm$ 6,0 mg; HS: 62,9$\pm$8,7 mg; HSM: 63,2$\pm$4,2 mg). Treatment with AR-C 118925XX attenuated the unloading-induced decline of muscle mass (HSA: 68,5$\pm$3 mg).

Unloading caused an increase in the ATP content in muscle (Figure 1A). Treatment with MRS2179 resulted in a significant decrease in the ATP content

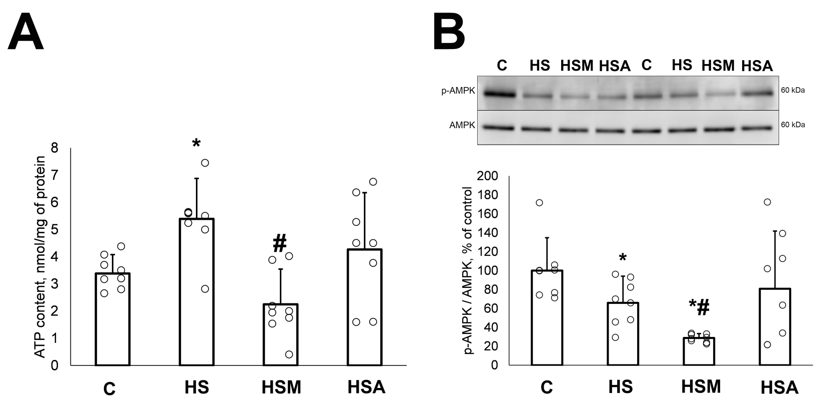

FIGURE 1

Evaluation of ATP (A) and phospho-AMPK (B) content in soleus muscles of control rats (C), rats with 3 days of unloading (HS), 3 days of HS with MRS2179 inhibitor (HSM), and 3 days of HS with AR-C 118925XX inhibitor (HSA). The ATP content in each sample was normalized to the level of total protein. The content of phospho-AMPK(Thr172) was normalized to the levels of total AMPK in each sample. N = 8. * indicates a significant difference from the control, # indicates a significant difference from the HS group, $p < 0.05$.

when compared HS group, while treatment with AR-C 118925XX completely prevented the increase in ATP content (Figure 1A).

Unloading caused a decrease in AMPK phosphorylation in soleus muscle (HS group; Figure 1B). Treatment with MRS2179 further decreased the phospho-AMPK content when compared with the level in the HS group (Figure 1B). Administration of AR-C 118925XX prevented the unloading-induced downregulation of phospho-AMPK content (Figure 1B).

Unloading increased the mRNA expression of MuRF1 (by 82%), MAFbx (by 151%), and ubiquitin (by 114%) in the HS group when compared with control muscle (Figures 2A, 2B and 2C). In rats treated with MRS2179 inhibitor, the decrease in MuRF1 mRNA expression in unloaded soleus muscle reached statistical significance, while MAFbx expression was not statistically

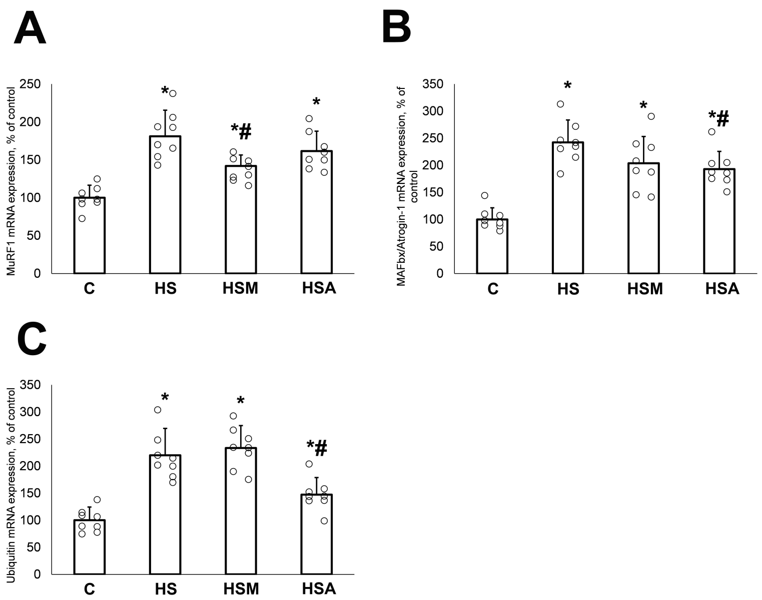

FIGURE 2

Evaluation of MuRF1 (A), MAFbx (B), and ubiquitin (C) mRNA expression in soleus muscles of control rats (C), rats with 3 days of unloading (HS), 3 days of HS with MRS2179 inhibitor (HSM), and 3 days of HS with AR-C 118925XX inhibitor (HSA). mRNA expression was normalized to the GAPDH mRNA expression in each sample. N = 8. * indicates a significant difference from the control, # indicates a significant difference from the HS group, $p < 0.05$.

different from the values in the HS group (HSM group; Figures 2A and 2B). In difference, in rats with AR-C 118925XX inhibitor administration, the decrease in MAFbx mRNA expression in unloaded muscle reached statistical significance, while MuRF1 expression was not statistically different from the values in HS group (HSA group; Figures 2A and 2B). Treatment with AR-C 118925XX inhibitor reduced the upregulation of ubiquitin mRNA expression in the unloaded soleus muscle when compared with the value in the HS group although it was still significantly higher than in the control muscle (Figure 2C).

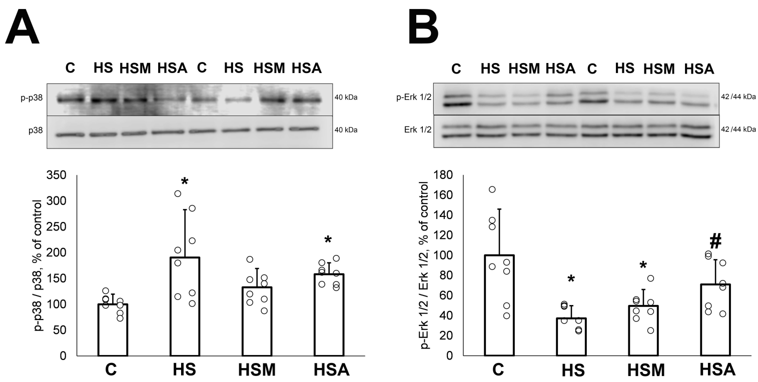

FIGURE 3

Evaluation of phospho-p38 (A) and phospho-Erk1/2 (B) in soleus muscles of control rats (C), rats with 3 days of unloading (HS), 3 days of HS with MRS2179 inhibitor (HSM), and 3 days of HS with AR-C 118925XX inhibitor (HSA). The content of phospho-p38(Thr180/Tyr182) and phospho-Erk1/2(Thr202/Tyr204) was normalized to the levels of total p38 and total Erk1/2. N = 8. * indicates a significant difference from the control, # indicates a significant difference from the HS group, $p < 0.05$.

Unloading increased the content of phospho-p38 (by 90%) and decreased the content of phospho-Erk1/2 (by 63%) in soleus muscle of the HS group (Figures 3A and 3B). Treatment with MRS2179 inhibitor prevented the unloading-induced increase of p38 phosphorylation (Figure 3A). The level of phospho-p38 in the HSM group was not different from the C group, while the level of phospho-p38 in the HSA group was 58% higher than in the control (Figure 3A). Administration of AR-C 118925XX inhibitor blocked the unloading-induced decrease of Erk1/2 phosphorylation (Figure 3B). At the same time, treatment with an MRS2179 inhibitor had no significant effect on the unloading-induced decrease of phospho-Erk1/2 (Figure 3B).

Discussion

The current study showed that muscle atrophy was attenuated after treatment with P2Y2 receptor inhibitor AR-C 118925XX. In P2Y2 knockout mice denervation-induced muscle atrophy is diminished when compared with the denervated muscle of wild-type mice (Chen et al., 2021). This correlates well with the data of the current study.

Increased accumulation of ATP in skeletal muscle during early stages of unloading is well described (Zaripova et al., 2021). In this study, ATP content was significantly upregulated in unloaded muscle without treatment. Treatment with either MRS2179 or AR-C 118925XX inhibitors diminished the increase of ATP content. This suggests that P2Y1 and P2Y2 receptors are critically important for the regulation of ATP levels in unloaded muscle.

AMPK is a key molecule regulating energy homeostasis (Vilchinskaya et al., 2018). The current study showed that phospho-AMPK level was downregulated in soleus muscle of the HS group. MRS2179 administration led to a more profound reduction in phospho-AMPK content, while treatment with AR-C 118925XX completely prevented this decline.

In this study, the mRNA expression of E3 ubiquitin ligases MuRF1 and MAFbx is increased in unloaded soleus muscle with and without P2Y receptor inhibitors. At the same time, the mRNA expression of MuRF1 in muscle of rats treated with MRS2179 was significantly lower than in HS group. Similarly, mRNA expression of MAFbx in unloaded muscle of rats treated with AR-C 118925XX was significantly lower than in HS group. Previous studies showed that the expression of both MuRF1 and MAFbx is increased after three days of unloading (Zaripova et al., 2021) and this can result in protein ubiquitination and muscle atrophy (Bodine, 2020). MAFbx is involved in the degradation of proteins that promote protein synthesis, while other muscle-degrading proteins (MuRF1, Trim32) catalyze ubiquitination and degradation of muscle structural proteins (Aweida and Cohen, 2021). The increased expression of ubiquitin mRNA was partially prevented only in the HSA group that also had preserved muscle mass. Protein ubiquitination is a marker for the subsequent protein degradation. Therefore, preservation of muscle mass in the HSA group could be associated with diminished ubiquitin mRNA expression.

Inhibition of phospho-38MAPK signaling during three days of unloading prevents muscle atrophy and unloading-induced upregulation of MuRF1 while showing no effect on the MAFbx (Belova et al., 2020). Treatment with MRS2179 diminished the content of unloading-induced phospho-p38MAPK. At the same time, in the soleus muscle of HS and HSA groups, phospho-p38MAPK content was still significantly higher than in the control muscle. Since the expression of MuRF1 was also decreased only in muscle of rats treated with MRS2179 this suggests the coordinated effect of the P2Y1 receptor inhibitor on phospho-p38MAPK content and the expression of MuRF1.

ERK1/2 phosphorylation was decreased in the HS group in the present study similar to previously reported data (Zaripova et al., 2021). Treatment with AR-C 118925XX blocked the unloading-induced decrease of phospho-ERK1/2, while treatment with P2Y1 receptor inhibitor MRS2179 had no effect. The expression of MAFbx was also decreased only in muscle of rats treated with AR-C 118925XX suggesting coordinated regulation of phospho-ERK1/2 content and MAFbx expression.

Conclusion

This study for the first time showed that treatment of rats with P2Y receptor inhibitors during three days of muscle unloading: 1) prevents the accumulation of ATP; 2) blocking of P2Y1 receptors prevents unloading-induced upregulation of phospho-p38MAPK and attenuates the increase of MuRF1 mRNA expression; 3) blocking of P2Y2 receptors attenuates muscle atrophy, an unloading-related decrease of phospho-ERK1/2, diminishes the increase in MAFbx and ubiquitin mRNA expression, and prevents the decrease of phospho-AMPK.

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Comparison of post-flight mission-critical tests between astronauts and bilateral vestibular patients

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Kuldavletova, O., Clément, G., Macaulay, T., Wood, S., Denise, P. Comparison of post-flight mission-critical tests between astronauts and bilateral vestibular patients.

Introduction

Astronauts returning from long-term missions report spatial disorientation and perceptual illusions; exhibit postural instability, impaired locomotion and manual control; and are affected by re-entry motion sickness and orthostatic intolerance (Moore et al., 2019; Mulavara et al., 2018; Reschke et al., 2017). These symptoms result from the deconditioning of the vestibular, somatosensory, muscular, and autonomic nervous systems by weightlessness. Before an adaptation takes place, astronauts' performance is impaired immediately after landing following spaceflight. These impairments occur during a critical part of the mission, because landing and egressing the spacecraft require taking control, making decisions, and acting rapidly and accurately. Functional, mission-critical tests such as tandem walk, sit-to-stand, and walk-and-turn have shown that astronauts' performance during these tests are altered for up to one week after landing following long-duration stays on board the International Space Station (Clément et al., 2022). On Earth, patients with bilateral vestibular loss (BVL) also exhibit spatial disorientation, postural instability, and gait disturbances. In these patients, the absence of vestibular input is compensated by a stronger reliance on visual and somatosensory inputs. Therefore, comparing the results of the astronauts and BVL patients can help to disentangle the role of the vestibular and somatosensory systems in the performance of these tests by astronauts.

Material and Methods

We administered three functional tasks: tandem walk (TW), Sit-to-Stand (StS) and Walk-and-Turn (W&T) in astronauts (TW and StS: n=22, 14M/8F, mean age 48.1; W&T: n = 11, 7M/4F; mean age 42.4) and BVL patients (n = 31, 14M/17F; mean age 61.4). WT was done with eyes closed and with eyes open and the percentage of correct steps was calculated based on video recordings of the performance. In StS test we evaluated the time to settle the posture after rapidly raising from a chair. In W&T subjects completed a small circuit with stepping over one obstacle and walking around a cone. We evaluated the time to complete the test and the peak angular velocity of walking around the cone. Astronauts were tested pre-flight, the day of the landing (R+0) and the day after (R+1).

Results

The time required for the BVL subjects to settle following StS was significantly longer than the astronauts' performance before flight; shorter than the astronauts' performance on R+0; and longer than the astronauts' performance on R+1 (Table 1). The time the BVL patients took to complete the W&T test not different from the astronauts' performance on R+1. The

TABLE 1: Per+formance (Mean ± SD) of astronauts preflight, at R+0, R+1 and BVL patients

	Astronauts			BVL
	Pre	R+0	R+1	
StS, time to settle, s	2.0 ± 0.5	5.0 ± 2.2	2.7 ± 1.0	3.4 ± 1.0
W&T, time to complete, s	9.2 ± 1.6	27.8 ± 12.0	13.6 ± 4.6	16.1 ± 7.3
W&T, turn rate, deg/ s	131.2 ± 21.7	60.5 ± 37.8	102.5 ± 29.7	73.4 ± 17.6
TW, eyes open, %	98.4 ± 7.6	40.8 ± 30.0	86.8 ± 20.1	44.8 ± 29.6
TW, eyes closed, %	76.1 ± 15.6	10.8 ± 11.8	27.7 ± 19.3	9.1 ± 20.7

turn rate in W&T test and the performance in TW both eyes open and closed of BVL patients was not different from the performance of astronauts R+0.

Discussion

All of the tasks degrade significantly after landing in astronauts. This degradation is multifactorial; however, regarding fast recovery, it is most likely to be due to neural adaptations. BVL patients take less time in StS and W&T tests. Somatosensation that is also deconditioned in astronauts might contribute to better performance of BVL patients in these tests. The turn rate in W&T test and the performance in TW both eyes open and closed are similar in astronauts R+0 and BVL patients. Improper functioning of the vestibular system might be the primary source of this deconditioning in astronauts.

Conclusion

Post-flight, astronauts show similar performance in functional tasks as BVL patients. TW and the rate of turn while walking around an obstacle are most sensitive to vestibular deconditioning.

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Examination of histone variant induction as a novel countermeasure for long term stay in space using mouse model

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Citation
Masuzawa, R., Kawano, F. Examination of histone variant induction as a novel countermeasure for long term stay in space using mouse model.

Introduction
Deep space exploration such as a manned mission to Mars will require a comprehensive medical care system to treat and maintain astronauts' health. The new generation of spacecraft that will be used for interplanetary missions limits a cargo capacity, which also restrict onboard exercise. In the future missions, gravitational unloading-induced loss of skeletal muscle mass and function will be more severe problem. Furthermore, in the case of Mars mission, crew is required to perform EVA tasks under partial gravity without the extensive rehabilitation support that was generally available after they return to Earth. Therefore, the development of new theory that prevents the loss of skeletal muscle mass and function is one of most urgent issues. Epigenetics is a current topic to understand the regulation of gene responsiveness to environmental stimuli. We previously reported using rats that tail suspension, a ground simulation model of spaceflight,

drastically decreased the distribution of histone variant H3.3 at the loci that were transcriptionally upregulated during disuse atrophy in skeletal muscle (Nakamura et al., 2017). Histone H3.3 is also known to accumulate in tissues during aging. Although incorporation of histone H3.3 into nucleosomes may affect the gene responsiveness via the conformational change of chromatin, the role of histone H3.3 in differentiated cells and organs is unclear. The purpose of the present study is to clarify the role of H3.3 in mouse skeletal muscle using aging and forced expression models.

Material and Methods

In Experiment 1, tibialis anterior muscles were sampled from male C57BL mice at 8, 32, 53, and 75 weeks of age. Histochemical properties of muscle fibers and the level of histones by western blotting were analyzed. RNA sequencing analysis was also performed to identify the genes up- or down-regulated by aging, and the genes were targeted to the chromatin immunoprecipitation (ChIP) analysis. In Experiment 2, young male C57BL mice (8-wk-old) were injected with adeno-associated virus vector expressing histone H3.3 under control of skeletal muscle-specific ACTA1 promoter or carrying non-cording sequence (stuffer). Motor coordination function was evaluated using a rotarod every 2 weeks until 32 weeks of age.

Results and Discussion

Experiment 1 showed that the tibialis anterior muscle weight relative to body weight significantly decreased after 53 weeks of age. Similar change was observed in the muscle fiber size. Myonuclear number was also decreased after 53 weeks of age. It was indicated that decline of tibialis anterior muscle was phenotypically detectable at middle age. Up- (n=15) and down- (n=14) regulated genes were selected by RNA sequencing. Interestingly, the expression of up-regulated genes gradually increased in association with age, although that of down-regulated genes significantly decreased at 32 weeks of age, suggesting that down-regulation of gene expression precedes age-related change in the transcriptome of skeletal muscle. The level of H3.3 relative to canonical variants H3.1/3.2 significantly increased in the tibialis anterior muscle during aging, although the increase of H3.3

level reached to peak at 53 weeks of age. Age-related H3.3 increase was correlated with H3K27me3 modification, not the other histone modifications. The histone distribution at the target loci analyzed by ChIP also showed the results similar to western blotting. It was suggested that transcriptionally repressive H3K27me3 accumulated in association with the increase of H3.3 incorporation. In Experiment 2, the mice expressing H3.3 showed longer latency to fall in the rotarod test compared to the stuffer group. These results suggest that H3.3 itself play a positive role in skeletal muscle function, although it is still unclear how H3.3 is modified by K27 trimethylation during aging.

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Preconditioning with mild hypergravity mitigates simulated microgravity-induced T cell dysfunctions

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Citation
Moser, D., Biere, K., Liemersdorf, C., Hemmersbach, R., Choukér, A. Preconditioning with mild hypergravity mitigates simulated microgravity-induced T cell dysfunctions.

Introduction

Microgravity (μ*g*) is considered to be among the main stressors which are responsible for emerging immune system dysregulations in space (Sonnenfeld, 2012; Luo et al., 2014). Monocytes for instance, show an increased pro-inflammatory potential (Buchheim et al., 2019). However, especially T cells have been shown to exhibit reduced activation capacities to antigenic and mitogenic stimuli both under real and stimulated µ*g* conditions (Chang et al., 2012). Artificial gravity (AG) encompasses both the restoration of Earth-like conditions in µ*g* and the induction of hypergravity in order to counteract µ*g*-induced deconditioning in space. Hypergravity was shown to have beneficial effects on the musculoskeletal and vascular system both as a countermeasure option for µ*g*-related deconditioning and as "gravitational therapy" on Earth (Tominari et al., 2019; Isasi et al., 2022). However, the impact on the human immune system, which can be severely affected by µ*g* was yet only sparsely explored. In the present study it was tested in a short-time *in vitro* approach, if an application of "mild" hypergravity (2.8*g*) is capable to counteract simulated (s-)µ*g*-induced dysregulations in monocytes and T cells.

Material and Methods

Activation capacities of monocytes and T cells were tested by an *ex vivo* whole blood incubation assay. For this, lithium-heparin anticoagulated whole blood of eight volunteers was diluted with an equal volume of RPMI 1640 cell culture medium containing the immunogenic antigens heat-killed *Listeria monocytogenes* (HKLM) or Pokeweed mitogen (PWM) and incubated for six hours at 37 °C. To elaborate the single impact of different *g*-grades, stimulation was performed either in 1*g*, simulated µ*g* (s-µ*g*) by clinostat

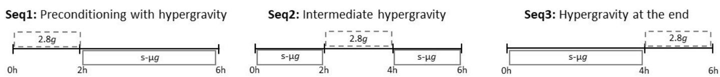

FIGURE 1
Different sequences to test the capability of hypergravity (2.8*g*) as a countermeasure option for s-µ*g* induced immune dysregulations. Seq1) preconditioning by application of hypergravity prior to s-µ*g*; Therapeutic action by application of hypergravity intermediately (Seq2) or at the end (Seq3) of s-µ*g* (from Moser et al., 2023).

rotation, or mild hypergravity of 2.8*g*. For testing potential gravitational countermeasure approaches, three different treatment sequences were designed: one preconditioning setting, where hypergravity (two hours) was applied before s-μ*g* exposure (four hours) and two therapeutic approaches in which hypergravity was set either intermediately or at the end of s-μ*g* (**Fig. 1**). Alterations in immune responses were determined by flow-cytometric assessment of surface activation marker expression on monocytes and T cells and quantification of secreted cytokines.

Results

In monocytes single *g*-grade exposure experiments revealed increased concentrations of the pro-inflammatory cytokines IL-1β and TNF when antigen stimulation was performed in s-μ*g* and an attenuated cytokine secretion after antigen-stimulation in hypergravity, compared to 1*g* controls (**Fig. 2**).

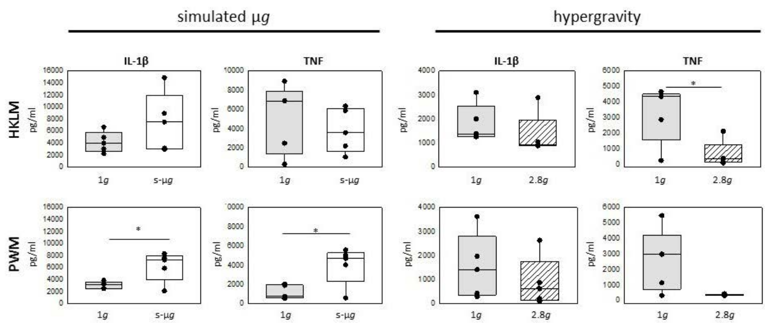

FIGURE 2
Concentrations (pg/ml) of the cytokines IL-1β and TNF after six hours incubation with HKLM or PWM in s-μ*g* (white boxes) or hypergravity (2.8*g*) (dashed boxes) compared to 1*g* controls (grey boxes). Boxes indicate median concentrations and interquartile range; whiskers represent minimum and maximum, dots represent single values (n=5). Differences between groups were calculated using unpaired two-tailed t test or Mann–Whitney *U* test, *p < 0.05 (from Moser et al., 2023).

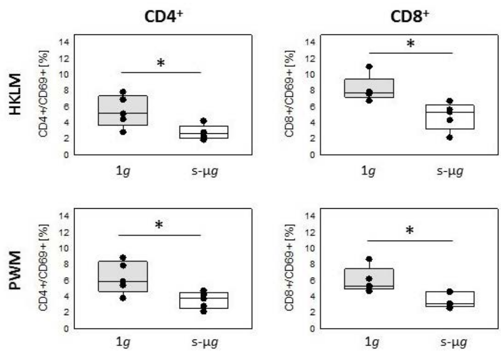

FIGURE 3
CD69 expression levels on the cell surfaces of $CD4^+$ and $CD8^+$ T cells after six hours stimulation with HKLM or PWM in 1*g* (co., grey boxes) and s-µ*g* (white boxes). Values represent percentages of $CD69^+$ cells among total $CD4^+$ or $CD8^+$ T cells. Boxes indicate median and interquartile range; whiskers represent minimum and maximum, dots represent single values (n=5). Differences between groups were calculated using unpaired two-tailed t test, *$p < 0.05$ (from Moser et al., 2023).

Both $CD4^+$ and $CD8^+$ T cells displayed reduced surface expression of the activation marker CD69 as well as IFNγ secretion in response to HKLM and PWM incubation in s-µ*g*, compared to the 1*g* control (**Fig. 3**).

All three countermeasure approaches applying hypergravity (**see Fig. 1**) did not alleviate the enhanced pro-inflammatory potential in monocytes. However, both in $CD4^+$ and $CD8^+$ T cells, the preconditioning approach (Seq1), in which the whole-blood-incubation samples were exposed to hypergravity before the s-µ*g* phase, restored antigen-induced CD69 expression and IFNγ secretion to levels similar to 1*g* controls (**Fig. 4**).

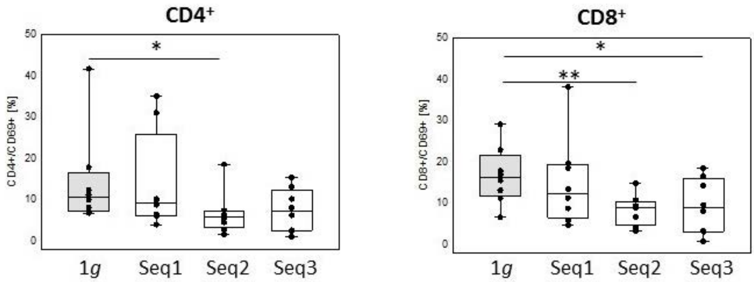

FIGURE 4
CD69 expression on the cell surface of CD4$^+$ and CD8$^+$ T cells after PWM incubation in s-μ*g* (white boxes) and hypergravity preconditioning (Seq1), therapeutic intermediate hypergravity (Seq2) or at the end of s-μ*g* (Seq3) and 1*g* control (grey box). Values represent percentages of CD69$^+$ cells among total CD4$^+$ or CD8$^+$ T cells. Boxes indicate median and interquartile range; whiskers represent minimum and maximum, dots represent single values (n = 8). Differences between groups were calculated using unpaired two-tailed t test or Mann–Whitney *U* test, *p < 0.05, **p < 0.01 (from Moser et al., 2023).

Discussion

A μ*g*-induced disruption of proper immune cell functionality represents a serious challenge to overcome for (long-term) spaceflights. Artificial gravity (AG) can be applied either to restore 1*g* conditions or to induce hypergravity by mechanical loading. Hypergravity was already demonstrated to reverse detrimental μ*g*-induced effects, such as bone and muscle loss in mice (Tominari et al., (2019)) and to even serve as a therapeutic option for vascular-based pathologies on Earth (Isasi et al., (2022)).

The underlying molecular mechanisms for altered immune cell functions have not been elucidated here. However, since gravitational alterations are known to induce structural changes of the cytoskeleton, these restructuring effects as well the associated impact on signal transduction pathways and cytokine production shall be investigated in distinct immune cell subsets in the future.

The present investigations have shown that an application of mild hypergravity of 2.8*g*, irrespective at which phase of s-μ*g* exposure doesn´t mitigate the enhanced pro-inflammatory cytokine secretion of monocytes in s-μ*g*. However, two hours preconditioning with mild hypergravity prevents loss of T cell activation capacities which can be observed after exposure to s-μ*g* (4 hours).

Thus, an application of preconditioning hypergravity should be considered as a potential preventive approach, not only for proper immune functions, but also to counteract musculoskeletal and cardiovascular deconditioning in space. Moreover, a hypergravity-inducted restoration of impaired T cell functions might have a positive effect for immune-depressed patients.

Conclusion

This *in vitro* study demonstrates a proof of concept that mild hypergravity represents a gravitational preconditioning option to avoid T cell dysfunctions which are induced by s-μ*g*.

Future approaches will focus on mechanistic insights as well as long-term effects and a more detailed resolution of the duration of hypergravity application.

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Is torpor slumbering in all of us? - A comparative cross-species analysis

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Moser, D. Is torpor slumbering in all of us? - A comparative cross-species analysis.

Torpor is a physiological state characterized by inactivity and an endogenous decrease of metabolic rate, which is followed by reduction of body temperature, heart rate and breath rate. A multi-day torpor is called hibernation. This state of metabolic depression enables mammals of diverse species and orders to overcome periods of harsh seasonal environmental conditions, such as cold temperatures or food scarcity. The ability of performing torpor is spread over a variety of different mammalian orders, however, torpor characteristics can differ strongly within the different species. Nevertheless, this suggests a common genetic program in all mammals which consequently might be also anchored in the human genome, which is underpinned by several analogies in adult and very young humans. For instance, every human experiences diurnal temperature changes in the range of 0.8 Kelvin with a decline of body temperature during sleep and in deep meditation, Yogi are able to suppress arbitrarily their metabolic rate by ~40%. Another set of evidence was provided by Bartsiokas and Arsuaga, who showed recently signs for human hibernation in hominins in Atapuerca (Spain), a half a million years ago (Bartsiokas and Arsuaga, 2020)). Moreover reports on expeditioners exist, who survived very unfavourable accidental conditions by downregulated body functions, which further supports the probability of human torpor. Newborn humans show pronounced similarities to torpid animals, such as a very low metabolic rate *in utero* or the chemical thermogenesis in brown adipose tissue.

As one of the first steps for shedding light on this putative common genetic torpor-program in mammals, transcriptomic analyses were performed on blood of the Djungarian hamster (*P. sungorus*), which is a model animal for daily torpor. A cross-species approach, encompassing a comparative literature research revealed that relevantly regulated genes at torpor nadir (deep torpor) in Djungarian hamsters show high similarities with gene expression regulations in other mammals during torpor/hibernation. This was shown to be independent of mammalian species and order, the organ analyzed, and the mode of torpor/hibernation of the respective animal.

Further analyses to decipher the key players in induction and execution of torpor are ongoing. A targeted induction of an endogenous hypometabolic state or achieving a torpid state in humans would have a game-changing character in various medical fields, such as transplantation medicine, in rehabilitation programs and in severe catabolic disorders. However, especially in space flight, research on torpor is of great relevance. Putting long-term space expeditioners into a torpid state would reduce spacecraft load as requirements for food and oxygen supply would be reduced. Beyond that detrimental effect of spaceflight on human health could be reduced, such as mental stress, musculoskeletal deconditioning and radiation damage. Thus human torpor/hibernation has –from an physiological point of view- the potential to enable human long-term exploration missions in the future, such as to Mars (Choukér et al., 2021).

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Duration judgment in astronauts and patients with idiopathic bilateral vestibular loss

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Citation
Morales, D.C.N., Kuldavletova, O., Quarck, G., Denise, P., Clément, G. Duration judgment in astronauts and patients with idiopathic bilateral vestibular loss.

Introduction
Time perception is critical during spaceflight activities such as docking, extra-vehicular activities, and landing. There is evidence that time perception is impaired in weightlessness. During parabolic flight, hypergravity and microgravity has been shown to affect temporal reproductions in dual tasks (Clément, 2018). During spaceflight, astronauts have higher rates and variability in motor timing (Semjen et al., 1998a, 1998b), and under-produce durations of one minute and intervals ranging from 2 to 38 s (Kuldavletova et al., 2023; Navarro Morales et al., 2023).

The vestibular system plays an important role in our perception of time. Previous studies have shown that during vestibular stimulation, subjective time contracts (Utegaliyev et al., 2022). Optokinetic stimulation, which interact with the vestibular system, causes an over-estimation of time (Vicario et al., 2007). Whole-body passive rotations affect the timing of sensory input

(Binetti et al., 2013) and motor timing (Binetti et al., 2010; Capelli & Israël, 2007). In addition, the perception of rotation duration is altered in patients with unilateral vestibular loss (Kwon et al., 2022).

We hypothesized that the misperception of time in astronauts is caused by the absence of constant otolith input in the vestibular system in weightlessness. According to this hypothesis, vestibular patients on Earth should exhibit alterations in time perception similar to those of astronauts during spaceflight. To evaluate this hypothesis, we tested two temporal tasks in astronauts before, during, and after spaceflight and compared their responses with those of patients with bilateral vestibular loss (BVL).

Materials and Methods

Participants

Ten crewmembers (9 males, 1 female; 44.1 $\pm$ 4.6 years old) were tested on board the International Space Station (ISS) and during pre- and postflight sessions in the Neuroscience Laboratory of NASA Johnson Space Center. The test procedures were approved by the European Space Agency Medical Board and NASA Johnson Space Center Institutional Review Board.

Thirty patients with idiopathic bilateral vestibular loss (13 males, 17 females; 60.6 $\pm$ 13.0 years old) were tested in the COMETE Laboratory at the University of Caen. The procedures were approved by the French Ethical Committee. Patients were diagnosed according to the criteria of the classification committee of the Barany society (Strupp et al., 2017).

Experimental Protocol

Participants wore a virtual reality headset (Oculus Rift, Oculus VR, Menlo Park, CA) and used a trackball. On the ISS, astronauts were free-floating; in the lab, all participants were seated upright. Participants were asked to produce a duration in a single task or a dual task. During the single task, participants were presented with an instruction on the screen with a specific duration. After clicking on "Start", a blue square appeared. Participants counted aloud for target durations ranging from 2 to 38 s. They clicked on the response panel when they felt the duration had elapsed. During the dual task, instead of counting aloud, participants were asked to read aloud numbers that were presented on the blue square while simultaneously estimating the target duration (Kuldavletova et al., 2023).

Study Schedule

The pre-flight test sessions with the astronauts occurred at launch minus (L-) 205 ± 51 days, L-149 ± 55 days, and L-116 ± 45 days. In-flight sessions were conducted on flight day (FD) FD17 ± 6, FD46 ± 8, FD71 ± 6, FD99 ± 7, FD134 ± 8, and FD164 ± 7. The post-flight session took place on the day after landing.

Statistical Analysis

We compared the error percentages in duration judgements between BVL patients and astronauts in each flight phase. There were no statistical differences between the responses during the 3 preflight tests and between the responses to the 7 in-flight tests, so there were pooled together. We conducted a permutation t-test with 5000 repetitions in R (R Core Team, 2022) using MKinfer package for permutation analysis (Kohl, 2023). The p-value were subsequently adjusted with FDR correction.

Results

In the astronauts, the errors in duration judgment during the single task were 5.83 ± 1.38%, -1.15 ± 1.02% and 0.46 ± 1.89% before, during, and after flight, respectively. The error in duration judgment of the BVL patients (1.67 ± 18.95%) was not significantly different from those of astronauts in any flight phase (Figure 1).

In the astronauts, the errors in duration judgment during the dual task were 20.90 ± 3.33%, 7.93± 4.66% and 17.33 ± 8.59% before, during, and after flight, respectively. The error in duration judgment of the BVL patients (0.35 ± 22.79 %) was significantly smaller than those of astronauts (Figure 2).

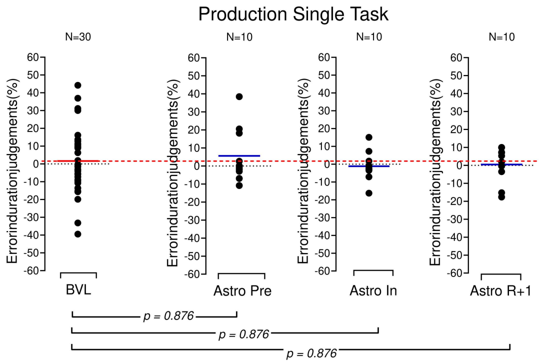

FIGURE 1

Individual errors in duration judgments during the production single task. Red bar: mean of patients with bilateral vestibular loss (BVL); blue bar: mean of astronauts before (Astro Pre), during (Astro in), and after spaceflight (Astro R+1). * $p < 0.05$.

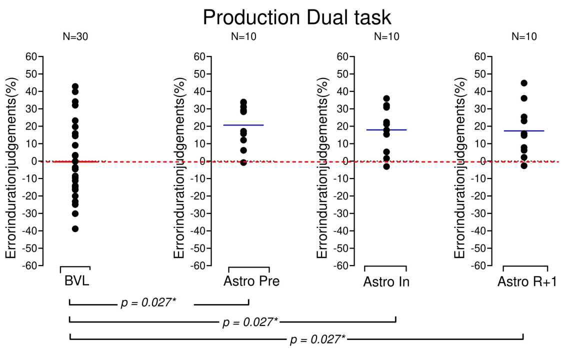

FIGURE 2

Individual errors in duration judgments during the production dual task. Red bar: mean of patients with bilateral vestibular loss (BVL); blue bar: mean of astronauts before (Astro Pre), during (Astro in), and after spaceflight (Astro R+1). * $p < 0.05$.

Discussion

Precision in Temporal Estimations

Vestibular patients made larger errors in duration judgments than astronauts in both production single and dual tasks. Age only partially explains this difference, since temporal impairments occur in subjects older than 75 years (Turgeon et al., 2016) and most our participants were younger. Because some brain structures (striatum, cerebellum) involved in time perception receive vestibular inputs (Dallal et al., 2015) no impairments in the accuracy or precision of duration reproduction using 20-s and 80-s peak-interval procedures were observed when both target durations were associated with the same lever response, but distinguished by signal modality (e.g., light or sound, we might infer that vestibular loss causes an irregular internal clock (Gibbon et al 1984). Another explanation would be variability as result of general cognitive deficits in BVL patients (Bigelow & Agrawal, 2015).

Accuracy in Temporal Estimations

Vestibular patients were quite accurate in their duration judgments. We expected that their perceived durations in the dual task would be over-estimated. Indeed, when less attention is attributed to time perception, we generally over-produce durations (Zakay & Block, 1996). This is what is observed in astronauts in their duration judgment in single task compared to dual task. It is known that vestibular patients have difficulties to perform dual tasks (Bigelow & Agrawal, 2015). Because difficulty leads to under-production of duration (Brown, 1997), the interplay of attention and difficulty might cancel each other out. Hence, results are the same in BVL patients' single and dual tasks. These results support the involvement of general cognitive deficits in vestibular patients.

Acceleration of Internal Clock

In a previous study, we observed that astronauts over-estimated the duration of one-minute (Navarro Morales et al., 2023). We hypothesized that the lack of static otolith signals in weightlessness might accelerate the internal clock (Kuldavletova et al., 2023; Semjen et al., 1998a). In the present study, the duration judgments of BVL patients during the single task were not different from those of astronauts. During the dual task, the duration judgments of BVL patients were smaller than those of the astronauts. This difference might be a clue for an acceleration of internal clock. However, due to significant cognitive participation in both tasks, it is impossible to elucidate this question.

Conclusion

Duration judgments are impaired in both astronauts during spaceflight and vestibular patients on Earth. This testifies a role of the vestibular system in time perception. Cognitive demands in temporal tasks are critical, and appear to impact more vestibular patients than astronauts.

Acknowledgments

The authors thank the *Association Française de Vestibulopathie Bilatérale Idiopathique* for recruiting the vestibular patients. The authors are also grateful to Emma Milot for her help in collecting data on the vestibular patients. The authors are grateful to François Derache, Timothy Macaulay, Maurice Marnat, and Scott Wood for their help in collecting data on the astronauts.

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Chronic increase in renin-angiotensin-aldosterone activity at steady state of microgravity simulated by dry immersion: Why and by which mechanisms?

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Citation
Navasiolava, N., Robin, A., Gharib, C., Custaud, M-A. Chronic increase in renin-angiotensin-aldosterone activity at steady state of microgravity simulated by dry immersion: Why and by which mechanisms.

Introduction

Renin-angiotensin-aldosterone axis plays a major role in regulating sodium and potassium balance as well as arterial blood pressure. When exposed to actual or simulated microgravity, the immediate response of the renin-angiotensin-aldosterone system (RAAS) is a suppression of plasma renin activity and plasma aldosterone due to relative central hypervolemia (Leach Huntoon *et al.,* 1998). However, in a chronic and stable state of microgravity, an increase in RAAS activity is often reported (Olde Engberink *et al.,* 2023; Drummer *et al.,* 2001; Sigaudo *et al.,* 1998; Custaud *et al.,* 2005), although the reasons and physiological role of this chronic increase are not entirely clear.

Material and Methods
We analyze data of our team on six dry immersion (DI) studies of different durations (3, 5, 7 days) without countermeasures, performed with the participation of healthy young volunteers at the Institute of Biomedical Problems (IBMP, Moscow, Russia) and at the Institute of Space Physiology and Medicine (MEDES, Toulouse, France) in 2006-2022:

- With authorized short daily raise (non-strict immersion):
    - 7-d DI performed at IBMP in 2006-2007 (n=8 males, 23±1 yrs, mean±SD) (Navasiolava *et al.,* 2011)
    - 5-d DI performed at IBMP in 2010 (n=14 males, 22±3 yrs) (Coupe *et al.,* 2013)
- Without periods of sitting or standing (strict immersion):
    - 3-d DI performed at MEDES in 2015 (n=12 males, 32±5 yrs) (De Abreu *et al.,* 2017)
    - 5-d DI performed at MEDES in 2018-2019 (control group, n=9 males, 33±7 yrs) (Robin *et al.,* 2020)
    - 5-d DI performed at MEDES in 2021 (n=18 females, 29±5 yrs) (Robin *et al.,* 2023)
    - 5-d DI performed at MEDES in 2022 (n=19 males, 28±4 yrs)

Water consumption was *ad libitum* in frames of 35-60 ml/kg/d for all protocols, except for 7-d DI with constant water consumption set at 50 ml/kg/d throughout the study - at baseline (B), during DI (D1-D7) and at recovery (R).

Blood sampling for renin and aldosterone assessment and hemoglobin and hematocrit determination was performed supine in bed in the morning before rising - before and after DI, and supine in bath - during DI. Plasma volume evolution was estimated by Hb-Hct Dill & Costill formula. Urinary sodium and potassium were assessed from 24-h urines. Water and sodium balances were determined as a difference between 24-h intake and urinary excretion (partial balances).

Results and Discussion
Data suggest that under DI, after initial drop, blood renin and aldosterone increase (Figure 1A, B).

Possible reasons and mechanisms for this sodium-retaining endocrine system activation can be considered:

- Reduction in intravascular volume (Figure 1C), which could be detected by stretch receptors of the aortic arch/carotid sinuses or by low-pressure baroreceptors of the vascular system.

- Increase of sympathetic activity might occur, e.g. in relation with baroreflex control. However urinary catecholamines are not systematically increased during microgravity (Olde Engberink *et al.,* 2023; Sigaudo et al., 1998; Coupe et al., 2013).

- Decrease in daily sodium supply cannot be completely excluded (Figure 1D), and it is difficult to estimate exactly the usual sodium consumption before protocols. However the efforts are made to keep it the same during the protocols - at Pre, During, and Post phases.

- Change in sodium regulation with a decrease in urinary sodium-to-potassium ratio (Figure 1E).

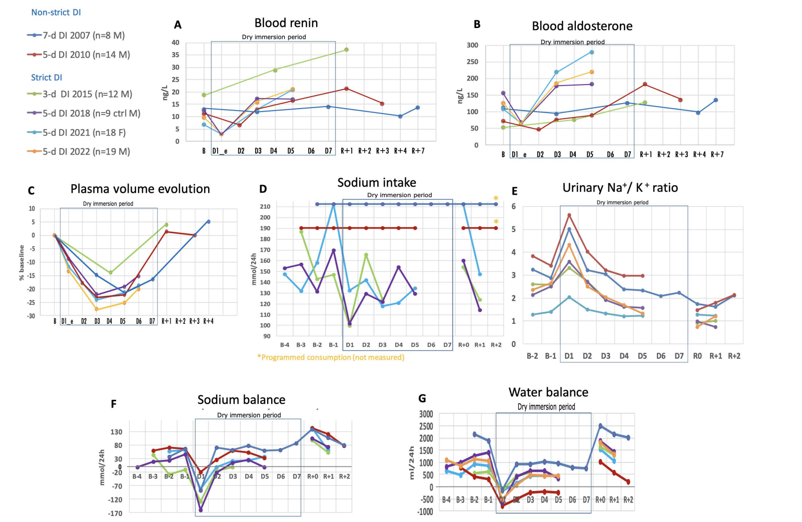

FIGURE 1
Blood renin (A) and aldosterone (B), plasma volume evolution (C), sodium intake (D), urinary sodium-to-potassium ratio (E), sodium balance (F) and water balance (G) during dry immersion (DI) studies of different duration. Data are presented as mean values for each DI protocol. B-baseline, D-immersion days, R – recovery, frame – dry immersion period. Blood sampling timepoint D1_e (evening) - about 8h after the onset of DI, R+1 – about 22h after the end of DI.

- Sodium retention in osmotically inactive form might occur in microgravity (Olde Engberink *et al.*, 2023, Drummer *et al.*, 2001), though location and reason for this retention are unclear. However our data on partial sodium balance show no evidence of sodium retention (Figure 1F), positive sodium balance seems maintained. Moreover, partial water balance decreases under immersion, however is also maintained positive (Figure 1G). To settle the question of sodium retention, insensible sodium and water losses should be taken in account.

- Reduction in renal perfusion pressure leading to excessive RAAS activation or changes in glomerulo-tubular balance. However 24-h blood pressure is not systematically seriously changed during dry immersion (Robin *et al.*, 2023).

Conclusion

The increased RAAS activity may indicate an increased physiological cost of maintaining a positive sodium balance. Furthermore, it could underlie vascular wall growth seen in microgravity as increased intima-media thickness, and thus participate in microgravity-induced vascular alterations.

Acknowledgments

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Dental care in space as an interdisciplinary procedure

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Citation
Neefs, D., Kijak, E., Singhal, I., Sampson, V., Kulesa-Mrowieck, M. Dental care in space as an interdisciplinary procedure.

Introduction

Space exploration and long-duration missions pose various challenges for astronauts, including the maintenance of their oral health and temporomadibular area. Dental care in space requires an interdisciplinary approach that integrates the expertise of dentists, physicians, nutritionists, and engineers. Addressing the unique challenges presented by the microgravity environment is vital to ensuring astronauts maintain good oral health and temporomadibular area, which is essential for their overall well-being and mission success. [1]

Material and Methods

The current short review examined 15 papers focusing on noninvasive studies of microgravity changes, oral health, human muscle tone of the temporomandibular area, interdisciplinary treatment, preventive dentistry in a long term space exploration and long-duration missions.

Microgravity Challenges

Space conditions present several obstacles to maintaining oral health. The absence of gravity in a microgravity environment affects saliva flow, making it difficult to effectively cleanse the mouth and remove debris and bacteria. Astronauts may also face difficulties in swallowing, which can contribute to oral hygiene issues. Furthermore, the limited space and weight restrictions on dental equipment necessitate innovation and the development of compact and lightweight solutions.

The Interdisciplinary Team

An interdisciplinary team is indispensable for effectively addressing these challenges. Dentists contribute their specialized knowledge in oral health, dental treatments, and diagnostics. Physicians collaborate closely with dentists to manage oral health issues and provide necessary medical guidance. Nutritionists play a crucial role in designing astronauts' diets, ensuring optimal nutrition for oral health. Physiotherapists contribute prophylaxis against temporomadibular disorders and spinal disfunctions. [2] Engineers contribute by developing innovative dental equipment that meets the unique requirements of space missions. Many specialist are responsible for dental care during preparations before long term spacemission (Fig. 1).

Interdisciplinary Strategies should take into account the leading directions for providing dental medical care during long-term space missions (Fig. 2).

Nutrition and Diet

Nutritionists work closely with astronauts to design diets that promote oral health. This involves selecting foods that are nutritious, easy to consume,

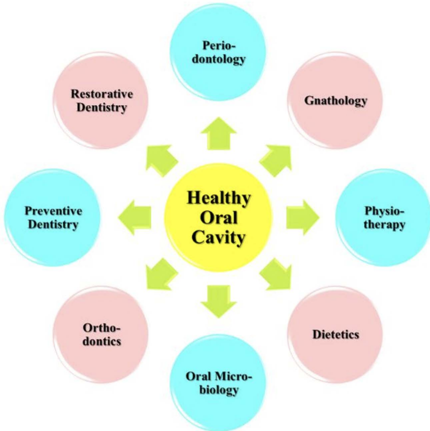

FIGURE 1
The interdisciplinary team in prophylaxis dentistry before long term spacemission.

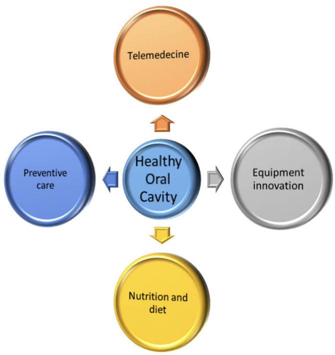

FIGURE 2
The leading directions for providing dental medical care during long-term space missions.

and minimize the risk of dental problems. Proper nutrition plays a vital role in maintaining strong teeth and gums while reducing the likelihood of tooth decay and gum disease [1,3].

Preventive Care
Emphasizing preventive measures is paramount in space. Astronauts receive education on proper oral hygiene practices, including regular brushing, flossing, and rinsing. Remote dental check-ups and analysis, including monitoring the oral microbiome and other biomarkers, are scheduled to identify and address early signs of dental issues before they escalate. [4]

Equipment Innovation
Engineers collaborate with dental professionals to develop compact and lightweight dental equipment suitable for space missions. These devices must meet stringent safety requirements, be easy to handle, and perform effectively in the microgravity environment. [4]

Telemedicine
Telecommunication technology enables remote dental consultations, allowing dentists on Earth to diagnose and guide treatments for astronauts in space. This approach minimizes the need for physical presence while ensuring timely and accurate dental care. [4,5]

Discussion
Many studies reporting physio-pathological changes deriving from aviation especially bone changes in bone tissues and oral microbiome.

The effect of microgravity in changes in bone mineral density are evident in weight-bearing bones. Bone mineral density (BMD) decreases in the lower extremities, but it tends to increase in the skull. (Bohra et al. 2019; Man et al. 2022). [2,6]

Nagaraj et al.2018 point out dental conditions, such as dental abscesses, periodontitis, deep carious lesions, as a oral health problem often resulting in severe pain. [7-9]

Studies by Schneider et al. (2015) [10] showed a significant decrease in tone in electromyographic (EMG) activity and stiffness of m. erector spinae, m. gastrocnemius, and tendo Achillis immediately after transition to 0G. De Abreu et al., 2017, and Demangel et al., 2017 also showed alterations in muscle viscoelasticity. Global leg muscle tone, immediately decreased by ~8-10% under immersion. What is important for temporomandibular area the tone of superficial muscles of the neck and upper trunk (m. trapezius and m. splenius) was unmodified, but six hours following the end of dry immersion, muscle tone was completely restored, except for m. masseter which tone unchanged, but increased at recovery, probably as the result of post-immersion postural disturbance [4,7].

Due to many changes connected with postural disturbance in space subsequent emergencies related to microgravity pointed by Pihut M. & Kulesa-Mrowiecka M. (2023) like myofascial pain syndrome, traumatic and inflammatory states of the temporomandibular joints, subluxation, and the consequences of intense occlusive parafunctions should be discussed in dental prophylaxis for astronauts. [9,11-15]

Conclusion

Dental care in space necessitates an interdisciplinary approach to tackle the unique challenges presented by the microgravity environment. By harnessing the expertise of dentists, physicians, physiotherapists, nutritionists, and engineers, astronauts' oral health can be effectively managed during long-duration space missions. Through the implementation of telemedicine, preventive care, tailored nutrition, and equipment innovation, the interdisciplinary team ensures that astronauts receive the necessary dental care for their well-being and mission success. As humanity continues to explore the frontiers of space, interdisciplinary collaboration will play a pivotal role in maintaining astronauts' oral health and overall physical and mental well-being.

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Psychological adaptation during a 3-weeks confinement in a space-like environment

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Introduction
Space missions are unique conditions that can deeply influence human psychology. To replicate and study these impacts, researchers often use 'analog environments' such as isolations. These are controlled environments that simulate multiple psychological stressors found in space, such as communication delays, isolation, psycho-social stress... (Kanas, 2015). When these stressors are combined and prolonged, they can modify the psychological adaptation of the individuals (Nicolas et al., 2021). Psychological adaptation involves multiple dimensions such as perceived stress, affective states, sleep quality, social functioning, or cognitive processing. Monitoring such diverse factors in space-like contexts is important as they are related to health, performance, and well-being of individuals.

In space-analog isolations, the research on psychological adaptation present varied findings. Studies about affective states and perceived stress are equivocal. Multiple reviews show that psychological responses to space-like environments can range from negative to neutral to positive, with a high variability between the studies and a lack of consensus (Alfano et al., 2018; Palinkas & Suedfeld, 2021).

Similarly, cognitive research in isolations shows contrasted results (Strangman et al., 2014). The impacts of these environments on cognition are unclear. There is also a difference between subjective experiences, which often refer to 'space fog,' and actual cognitive functioning. While people may report cognitive difficulties, these reports do not always correspond to measured performance.

Many studies show that sleep is often disturbed by space-like conditions. Issues like reduced sleep quality or duration, increased fatigue, or disrupted sleep cycles are common (Basner et al., 2013).

Finally, being in space-like contexts can change social dynamics. There can be more interpersonal tensions, loss of cohesion, subgrouping... (Landon et al., 2018).

The impacts of space isolations on psychological adaptation are not constant throughout the missions. There are particular phases such as the beginning of isolation, which tends to be particularly challenging as participants adapt to an unfamiliar environment and new stressors (Dunn Rosenberg et al., 2022).

There are a limited number of studies in space conditions that consider simultaneously a wide range of psychological adaptation dimensions. To our knowledge, there is no research that uses such a global perspective to describe the psychological adaptation to the beginning of a mission.

In our research we proposed to study the time-course of several factors that can account for psychological adaptation during a 3-week space-analog isolation. We expected modifications at the beginning of the mission because of adaptation processes. We considered multiple dimensions of psychological adaptation, such as perceived stress, affective states, social functioning, sleep quality and cognitive functioning.

Material and Methods

Our research took place at the Mars Desert Research station (MDRS) in the desert of Utah, United States, in 2021. Two groups were successively isolated for 21 days in a multi-compartment facility replicating a Martian station. This environment simulated multiple aspects of space conditions such as isolation, living and working conditions, monotony, Mars analog landscape, social deprivation, and Extra-vehicular activities.

The participants were students from the Superior Institute of Aeronautics and Space, in France. The first crew involved six subjects (4 males/2 females), with a mean age of 22,5 (+/- 1,05). The second crew involved seven subjects (2 males/5 females), with a mean age of 22,3 (+/- 1,25).

Our study involved multiple psychometric tools:

- the Isolated and Confined Environments Questionnaire (ICE-Q; Nicolas et al., 2019) that investigates how individuals adapt to space-analog conditions across emotional, occupational, physical, and social subscales;

- the Stress Overload Scale (SOS; Amirkhan, 2012) which assesses the perceived stress of the subject (two subscales : environmental load (EL) and personal vulnerability (PV));

- the State-Trait Anxiety Inventory (STAI-YA; Spielberger, 2010) to quantify anxiety states;

- the Profile Of Mood States (POMS; Cayrou et al., 2003) that provides an overview of the current mood state;

- the Self-Assessment Manikin (SAM; Bradley & Lang, 1994) that evaluates the valence and arousal levels of affective states;

- the Pittsburgh Sleep Quality Index (PSQI; Ait-Aoudia et al., 2013) to assess the quality of sleep.

Our protocol also included computerized cognitive tasks from the 'Test of Attentional Performance' battery (TAP; Zimmerman & Fimm, 1994). These tasks evaluated attentional (Alertness, Divided attention, Sustained attention) and executive functions (Flexibility, Incompatibility/Inhibition, Working memory/Updating). The dimensions of psychological adaptation and their related parameters are detailed in Table 1.

The experimental procedure was carried out 5 times during the mission (Mission Day (MD) 3, MD7, MD11, MD15, MD19). The protocol lasted approximately 1h30. First, the participants had to complete the psychometric tools. Then, they had to perform the cognitive tasks.

We used a Friedman statistical test to study the effect of time on our data.

Results

All the results from our study are detailed in Table 2, Figure 1, Figure 2 and Figure 3 for psychometric measurements and Table 3, Figure 4 and Figure 5 for cognitive measurements.

We observed significant time effects for the three scales of the SOS (PV: $X^2(4) = 20.664$, $p < 0.001$***; EL: $X^2(4) = 14.379$, $p = 0.006$**; Total: $X^2(4) = 22.924$, $p < 0.001$***), which appeared to decrease across time. A time effect was also present for the occupational dimension of the ICE-Q ($X^2(4) = 11.649$, $p = 0.020$*) which increased, while the emotional dimension significantly decreased throughout the isolation ($X^2(4) = 11.646$, $p = 0.020$*). Three POMS scales showed time effects and decreased through time: Tension/Anxiety ($X^2(4) = 13.234$, $p = 0.010$*); Depression/Dejection ($X^2(4) = 12.816$, $p = 0.012$*); and Anger/Hostility ($X^2(4) = 15.598$, $p = 0.004$**).

Multiple cognitive parameters showed time effects. For the Flexibility task, the median of reaction times (RT) significantly decreased through time ($X^2(4) = 16.333$, $p = 0.003$**) while the total performance index increased ($X^2(4) = 11.271$, $p = 0.024$*). For the Alertness task, we observed a significant time effect for the RT on the intrinsic alertness condition ($X^2(4) = 15.734$, $p = 0.003$**) and

TABLE 1: Dimensions of psychological adaptation and their related parameters. SOS = Stress Overload Scale; ICE-Q = Isolated and Confined Environments Questionnaire; POMS = Profile Of Mood States; SAM = Self-Assessment Manikin; STAI = State-Trait Anxiety Inventory; PSQI = Pittsburgh Sleep Quality Index; RT = Reaction Time; SD = Standard Deviation; ms = Milliseconds

Psychological adaptation dimensions	Measured parameters
Perceived stress	*SOS – Personal vulnerability*
	SOS – Event Load
	SOS – Total
	ICE-Q – Occupational dimension
Affective states	*POMS – Tension/Anxiety*
	POMS – Depression/Dejection
	POMS – Anger/Hostility
	POMS – Vigor/Activity
	POMS – Fatigue/Inertia
	POMS – Confusion/Bewilderment
	SAM – Arousal
	SAM – Valence
	STAI-YA – Anxiety state
	ICE-Q – Emotional dimension
Social functioning	*ICE-Q – Social dimension*
Sleep quality	*PSQI – Sleep quality*
	PSQI – Sleep latency
	PSQI – Sleep duration
	PSQI – Habitual sleep efficiency
	PSQI – Sleep disturbance
	PSQI – Daytime dysfunction
	PSQI – Global score
	ICE-Q – Physical dimension

(*Continue*)

(*Continue*)

Cognitive functioning – Executive functions	*Incompatibility – Errors* *Incompatibility – RT indice* *Working memory - Omissions* *Working memory - Errors* *Flexibility - Errors* *Flexibility – Median RT (ms)* *Flexibility – Total performance indice*
Cognitive functioning – Attentional functions	*Alertness – RT intrinsic alertness (ms)* *Alertness – SD intrinsic alertness (ms)* *Alertness – phasic alertness indice* *Alertness – SD phasic alertness (ms)* *Alertness – Anticipations* *Alertness – Outliers* *Divided attention – Omissions* *Sustained attention - Omissions* *Sustained attention - Errors*

the standard deviation (SD) on the phasic alertness condition ($X^2(4) = 11.404$, $p = 0.022$*). Both decreased through time. The number of anticipations also presented a time effect during the mission ($X^2(4) = 10.090$, $p = 0.039$*).

Discussion

In our study, we observed higher stress factors and negative affective states at the beginning of the mission. As in other studies, we conclude that this period induce stress on individuals ("eustress of initial adaptation" - Dunn Rosenberg et al., 2022).

Despite this challenge, the individuals globally adapted well to the isolation. The positive affective states were constant and higher than negative ones all along the mission. Sleep quality and social functioning remained stable. The stress factors

TABLE 2: Psychometric measures. SOS = Stress Overload Scale; ICE-Q = Isolated and Confined Environments Questionnaire; POMS = Profile Of Mood States; SAM = Self-Assessment Manikin; STAI = State-Trait Anxiety Inventory; PSQI = Pittsburgh Sleep Quality Index; MD = Mission Day. Descriptive statistics: Mean ± Standard deviation by mission day (horizontal). Population N = 13

Variable	MD-3	MD-7	MD-11	MD-15	MD-19	Friedman test
SOS – Personal vulnerability	**22.085.63**	**23.587.52**	**19.005.13**	**18.504.58**	**17.334.58**	**$p <$ 0.001*****
SOS – Event Load	**35.9011.31**	**31,7010.91**	**31.2010.74**	**28,1012.56**	**28.2011.45**	**$p = 0.006$****
SOS – Total	**58.6016.45**	**55.5016.29**	**50.2015.24**	**46.7016.61**	**45.5015.69**	**$p <$ 0.001*****
ICE-Q – Occupational dimension	**14.152.97**	**15.313.17**	**15.693.45**	**16.543.64**	**16.383.97**	**$p = 0.020$***
ICE-Q – Social dimension	22.832.62	23.003.07	22.422.91	22.173.10	22.753.11	$p = 0.927$
ICE-Q – Emotional dimension	**23.084.64**	**22.503.50**	**21.753.41**	**20.083.60**	**20.252.67**	**$p = 0.020$***
ICE-Q – Physical dimension	19.833.93	20.172.89	18.503.03	20.172.59	19.83.61	$p = 0.488$
POMS – Tension/Anxiety	**10.504.27**	**10.505.28**	**8.404.19**	**6.603.10**	**6.803.64**	**$p = 0.010$***

(*Continue*)

(Continue)

POMS – Depression/ Dejection	**7.544.16**	**9.455.39**	**6.183.92**	**5.273.80**	**4.733.77**	***p* = 0.012***
POMS – Anger/ Hostility	**7.824.26**	**9.825.44**	**8.276.18**	**5.094.59**	**3.912.84**	***p* = 0.004****
POMS – Vigor/ Activity	19.272.76	18.093.67	17.274.82	17.364.03	17.453.75	*p* = 0.189
POMS – Fatigue/Inertia	10.363.96	9.732.94	11.092.21	10.273.10	9.913.99	*p* = 0.630
POMS – Confusion / Bewilderment	7.092.91	6.912.95	7.823.22	6.732.28	5.642.20	*p* = 0.130
SAM – Arousal	5.002.45	4.002.24	4.381.89	3.541.61	4.151.86	*p* = 0.200
SAM – Valence	6.771.42	6.691.11	6.621.45	6.541.33	7.081.44	*p* = 0.541
STAI-YA – Anx-iety state	35.276.69	35.827.68	35.367.98	35.643.98	31.557.02	*p* = 0.181
PSQI – Sleep quality	1.150.69	1.080.64	1.230.73	0.850.55	1.080.49	*p* = 0.201
PSQI – Sleep latency	0.620.96	0.460.66	0.771.01	0.460.52	0.620.87	*p* = 0.769

(Continue)

(*Continue*)

PSQI – Sleep duration	0.150.38	0.080.28	0.150.38	0.230.44	0.310.48	p = 0.577
PSQI – Habitual sleep efficiency	0.230.60	0.230.44	0.230.60	0.460.52	0.620.77	p = 0.073
PSQI – Sleep disturbance	1.000.41	1.080.64	1.150.38	1.150.38	1.150.38	p = 0.469
PSQI – Daytime dysfunction	1.000.58	0.920.64	0.850.38	1.000.58	1.080.64	p = 0.736
PSQI – Global score	4.151.86	3.851.77	4.382.06	4.151.57	4.851.91	p = 0.149

TABLE 3: Cognitive measures. RT = Reaction Time; SD = Standard Deviation; ms = Milliseconds; MD = Mission Day. Descriptive statistics: Mean± Standard deviation by mission day (horizontal). Population N = 13

Variable	MD-3	MD-7	MD-11	MD-15	MD-19	Friedman test
Incompatibility – Errors	1.15 ± 1.95	0.85 ± 1.41	0.62 ± 1.66	0.69 ± 1.11	1.08 ± 1.32	$p = 0.721$
Incompatibility – RT indice	104.86±7.03	107.71±7.70	107.93±7.86	106.49±9.77	106.80±7.79	$p = 0.787$
Working memory - Omissions	0.33±0.65	0.75±0.97	0.25±0.62	0.42±0.51	0.33±0.89	$p = 0.481$
Working memory - Errors	0.50±0.67	0.42±0.67	0.42±0.51	0.58±0.67	0.17±0.39	$p = 0.459$
Flexibility - Errors	2.42±2.23	3.25±2.26	2.25±2.09	2.42±1.68	3.25±2.14	$p = 0.354$
Flexibility – Median RT (ms)	**491.17±115.61**	**468.17±106.25**	**432.58±61.51**	**423.92±67.29**	**422.42±95.33**	**$p = 0.003$****
Flexibility – Total performance indice	**9.37±6.78**	**8.54±5.48**	**12.55±4.69**	**13.08±5.83**	**12.02±5.30**	**$p = 0.024$***

(*Continue*)

(Continue)

Alertness – RT intrinsic alertness (ms)	**204.54±15.30**	**205.23±15.94**	**203.31±12.43**	**198.23±17.14**	**195.23±11.41**	***p*=0.003****
Alertness – SD intrinsic alertness (ms)	31.46±13.40	29.69±17.67	27.77±26.81	33.62±19.94	27.69±22.51	*p* = 0.711
Alertness – phasic alert-ness indice	0.00±0.05	0.00±0.05	-0.01±0.04	0.00±0.04	0.00±0.04	*p* = 0.787
Alertness – SD phasic alertness (ms)	**24.15±6.49**	**21.15±6.85**	**24.38±7.75**	**21.69±7.11**	**18.77±6.03**	***p* = 0.022***
Alertness – anticipations	**1.92±1.19**	**2.15±1.41**	**0.92±1.04**	**1.92±1.80**	**1.54±1.20**	***p* = 0.039***
Alertness – outliers	2.38±0.96	2.46±0.66	2.54±0.97	2.85±1.07	2.38±0.96	*p* = 0.844
Divided attention – omissions	0.69±0.85	0.69±0.95	0.54±0.66	0.46±0.52	0.31±0.63	*p* = 0.458
Sustained attention - omissions	2.58±2.97	2.00±2.34	2.83±2.92	2.33±1.97	4.00±4.73	*p* = 0.777
Sustained attention - errors	0.83±1.03	1.00±1.28	1.17±0.83	0.92±1.08	1.08±1.16	*p* = 0.825

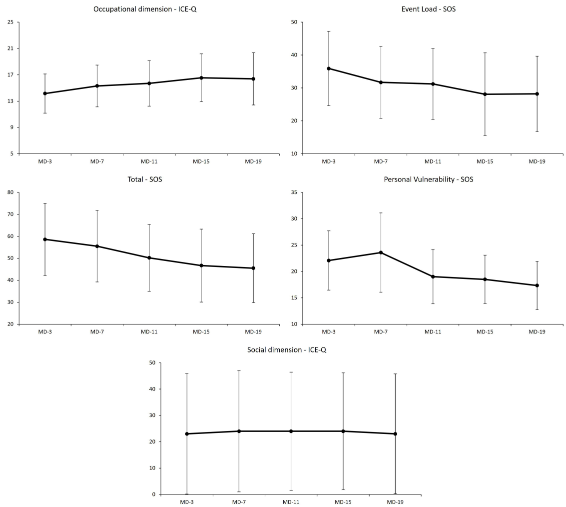

FIGURE 1

Graphic representation of perceived stress and social functioning parameters throughout the isolation. SOS = Stress Overload Scale; ICE-Q = Isolated and Confined Environments Questionnaire; MD = Mission Day.

decreased during the mission, which indicates an improvement of the relation to workload and environmental constraints. The negative affective states decreased similarly. We associate this result with the previous one, because a reduction in stress factors could lead to an improvement in affective states. Such positive adaptation to space conditions is not a rare phenomenon and has been observed in previous longer missions (Basner et al., 2014; Nicolas & Gushin, 2015).

We observed a degradation of emotional adaptation in the ICE-Q, which might seem incoherent with the other affective measures. However, the items of this subscale are all about boredom and how activities are interesting for individuals (Nicolas et al., 2019). This result should be considered with the perceived stress measures. As the participants adapt to workload during the mission, routine might appear, which may induce a feeling of boredom. But the overall affective states remained positive during the isolation.

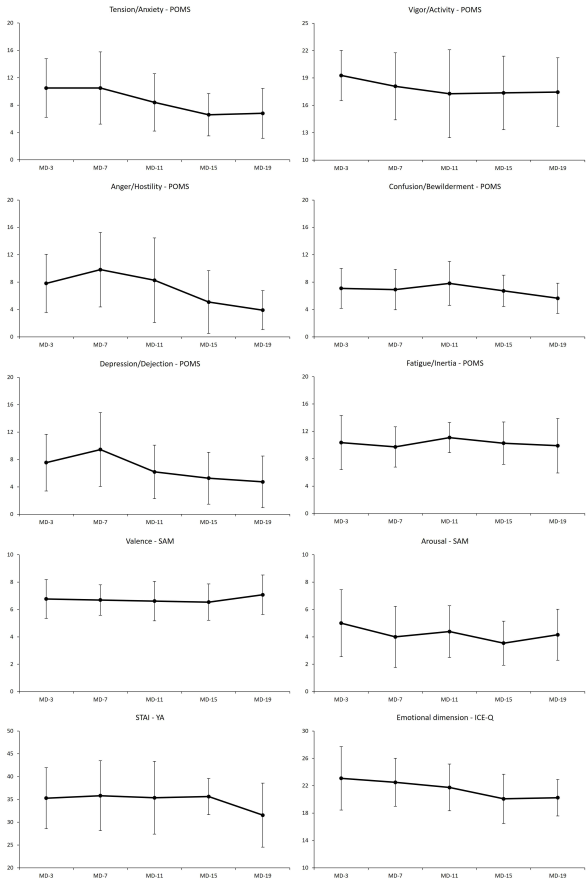

FIGURE 2

Graphic representation of affective states parameters throughout the isolation. ICE-Q = Isolated and Confined Environments Questionnaire; POMS = Profile Of Mood States; SAM = Self-Assessment Manikin; STAI = State-Trait Anxiety Inventory; MD = Mission Day.

The changes in cognitive functioning are all indicating an improvement of performance in the flexibility and alertness tasks. This modification is likely due to a retest effect (Scharfen et al., 2018) and a motivational factor, as participants saw the tasks as a game and competed against each other. We do not interpret this as a cognitive improvement associated with the mission conditions.

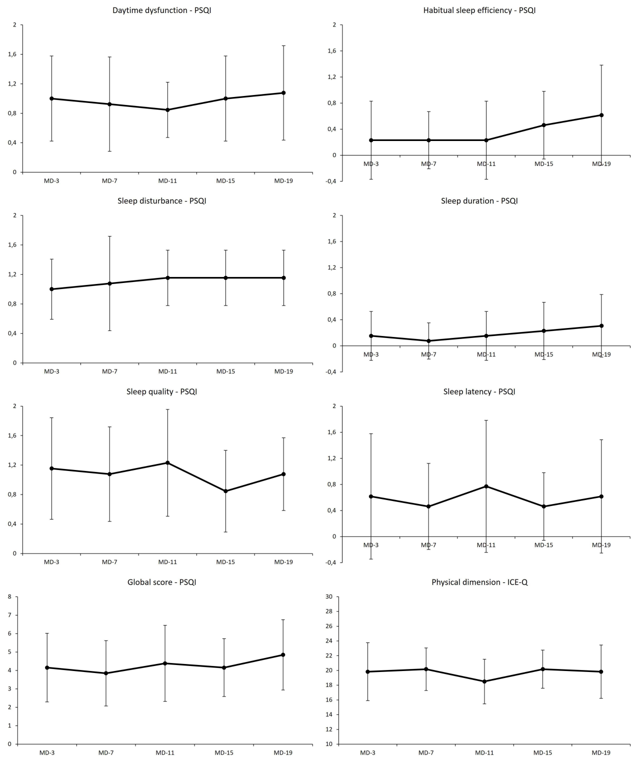

FIGURE 3

Graphic representation of sleep quality parameters throughout the isolation. ICE-Q = Isolated and Confined Environments Questionnaire; PSQI = Pittsburgh Sleep Quality Index; MD = Mission Day.

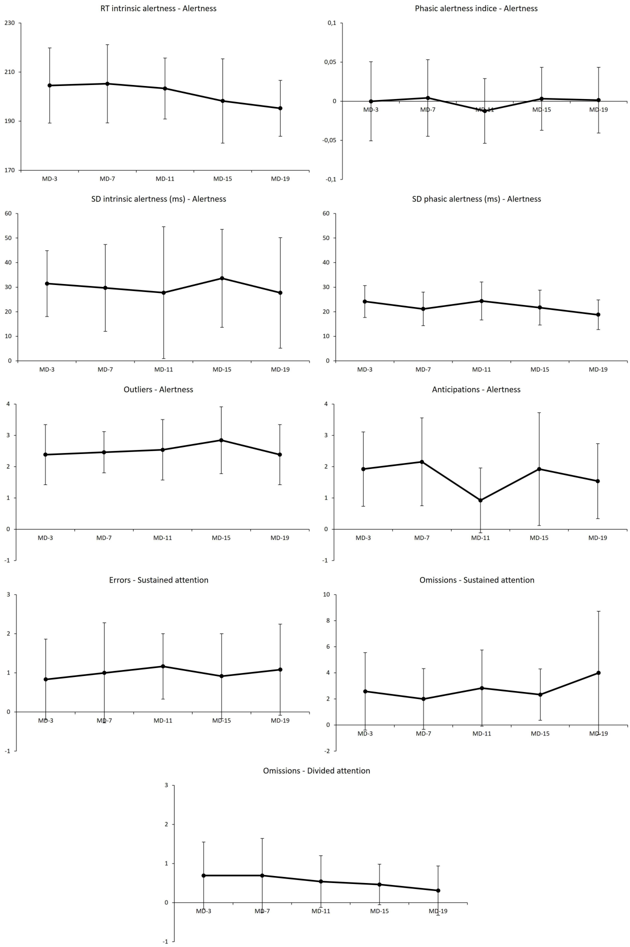

FIGURE 4
Graphic representation of attentional functions parameters throughout the isolation. RT = Reaction Time; SD = Standard Deviation; ms = Milliseconds; MD = Mission Day.

Our study has multiple limitations. The participants saw a tourist car once during their mission, which breaks the feeling of isolation. Pre- and post-mission measurements were removed from our study due to poor quality. The absence of a control group does not allow for a comparison with casual earth conditions. Finally, the MDRS station does not replicate all the stressors of actual spaceflights (microgravity, danger, distance, duration…).

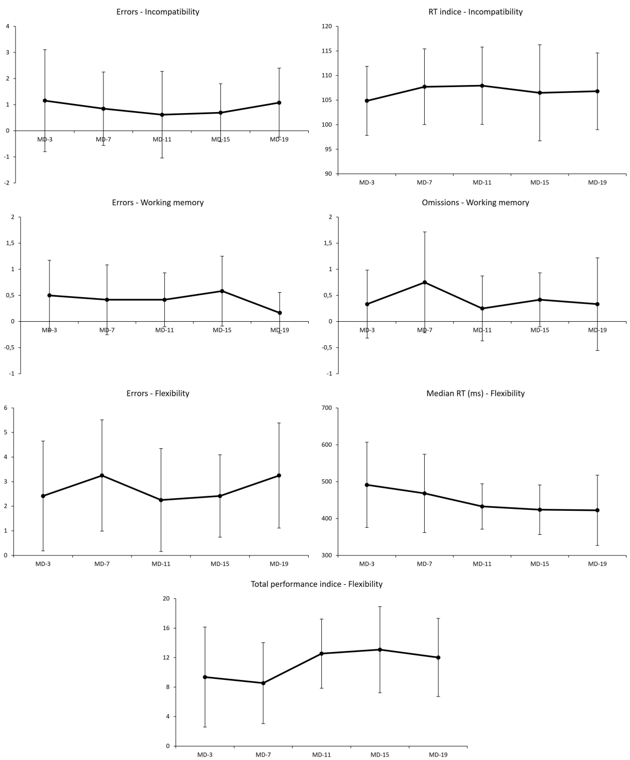

FIGURE 5

Graphic representation of executive functions parameters throughout the isolation. RT = Reaction Time; SD = Standard Deviation; ms = Milliseconds; MD = Mission Day.

Conclusion

Our study aimed to monitor multiple dimensions of psychological adaptation during a space-analog isolation. While the start of the mission was stressful, the participants adapted well to the environment and generally had positive experiences. We encourage the assessment of heterogeneous factors in space conditions to have an overall perspective on psychological adaptation. Future works should reproduce this kind of study during longer isolations and different kinds of space-like missions. It might also be interesting to consider the individual responses to the stressful beginning of space-missions, because it might be an indicator of the overall psychological adaptation during the mission (Nicolas et al., 2021). Finally, we encourage the regulation of workload at the beginning of space missions, as astronauts need time to adapt to the environment and be fully operational.

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Effect of 3-day exposure to dry immersion on veno-arteriolar reflex

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Robin, A., Navasiolava, N., Larina, I., Pastushkova, L., Tomilovskaya, E., Custaud, M-A. Effect of 3-day exposure to dry immersion on veno-arteriolar reflex.

Introduction
Veno-arteriolar vasoconstriction reflex (VAR), a local reflex activated by acute venous congestion leading to localized vasoconstriction, is important to maintain upright position. VAR impairment could contribute to microgravity-induced orthostatic intolerance. VAR state following actual or modeled

microgravity remains unclear. Thus, Gabrielsen and Norsk observed an unchanged VAR after 4-6.5 months of actual microgravity (Gabrielsen and Norsk, 2007), while 20 and 14-day head-down bedrest led to increased (Gabrielsen et *al.,* 1999), or attenuated (Wilson et *al.,* 2003) VAR. We previously demonstrated increase in upright calf skin vascular resistance following 7-day dry immersion (DI), while cardiovascular autonomic control was not modified, suggesting increased VAR (Navasiolava et *al.,* 2011). However the direct effect of DI on VAR was not measured. We aimed to evaluate the effect of 3-day exposure to DI on VAR.

Material and Methods

14 healthy men were studied before (B1) and at day 3 of non-strict 5-day DI (with allowed 15-min daily raise for hygiene). Measurement scheme is shown in Figure 1.

To block neurally mediated skin vasoconstriction (hence, VAR), EMLA anesthetic cream (Eutectic Mixture of Local Anesthetics, 2.5% lidocaine and 2.5% prilocaine, Astra-Zeneca, Wilmington, DE) was used. EMLA was placed 2h before the measurement to 3x3 cm area of calf and forearm skin and covered with a Tegaderm dressing. Prior to measurement, the dressing and the EMLA cream were removed, and the effectiveness of the anesthesia was assessed by testing of tactile sensation blockade.

To induce the forearm and lower leg VAR, venous congestion by proximal cuff inflation up to 40 mmHg was applied (Okazaki *et al.,* 2005). The cuff remained inflated at this pressure for 3 min. Cuff was placed around the upper arm and around the thigh. Skin blood flow (SkBF), accessed via laser-Doppler flowmetry (Perimed, Sweden), was measured at two sites on the forearm and at two sites on the calf (EMLA sites - with previous EMLA application, and control sites - forearm and calf intact skin without EMLA). "EMLA" laser-Doppler flow probes were placed at the outer surface of the leg/forearm, and "control" probes – at the inner surface, with the same topography in all subjects. To assure the same placement of probes for two subsequent measurements, their position was marked on the skin.

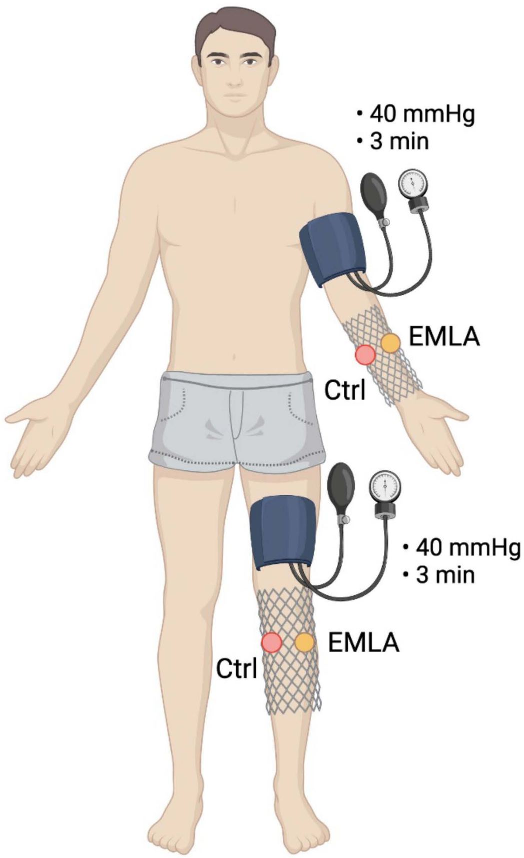

FIGURE 1
Measurement scheme. Subject supine on the couch at B1, supine in dry immersion bath at D3. Venous occlusion at 40 mmHg for 3 min was performed using manual pneumatic cuffs. Skin blood flow was assessed via laser-Doppler flowmetry at 2 sites of each extremity (Ctrl site, and EMLA site - with topical application of EMLA anesthetic cream 2h before venous occlusion in order to block VAR).

Forearm and leg skin temperature was controlled with thermocouples 5 cm apart the probes, connected to electronic thermometer (SKT 100C, Biopac Systems, Inc., USA).

Finger blood pressure wave (Finapres, Ohmeda, Englewood, Col.) and standard ECG (Biopac, ECG 100C, USA) were recorded continuously with the data acquisition system (Biopac MP 150, USA). Mean blood pressure (MBP) and heart rate (HR) were extracted from the signal using a software package (Acknowledge® 3.9.0). Stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) were estimated from the blood pressure signal using the modelflow® method (Beatscope® software, TNO, the Netherlands).

Baseline data were collected for 10 min. Then leg cuff inflation was applied for 3 min. After leg cuff depletion and 2-min recovery period, arm cuff was inflated for 3 min. The stable 120s of data for each measurement were averaged and used for analysis.

SkBF diminution during cuff inflation (%ΔSkBF) was calculated as percent change from resting baseline:

$$\%\Delta SkBF = [(SkBF_{infl} - SkBF_{bas}) / SkBF_{bas}] * 100\%,$$

where $SkBF_{bas}$ is mean resting skin blood flow, and $SkBF_{infl}$ is mean skin blood flow during cuff inflation.

The magnitude of VAR before immersion and on D3 was estimated as a difference in SkBF diminution to cuff inflation between Control and EMLA sites:

$$\text{VAR magnitude} = \%\Delta SkBF_{ctrl} - \%\Delta SkBF_{EMLA}$$

Results

Parameters of resting central hemodynamics (HR, BP, SV, CO and TPR) remained within normal limits and were not significantly modified on D3 of immersion (Table 1). During measurements, HR and BP remained stable. Calf and forearm skin temperature were about 33°C without significant difference between two measurement days (Table 1).

Baseline SkBF at D3 significantly decreased at forearm, but not at calf level (Table 1).

SkBF in response to cuff inflation decreased more at calf than at forearm both before DI and at D3 (Figure 2).

EMLA attenuated SkBF diminution in response to cuff inflation both before and at D3 (significantly at leg level) (Figure 2).

VAR magnitude tended to increase under immersion, especially for lower limb (arm: -4±11% - before- vs. -9±9% - at D3, leg: -11±5% - before- vs. -19±5% - at D3), though without reaching statistical significance (Figure 2).

Discussion and Conclusion

The possible mechanisms of SkBF decrease in response to cuff inflation with infra-diastolic occlusion level are discussed in Figure 3. SkBF decrease might

TABLE 1: General hemodynamic conditions (MAP, HR, SV, CO, TPR), skin blood flow, and skin temperature at resting baseline. Data are mean±SEM. *$p \leq 0.05$ vs. B1

	B1	**Day 3**
MAP, mmHg	81±3	88±3
HR, bpm	62±2	61±3
SV, ml	86±3	92±3
CO, L/min	5.67±0.34	5.59±0.25
TPR, AU	0.885±0.046	0.965±0.041
SkBF arm (ctrl site), AU	27.1±4.7	16.9±2.9*
SkBF leg (ctrl site), AU	9.4±1.2	8.2±1.2
SkT arm, °C	32.9±0.4	32.9±0.2
SkT leg, °C	32.9±0.3	33.2±0.2

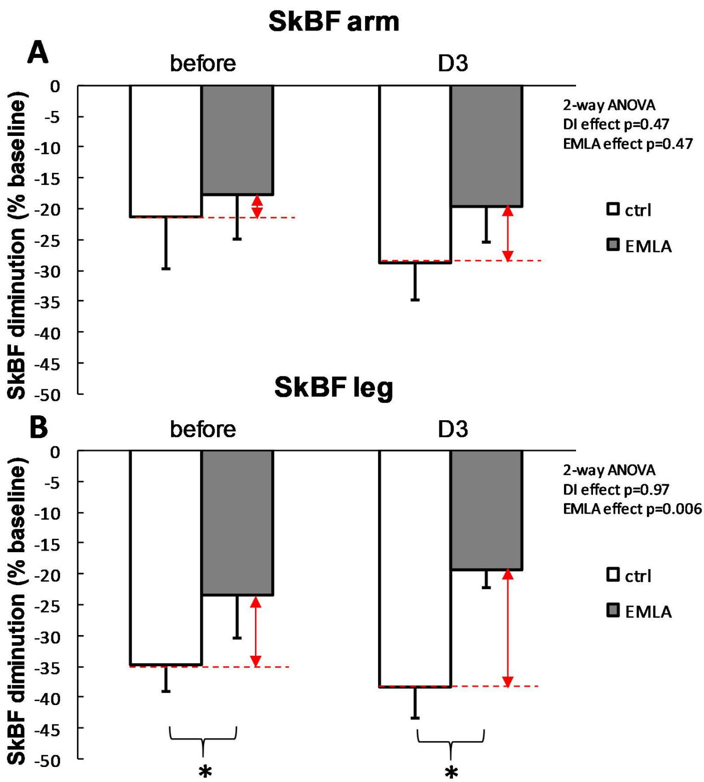

FIGURE 2
Percent change in skin blood flow in forearm (A) and calf (B) during cuff inflation to 40 mmHg at the control sites and EMLA-treated sites. n=14 subjects. Values are means $\pm$ SEM; *$p \leq 0.05$ vs. Ctrl; arrows reflect magnitude of VAR as a difference in SkBF diminution to cuff inflation between Ctrl and EMLA sites.

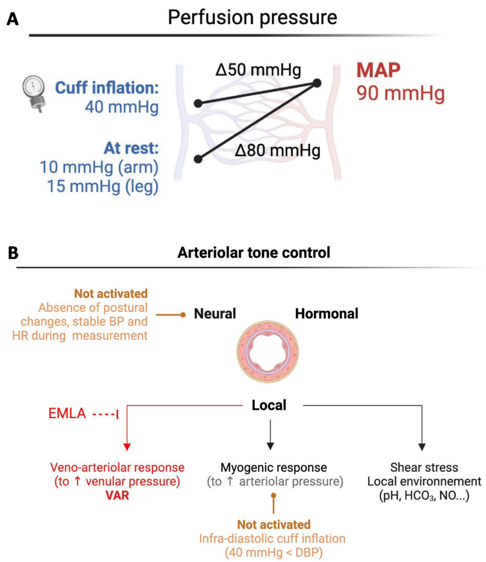

FIGURE 3
Possible mechanisms of SkBF decrease in response to cuff inflation: effect on perfusion pressure (A); arteriolar tone control mechanisms (B).

be explained mainly by decrease in perfusion pressure and by VAR-induced vasoconstriction. As subjects remained supine during measurement, with no limbs dependency, and central hemodynamic remained stable, neural vasoconstriction and myogenic response should not be activated. At EMLA sites, neurally-mediated VAR was blocked.

Our results suggest that veno-arteriolar response at leg and arm skin level is not attenuated by non-strict DI. It seems that VAR does not need sustained daily orthostatic stimulation to maintain efficiency. Preserved VAR might compensate insufficient vasoconstriction of other vascular beds following exposure to microgravity. Different topography of Control and EMLA sites might be responsible for the failure to demonstrate significant increase in VAR following DI. This might also explain the absence of significant changes to EMLA (i.e. VAR) at the arm level, contrary to the results of Okazaki *et al.* (2005).

Trend to exaggerated VAR under DI could be due to the chronic squeezing effect of the water mass around the body, acting like elastic compression therapy for venous insufficiency, which is known to improve impaired VAR (Mancini et al., 2003; Belcaro et al., 1988, 1993).

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"Space dentistry"-An invention or a modern necessity?

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Introduction

During space missions, space crews undertake complex tasks in remote, hostile, and hazardous environments exacerbated by microgravity and heightened levels of radiation. These conditions subject astronauts to various physiological stresses, both during short-duration and long-duration space trips. [1] Notable effects include bone loss, muscular atrophy, cardiac dysrhythmias, altered spatial orientation, and psychological well-being. There is limited research however regarding the impact of factors such as UV rays, microgravity, and other space-related conditions on the oral cavity. [2] By investigating the effects of space-related conditions on the oral cavity, aeronautical dentistry aims to develop effective protocols and treatments to maintain astronauts' oral health during missions. This knowledge is crucial for ensuring the well-being and performance of astronauts in the demanding and challenging environment of space travel [3,4].

Review

The oral cavity plays a critical role in numerous physiological processes including taste perception, mastication, solubilization of nutrients, and digestion. Moreover, it contributes to innate and adaptive immunity, respiration, speech, and other vital bodily functions. [2,4] Alterations in saliva composition and rate of production of saliva can profoundly affect oral tissues and overall health. Aeronautical dentistry, also known as space dentistry, focuses on studying the pathophysiology of the oral cavity in airborne and extra-terrestrial conditions. Understanding this topic is of paramount importance due to the potential oral health implications in extra-terrestrial environments. [3] The review article will delve into the significance of comprehending the oral health impacts of space travel and proposing potential strategies for managing dental crises in space.

<u>Effects of the space environment on the oral cavity:</u>

- Microgravity: It is the condition in which people or objects appear to be weightless. The effects of microgravity can be seen when astronauts and objects float in space. Microgravity can be experienced in other ways, as well. It affects almost all the systems and physiological activities including the cardiovascular system, skeletal system, renal system, nervous system, respiratory system, hormonal imbalance, oral cavity, and temporomandibular joint. The effect of microgravity on teeth results in dental caries, pulpitis, defective tooth restorations, apical periodontitis, pulp necrosis, vertical root fracture, and impacted teeth. These results suggest that the reversible effect of microgravity is edema of the face, change in taste, abnormal expression of the face, tooth pain, and xerostomia. [4,5]

- Radiation: Radiation exposure is one of the many health concerns that astronauts face when traveling and working in space. While the effects of radiation on the oral cavity specifically may not be as extensively studied as some other aspects of space radiation exposure, it can still have several potential impacts. Here are some effects of radiation in the oral cavity on astronauts: [2,4]

- Caries: Radiation exposure can weaken tooth enamel and increase the risk of dental caries. The reduced protection of tooth enamel can make astronauts more susceptible to oral health issues, especially if they consume sugary or acidic foods and beverages. [4]

- Periodontitis: Radiation can also affect the gingiva, alveolar bone, and oral mucosa. Astronauts may experience gingival inflammation, bleeding, or even ulceration because of radiation exposure. Proper oral hygiene practices are crucial to mitigate these issues. [4]

- Tissue Damage: High levels of radiation can damage oral tissues, including the mucous membranes and salivary glands. Reduced salivary flow can lead to xerostomia, which not only increases the risk of tooth decay but also makes it uncomfortable for astronauts to eat and speak. [1,4]

- Oral Lesions: Radiation exposure may increase the risk of developing oral lesions, such as ulcers or leukoplakia. These can be painful and require special attention and treatment as they can advance to oral cancer. [1,2,4]

- Increased Cancer Risk: Prolonged exposure to space radiation, which includes cosmic rays and solar radiation, can elevate the risk of cancer, including oral cancer. The oral cavity is not immune to the potential carcinogenic effects of ionizing radiation. [1,4]

To mitigate these effects, astronauts receive training on oral hygiene practices and are encouraged to maintain good dental health before and during their missions. Regular dental check-ups and dental care supplies are also provided on the International Space Station (ISS) to help astronauts maintain their oral health. Also, space agencies are continually researching and developing ways to protect astronauts from radiation, both in terms of shielding spacecraft and monitoring radiation exposure levels. [1,4]

Dental emergency in space:

A dental emergency in space can be a challenging situation due to the unique environment of microgravity and limited access to comprehensive dental care. Here are some key considerations and steps that astronauts and mission control might take when facing a dental emergency in space [6-8]:

1. Identification of the Problem:

- Astronauts are trained to recognize dental issues, such as severe toothaches, broken dental prosthetics (like crowns or fillings), gum infections, or abscesses.

- Communication with mission control is crucial to relay the nature and severity of the dental emergency.

2. Immediate Pain Management:

- Astronauts have access to a basic medical kit, which may include pain relievers. These can help manage pain temporarily until further action can be taken.

- Topical oral analgesics (gels or swabs) may be used to alleviate oral pain and discomfort.

3. Consultation with Mission Control:

- Astronauts communicate with medical professionals on Earth (mission control) to discuss the situation and receive guidance.

- Telemedicine consultations can be conducted to assess the issue and determine the best course of action.

4. Emergency Dental Kit:

- The spacecraft or space station may have an emergency dental kit containing basic dental tools and materials, such as dental mirrors, forceps, and temporary filling materials.

- Astronauts with some dental training may use these tools to address minor issues, such as loose dental fillings.

5. Stabilization and Temporary Solutions:

- Astronauts may be instructed to stabilize loose dental prosthetics or to place temporary fillings or crowns if they have the necessary training and equipment.

- The goal is to provide temporary relief until the astronaut can return to Earth, where more comprehensive dental care is available.

6. Possible Medication:

- If there is an infection, antibiotics may be prescribed to control it until the astronaut can receive appropriate dental treatment.

7. Return to the Earth:

- In serious cases or when the dental issue cannot be adequately managed in space, a decision may be made to return the affected astronaut to Earth.

- The decision to return to Earth would depend on the severity of the dental emergency, the mission's duration, and the astronaut's overall health.

It is important to note that dental emergencies in space are rare due to the rigorous medical evaluations that astronauts undergo before their missions. Astronauts are trained to manage minor dental issues, and mission control can provide guidance and support from Earth. Additionally, preventive dental care is a crucial aspect of astronaut training to minimize the risk of dental emergencies during space missions.

Management of oral problems on space missions: [6,8-10]

Every mission has 2 Crew Medical Officers who are trained to perform both medical and dental emergency procedures. They are authorized to treat with antibiotics and analgesics, administer anesthetics, place temporary dental fillings, replace a crown with temporary cement, treat exposed pulp, and as a last resort, extract teeth. Any emergency treatment would include communication with ground support flight physicians, as the CMOs are not necessarily physicians or dentists themselves. Besides this, a Dental Emergency Kit is also provided which should be lightweight, non-toxic, and meet relevant packaging criteria for the retention of the instruments under gravity-free and thermal-free conditions.

Duties of aeronautic dentists: [9]

Aeronautic dentists are individuals who manage dental issues while flying or dealing with the impact of altitude changes on the oral health of pilots, astronauts, and other space crew members. Their duties are to design and conduct ground-based studies in aeronautic dentistry with the purpose of determining the effects of acceleration on the system under investigation, preparing for real microgravity experiments on orbiting spacecraft, sounding rockets, and parabolic flight aircraft, identifying the parameters that might be changed under real micro weight conditions, examining the interaction of a system under study in relation to the hardware being used in a real micro weight experiment, and investigating the effect of launch accelerations and vibrations on the system under study or in combination with the utilized hardware.

Dentists are integral members of the astronaut healthcare team, and their expertise in oral health is essential for maintaining the physical and psychological well-being of astronauts during space missions. Dentists may also be involved in research and development efforts related to dental care in space, including the design of dental instruments and treatments suitable for the space environment. By addressing oral health concerns and providing preventive care, dentists contribute to the overall success and safety of space exploration missions. [10]

Conclusion

The role of a dentist is very crucial in further space research. Dental emergencies are very painful and can jeopardize million-dollar missions. Treatment of dental problems in space remains an incredibly challenging area. Accordingly, much more dental research must be performed in the planning of the future for long-duration crewed missions in space.

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New commercial applications for space physiology and human spaceflight

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Introduction

Human spaceflight is becoming more common, accelerated by commercial activities, like the Axiom Space private astronaut missions. These advancements are providing new opportunities to use, test and validate innovative solutions for research in space physiology as well as health monitoring in space.

This new chapter opens doors to a wide range of opportunities, technologies, models and procedures, at the intersection of space and non-space activities. The adoption of Artificial Intelligence (AI) and Machine

Learning (ML) techniques in space can result in important steps forward for a wide range of human health research and applications. These include real-time Big Science Data processing, AI-powered assistants for medical care, research, evaluation and support of crew activities and performance, opening new portals into what future human spaceflight missions could look like.

Health Applications for Research On-board the International Space Station
During the RAKIA mission on Axiom-1, the International Commercial Experiment (ICE) Cubes Service had the opportunity to support the implementation of several health applications solutions involving tablet processors computers used to perform a series of physiological and psychological tests on-board the International Space Station (ISS) (ICE Cubes Journal, 2022).

One activity from the RAKIA mission had the objective to conduct an albumin to creatinine ratio (ACR) urinalysis test in space, with the aim to help astronauts measure kidney function and get immediate diagnostic results. This involved the use of a tablet applications running a customized (space edition) kidney test protocol. While the topic of kidney function in space has already been researched, previous studies required urine collected in space to be returned frozen back to Earth for post-flight analysis. The use of this new tablet application enabled the astronaut crew to obtain immediate medical diagnostic information in-situ, which is particularly relevant as missions to space become longer.

Living in space can also affect eyesight and cause spaceflight associated neuro-ocular syndrome (SANS). The RAKIA mission also included the utilization of a different tablet application that provided real-time evaluation of eye fitness before, during, and after a task, with the aim to investigate spaceflight effects on visual function, the early onset of SANS, and the recovery of visual functions.

With increased human presence in space, and new space stations and habitats being developed to expand the scope of space missions, astronauts will need a variety of modern diagnostic tools to screen and monitor their health in the space environment. These tools and applications can be tested, validated and demonstrated on-board the ISS, and future space stations, in a fast-track and direct manner through commercial services like the ICE Cubes Service. Such tools can include customized questionnaires and applications running on tablets or other mobile devices, games and interactive applications, wearables, sensors, diagnostic and imaging tools, health and fitness gadgets, AR/VR, AI/ML, commercial-off-the-shelf components (COTS), as well as customized devices and solutions.

Artificial Intelligence for R&D in Space
In modern space research, AI is already playing a crucial role in providing in-situ, fast, real-time processing of big data generated by a variety of experiments, measurements and activities, either in crewed or fully-automated spacecraft. One can therefore predict that the application of AI/ML assets will support scientists and operators in more efficiently understanding and monitoring the effects of the space environment and microgravity in all its facets, by deciphering models and patterns that are not easily observable. This opens new avenues for R&D in life sciences, and may lead to important new developments in industries including biotechnology, pharmaceutical, agriculture, and food technology. In the realm of human health monitoring and protection, the use of AI/ML assets with a boost in computational capability may lead to improved methods for data analysis, problem solving and automation of complex tasks.

The effects of long-term exposure to spaceflight conditions manifest in many areas of human physiology and health: neurology, ophthalmology, cardiovascular, pulmonary, gastrointestinal, urinary, musculoskeletal, hematology, immunology, oncology, and psychological stress (Haney et al., 2020). AI/ML techniques can enhance in-space research in any of these areas, at different scales and under different setups, by combining and analyzing data stemming from specific individual processes of sub-systems. These include studies at the organism level through human physiology studies (e.g. astronauts as health research subjects), and research at the cellular or tissue level though *in vitro* research studies (e.g. organoids, spheroids, tissues, genomics via lab/organ-on-chip research) (ICE Cubes Journal, 2022). Moreover, AI could play an increasingly important role in personalized health monitoring and assistance for crew healthcare, emergency medical procedures, and generally support fusing, processing and analyzing data generated by multiple sensors and devices (ICE Cubes Journal, 2022).

Future Applications

Commercial spaceflight comes with a different approach to conducting activities in space, tapping into the solutions already available on different non-space/terrestrial markets. In recent years, the rapid development of technology on Earth offers a wide range of tools, applications and off-the-shelf solutions that can be quickly adapted for utilization in space. This not only allows for more advanced activities and processes, but also significantly shortens the time required for integration, implementation of R&D activities, data retrieval and analysis. Such solutions include wearable devices, biosensors, diagnostic imaging, augmented and virtual reality (AR/VR) tools.

The empowerment of such applications with AI/ML capabilities can pave the way for the implementation of innovative health monitoring and evaluation solutions. The validation and demonstration of these solutions in space can then become part of the health check protocols and evaluation process of space crews, enabling automatic data analysis in-orbit, while also providing real-time connection for data and results comparison and cross-checking by the medical staff on ground.

Combining research payloads and devices with edge computing capabilities in-orbit offers the opportunity to test and benchmark new solutions and applications required by future long-term deep space exploration missions, where (near) real-time communication with ground (Earth) is not possible. Such solutions may include machine vision applications, in-situ processing and analysis of data via AI/ML powered visual inspection, development of smart devices, robotic assistants and habitats, as well as AR/VR-based applications for future space activities and operations (in-orbit and on-ground).

Conclusion

Commercial space activities are enabling research in space by providing easier, faster, and lower cost access the ISS and other platforms. As more humans will be living and working in space in the future, it will be necessary to develop improved tools to monitor human health, support crew activities and deliver quality medical care in low Earth orbit and beyond. The advent of AI/ML is having a significant impact on technology development on Earth, and offers unique opportunities for space exploration. Crew health monitoring studies conducted during private astronaut missions to the ISS have provided initial contributions for exploring these opportunities. Future work the through integration of new technologies including edge computing, machine vision applications, in-situ data processing and analysis will likely generate significant benefits for space exploration, as well as for healthcare and industries on Earth.

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Reimagining the horizons: Space agencies' goals for human space exploration

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Introduction

Human space exploration, a remarkable achievement, showcases our curiosity and commitment to innovation. From gazing at stars to advanced space travel, originating in the Cold War era, the Space Race began with the Soviet launch of Sputnik 1 in 1957 [1]. Yuri Gagarin's orbit in 1961 and Apollo 11's Moon landing in 1969 marked iconic moments [2,3]. Advancements continued with space shuttles, stations, and global cooperation like the International Space Station (ISS). Milestones include the Space Shuttle program, Hubble Telescope, and robotic exploration of planets. Technologies developed benefit life on Earth. Looking forward, human space exploration aims for Mars missions, commercial space travel, and exploration of distant moons and asteroids [4]. The quest for knowledge ensures an ever evolving and captivating chapter in humanity's story.

Driven by human curiosity and the pursuit of the unknown, space exploration finds its rationale in scientific, economic, and existential motivations. Scientifically, space exploration provides a unique point of view to study celestial bodies, their compositions, and its effect on human physiology [5–12]. Economic prospects include resource utilisation and technological advancements, with the potential for mining valuable minerals from asteroids or the Moon [13–16]. This addresses resource shortages and fosters economic growth on Earth.

In recent years, a profound shift has driven human space exploration to the forefront of global priorities. This transformation, driven by technological advances and the commercialization of space travel, has turned what was once a niche pursuit into an unprecedented focus for public and private investment [17]. The shift towards prioritising human space exploration also arises from a heightened awareness of Earth's finite resources and the imperative for sustainable alternatives [18,19].

International collaborations, exemplified by organisations like NASA, ESA, and private entities forming global partnerships, have further reshaped the narrative around space exploration. This collaborative spirit not only expands the scale of space missions but also promotes unity and shared responsibility beyond geopolitical boundaries. Governments, private enterprises, and global alliances continue to prioritise human space exploration, setting the stage for a defining chapter in the story of human progress and resilience.

Objectives and Milestones of Prominent Space Agencies
Space agencies, such as NASA (USA), CSA (Canada), and ESA (Europe, including CNES, DLR, and ASI), aim to explore and understand the universe. Objectives include scientific research, satellite deployment, and planetary exploration. Milestones include successful satellite launches, Mars rover missions, and collaborative projects like the ISS.

1. **Astronaut programs and human spaceflights** Space agencies conduct astronaut programs to train individuals for space missions. Human spaceflights, like those conducted by NASA's Apollo program or SpaceX's Crew Dragon missions, contribute to scientific research, technology testing, and the long-term goal of human settlement beyond Earth.

2. **Gender equality** Achieving gender balance in space exploration is a priority. Efforts are being made to increase female representation in astronaut programs and leadership roles[20]. Space agencies have actively promoted diversity, recognizing the importance of diverse perspectives in advancing space exploration.

3. **International Space Station collaboration** The ISS represents a significant collaborative effort involving space agencies from the USA, Europe, and Canada. This collaboration promotes scientific research, technological advancements, and international cooperation in space exploration, serving as a model for future joint ventures.

4. **Lunar and Martian exploration ambitions** Space agencies have set ambitious goals for lunar and Martian exploration. Missions like NASA's Artemis program aim to return humans to the Moon, while aspirations for crewed missions to Mars involve extensive planning and international collaboration.

5. **International partnerships and cooperation** International partnerships are crucial for sharing resources, expertise, and costs in space exploration. Collaborations like the European Space Agency's involvement in various

missions exemplify the global nature of space exploration, with countries pooling their strengths for common objectives.

6. **Public-private partnerships and commercial space ventures** The rise of private space companies, like SpaceX and Blue Origin, has transformed the space industry. Public-private partnerships between these companies and space agencies facilitate cost-effective space missions, technology development, and the commercialization of space, opening new avenues for exploration and innovation.

7. **Advancing scientific knowledge and technological innovation** Space agencies contribute significantly to advancing scientific knowledge and technological innovation. Missions exploring exoplanets, studying cosmic phenomena, and developing cutting-edge technologies benefit both space exploration and contribute to broader scientific understanding.

8. **Propelling technological innovation and global competitiveness** The space sector engages technological innovation, propelling advancements in materials, robotics, telecommunications, and more. Nations investing in space exploration enhance their global competitiveness by promoting industries that drive economic growth and technological leadership on Earth and beyond.

Conclusion

In conclusion, space agencies have evolved significantly, navigating a dynamic landscape of science, technology, and ethics. Balancing knowledge pursuit, technological progress, and ethical responsibilities is crucial for global benefit. Ethical dimensions like space debris management and fair benefit distribution are prominent. The collaborative nature of space exploration stresses international cooperation for collective problem-solving. Amid remarkable possibilities and responsibilities, ongoing reflection showcases human ingenuity. This collective wisdom will guide future endeavours, ensuring space exploration remains an enduring inspiration for generations.

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Role of exercise-induced H3K27me3 for the gene response to exercise in mouse skeletal muscle

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Shimizu, J., Kawano, F. Role of exercise-induced H3K27me3 for the gene response to exercise in mouse skeletal muscle.

Introduction
Physical exercise is undoubtedly beneficial to prevent the loss of skeletal muscle mass during long-term space stay. Deep space exploration, such as a manned mission to Mars, however, presents a challenge due to limited payloads for onboard exercise. Therefore, it is crucial to develop effective onboard exercise procedures and preconditioning methods on Earth to promote minimizing to cause muscle dysfunction or maximizing skeletal muscle adaptation to exercise. The adaptability of skeletal muscle to exercise, on the other hand, can significantly vary across individuals both in space (Fitts *et al*., 2010) and on Earth (Bamman *et al*., 2007). The mechanism to determine the thresholds of adaptation is still unknown, which could be hindering the development. We previously reported that histone modifications, which can control the transcriptional responsiveness to certain stimulation, significantly altered in response to acute and chronic

exercise at gene loci whose genes are upregulated by exercise in human skeletal muscle (Lim and Shimizu *et al*., 2020). This newly proposed mechanism suggests that pre-existing exercise levels in individuals determine the threshold for responsiveness to the following exercise, ultimately leading to individual differences. Histone H3 trimethylation at lysine 27 (H3K27me3) is a transcriptionally repressive histone modification that promotes heterochromatin formation. Interestingly though, exercise can also increase H3K27me3 at loci where gene transcription is upregulated (Lim and Shimizu *et al*., 2020). However, the roles of exercise-induced H3K27me3 in altering gene responsiveness to a single-bout of exercise, its dynamics during exercise training, and the persistence of distribution shifts after leaving training, remain unclear. Therefore, this study aimed to investigate the effects of exercise training and detraining on H3K27me3 dynamics and gene responsiveness to exercise in mouse skeletal muscle.

Material and Methods

To achieve the aim of the study, we conducted two phases of experiments using male C57BL/6J mice (8 weeks old). During the first phase, the mice underwent acute treadmill running at a speed of 15 m/min for 30 minutes, after which we collected Tibialis anterior muscles 2 hours after the end of the exercise. We performed RNA sequencing on the sampled muscle tissue to differentiate between upregulated genes in response to exercise and non-responsive genes. Additionally, we conducted chromatin immunoprecipitation followed by next-generation sequencing analysis (ChIP-sequencing) to analyze the distribution of histone modifications of H3K27me3, H3K4me3, and RNA polymerase to clarify each region's specific features. During the second phase, we organized an experiment to directly observe the effects of training and detraining on histone modification and gene expression responsiveness. The mice underwent exercise training at the same magnitude as in the acute study for four weeks (5 days per week). Half of the trained mice were detrained, i.e.sedentary for 4 weeks after the end of the training period. Mice performed a single bout of running exercise 3 days after the last exercise session in the training study or at the end of the detraining study. Sedentary mice who neither underwent training nor detraining were also tested to run with the same schedule as the control. 2 hours after the end of acute exercise, we took samples of the tibialis anterior muscles from each group, and analyzed gene expression with RT-qPCR and histone distribution with ChIP-qPCR at the loci of 20 selected genes that were transcriptionally upregulated in response to acute exercise in a previous study (Ohsawa and Kawano, 2021).

Results

The results obtained from the ChIP sequencing showed that non-responsive loci, whose genes never responded, were marked only by H3K4me3 and

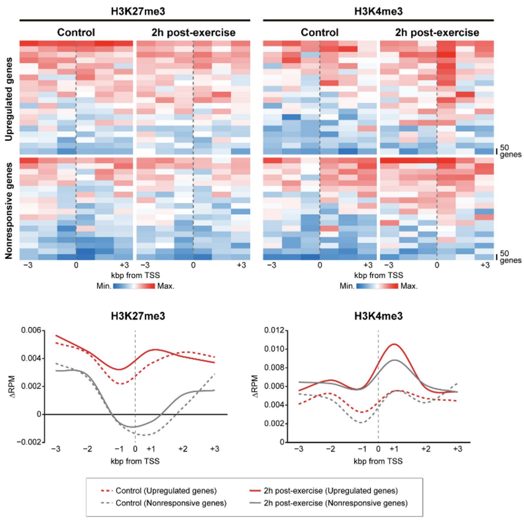

FIGURE 1

Responses of H3K27me3 and H3K4me3 distributions at the target loci to exercise. In heat maps, a single block showing the occupancy in every 50 genes was aligned by the order of RNA polymerase II distribution, e.g. top row is most prevalent. Mean occupancies in all upregulated (n=1,100) or non-responsive (n=935) genes are plotted in the bottom graphs.

lacked H3K27me3. However, upregulated loci, whose genes significantly increased (more) in response to exercise, were marked by both H3K27me3 and H3K4me3. Moreover, the levels of H3K27me3 and H3K4me3 were further augmented in response to acute exercise at those loci, as demonstrated in Figure 1 (also published in Shimizu and Kawano, 2022).

Exercise training also supplied ed the remarkable accumulation of both H3K27me3 and H3K4me3 at the 20 target loci. In the trained group, the genes further upregulated in response to a single bout of exercise. Both H3K27me3 and H3K4me3 distributions in the trained mice decreased by a single bout of exercise. Detraining, however, did not result in any discernible changes in the accumulation levels of H3K27me3 or H3K4me3 compared to the control group. Furthermore, in the detrained muscle, we noted suppressed gene response to a single bout of exercise, and there was no significant change in the level observed in either H3K27me3 or H3K4me3.

Discussion
Pharmacologically strengthening exercise-induced H3K27me3 significantly promoted exercise adaptation (Shimizu and Kawano., 2022). Taken together with the results of the present study, exercise-induced H3K27me3 associates with H3K4me3, and can play an active role in transcription in skeletal muscle. In this study, exercise-induced H3K27me3 accumulated during exercise training, enhancing gene responsiveness, suggesting that skeletal muscle acquires exercise responsiveness through H3K27me3 accumulation, where the accumulation level can be crucial for an individual's threshold of exercise responsiveness or adaptation. The accumulation and enhanced gene response, on the other hand, did not persist during the detraining period in the current exercise protocol, indicating that detraining can reverse the ability of acquirement to exercise adaptation.

Conclusion
The findings of this study are that exercise training leads to an accumulation of exercise-induced H3K27me3 at transcriptionally active sites, thereby promoting gene responses to exercise in mouse skeletal muscle.

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Impact of honey bee (*Apis mellifera*) queen exposure to hypergravity on colony development – Case study

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Stasiowska, D. Impact of honey bee (Apis mellifera) queen exposure to hypergravity on colony development – Case study.

Introduction

Since the beginning of the space age, the issue of the impact of hypergravity on living organisms has been raised. A relatively low number focus on insects, which may become an important part of the food production system as extraterrestrial crops pollinators. Such a species is honey bee (*A. mellifera*), first studied by NASA in the 1980s. The first experiment, performed in 1982, focused on flight patterns of moth, house fly, and honey bee in microgravity (Onboard STS-3, 1982). During the observation, house flies adapted the best to microgravity conditions, while bees were unable to move properly. However, such a situation could have been caused by lack of good quality food or lack of relative motion stimuli. Later, in 1984, Vandenberg et al. checked honey bees' abilities to construct honeycomb in microgravity (Vandenberg et al., 1985). Combs constructed in microgravity had a geometry similar to the ones from Earth, except angling downwards. In 2009, a Chinese team examined microgravity's impact on the drones' semen quality, proving that the lifespan of queens inseminated with the semen stored in microgravity is shortened (Jun et al., 2009).

The usefulness of *A. mellifera* as extraterrestrial pollinator will depend on the correctness of the colony development after space travel. This article presents a case study of the development of the honey bee colony with the queen bee exposed to a rocket launch acceleration pattern generated by the large-scale centrifuge.

Materials and Methods

Four artificially inseminated sister honey bee queens (*A. mellifera carnica*) were given to the acceleration pattern of the launch of the *Soyuz*-type rocket in the Human Training Centrifuge at the Military Institute of Aviation Medicine in Warsaw, Poland. For experiment time, both the test and control sample queens were enclosed in separate queen mailing cages with their attendants and placed in specially designed devices that enabled their observation and ensuring identical, stable conditions throughout the centrifugation time. After centrifugation, along with four noncentrifuged sister queens from the control sample, they were given to the experimental hives for observation and data gathering. During regularly performed controls, from August 2021 to April 2022, data on the number of cells occupied by food stores, eggs, larvae, and

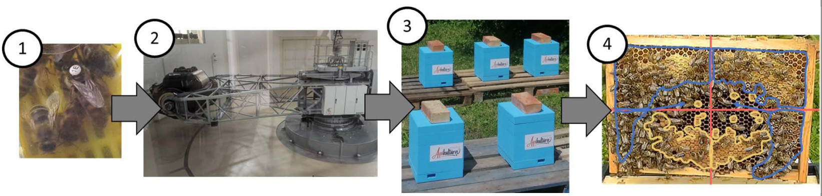

FIGURE 1
Experiment main events scheme. 1: Obtaining artificially inseminated sister queens and placing them in separate queen mailing cages with attendants; 2: Dividing queens into test and control samples, placing them accordingly into the experimental device, and centrifugation of the test sample to the acceleration pattern of the *Soyuz* rocket launch; 3: Transferring all the queens to experimental hives; 4: Observation and collection of data on the number of cells occupied by food stores, eggs, larvae, and pupae.

pupae were gathered using the variation of the *Liebefeld method* (Imdorf et al., 1987). Before the overwintering period, the colonies were fed invert syrup and weighted for two consecutive weeks to obtain the appropriate weight. Fig. 1 shows the most important stages of the experiment performance.

Results

The total observation time of colony development was 270 days. Only two out of four queens from each sample developed colonies suitable for analysis. The rest of the queens did not start laying eggs or were not accepted into the colony (both for the test and for the control sample). All colonies followed a similar development pattern, as shown in Fig. 2.

Some differences are visible for test sample 1 - the number of eggs increased more rapidly, and more drone pupa were noticed. Furthermore, a sudden drop in the number of stores was observed during the last control before the overwintering (Fig. 3), while keeping the colony mass comparable to the other.

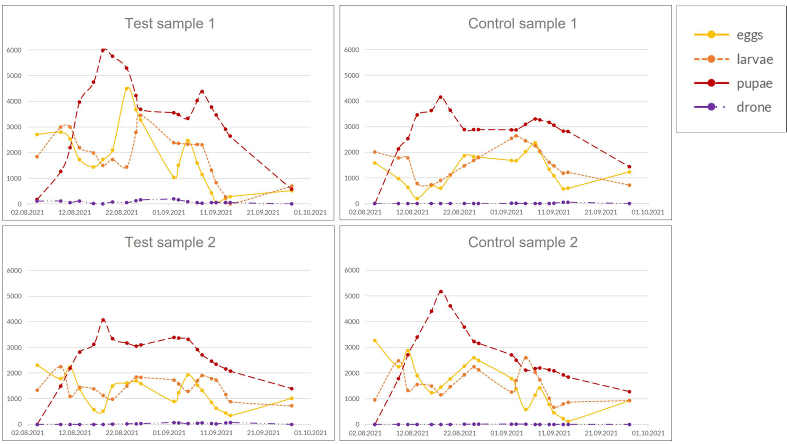

FIGURE 2

Total number of honeycomb cells in the hive occupied by various stages of the development of honey bees (x-axis: time, y-axis: number of cells occupied).

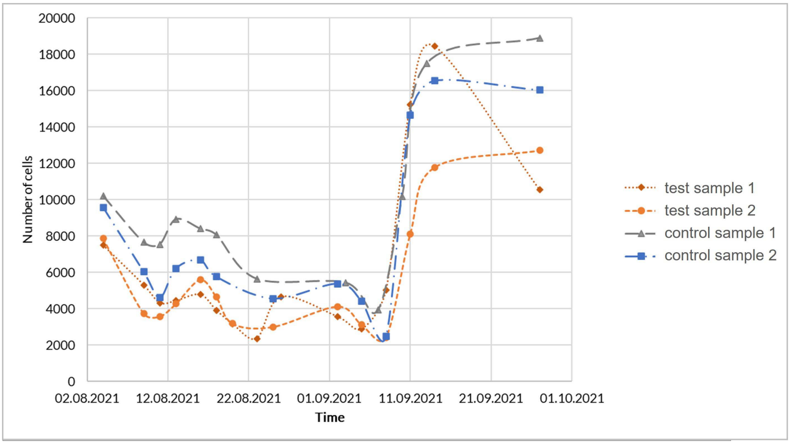

FIGURE 3

Total number of honeycomb cells in hive occupied by food stores - visible decrease for test sample 1 .

All colonies successfully survived overwintering and the queens started egglaying in the spring.

Discussion

Described experiments were the first to focus on the hypergravity link to the later condition of honey bee colony (*A. mellifera)* and its development. As such, research was not without flaws, which, in analogous experiments realised in the future, should be eliminated.

Apiary Organisation and Procedures

All hives prepared for settling by examined specimens, while not composed only of empty combs, should have precisely noted numbers of eggs, larvae, and pupae and the number of food stores present in the hive at the beginning of the research. Such data enables reliable analysis of the composition of the hive with exclusion of the “settling bias”. Whenever possible, beekeeping operations, such as hives weighting, should be automated, saving time and enabling required measurement frequency. The number of controls performed on each colony, according to the *Liebefeld method* (Imdorf et al., 1987; van Bargen et al., 2020), can be as low as 1 every 3 weeks.

Sample Size & Observation Time

A small number of tested queens in the presented research prevented one to draw statistically significant conclusions from the research. However, as the *BeeO!Logical* payload was found useful for honey bee hypergravity testing, the device should be enlarged or its several copies should be used in the future to ensure stable experiment conditions for all the examined samples. The matter is more important as queens may not be accepted to the colonies because of the experienced stress. Notes on all the rejections, their exact times and probable reasons should be made throughout the apiary observation time. Rejected queens may be given to the tests impossible to perform on healthy specimens, such as ovaries examination requiring killing specimens prior to testing. Analogous tests may be performed for all rejected queens and for all the queens after the required observation period.

As the beekeeping season in 2021 started late, artificially inseminated queens were available with a significant delay, which caused shortened observation time. Seasonality impact could be mitigated by conducting research in latitudes with more stable weather conditions or in an artificial environment such as an enclosed greenhouse.

Acceleration Pattern

The *Soyuz*-type rocket acceleration pattern was used because it was the most popular model for space transportation at the time. However, as the final choice of the actual transportation device may change because of various reasons, ideally it would be to use a probable pattern of a rocket with a Moon/Mars flight range. Another option is to examine subsequent G values separately. In such a way, it could be possible to establish boundary conditions that may permanently impair *A. mellifera* reproductive traits. Another approach worth considering could be examining specimens for specific flight event values, predicted in mission handbooks prepared by space agencies such as NASA.

Conclusion

All tested specimens which started laying eggs were able to develop fully functional colonies. However, test queen 1 was less consistent with the egg-laying ratio and changes in its activity were more rapid and extreme – further investigation on the reason for such behaviour is required. Moreover, accelerated queens started to lay drone eggs earlier than nonaccelerated sisters. Such an observation might suggest that the reproductive traits are somehow altered by the hypergravity, causing such behaviour to occur. The reason for the inactivity of the queens excluded from the analysis remains an open question. Due to the exclusion of the same number of specimens from both samples, it was assumed that it was caused by individual variability; however, further investigation might prove otherwise, finding signs of trend for the species.

The research enabled identification of important areas of special attention for future similar experiments. One of the critical areas is the observation of the colony's introduction into the subsequent season, the strength of the colonies after overwintering, and the verification of its propensity to enter the swarming state. Addressing the recognised issues would affect positively obtained results, leading to more conclusive and reliable data production.

Acknowledgements

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The implementation of the educational programs for space medicine in Japan

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Abstract
Recently, space agency, such as NASA and JAXA, has the plan to send human to Moon or Mars. And, astronauts must spend time in closed environment during human space mission. So, we need to know the physical and mental effects during space mission. Therefore, it is very important to understand how we are affected by the space environment (such as microgravity, space radiation etc.) during space staying and what we should do for staying in other planets. To recognize the important factors for future space mission, we established the educational programs for space medicine, and we are carrying out.

This educational program consists of the lectures and the hands-on training. In the lectures, student can learn the physiological effects on the human body during the spaceflight. The lectures include many topics such as the muscle atrophy, bone loss, motion sickness, fluid shift, mental stress, space radiation and so on. In space, the microgravity induces the muscle atrophy

and bone loss. Therefore, astronauts must perform the exercise for about 3 hours/day in International Space Station (ISS). By the understanding of these topics, students will learn the common factors of the physiological changes on ISS and the phenomena of aging on the earth. In the hands-on training, we will provide the opportunity of performing the bedrest study. In the bedrest, subjects will have various physiological changes in the reality. They may have the muscle atrophy and bone loss, cardiovascular effects, fluid shift and so on. In the bedrest study, student can learn the various physiological changes by the decreasing of the mechanical stress on the leg or body.

In our educational programs for space medicine, students can learn many necessary knowledges for future human space mission. Therefore, this program must be very important opportunity to inspire the students to be interested in space medicine.

Introduction

More than 600 astronauts have carried out space missions through the Apollo program, the Space Shuttle program, and the International Space Station (ISS) program. there is Space businesses such as private space travel are also becoming a reality. Under such circumstances, it is a basic human right for human beings to lead a healthy life, and the same is true in the space environment. In order for people with various backgrounds to stay in space safely and securely and return to Earth, many technologies and human support are essential. Support from a wide range of fields such as doctors, physiotherapists, and laboratory technicians is required. For the health care of human beings in space, it is necessary to develop human resources who can understand the peculiarities of the space environment and can practice medical knowledge and technology. In addition, the psychophysiological support and ethical perspectives is essential, and it is necessary to provide comprehensive and complex education.

The reality is that there are few medical practitioners and researchers involved in space medicine in Japan, but many young people have a desire to learn space medicine. Therefore, it is necessary to establish a space medicine education program and work on human resource development related to space medicine. In this program, through the implementation of space medicine training and research on a large scale of human subjects, consideration of ethical aspects, acquisition of research methods, data analysis, and summary of results will be experienced, and more practical knowledge and skills will be acquired. In this way we will implement an educational program to develop human resources who will be ready to work in support of future manned space activities.

Educational Program Content

Specifically, in this educational program, 1) Learning the effects on the human body that occur in a microgravity environment through space medicine lectures and countermeasures, 2) Through simulated microgravity exposure experiments, practical understanding and measurement of the effects on the human body, and acquisition of methods and analysis methods, 3) Research exchange by visiting space medicine laboratories nationwide, 4) Construction of a space ethical perspective centered on bioethics, 5) Exchange with space-related academic societies, companies, and institutions through practical human exchanges.

In this program, we aim to further revitalize young human resources in the field of space medicine and promote cooperation with fields other than medical fields, and build strong collaboration with academia. The results of this program will be actively presented at annual conferences of each academia, enlightenment of space medicine research and medical education, and broadening and solidifying the foundation of the field of space medicine that supports Japan's manned space development.

Summary of Implementation Contents

We constructed the lecture "Manned Space Medicine" as a graduate school interdisciplinary course at Kyoto University. Humankind's expansion into space is becoming more and more active, especially with technological development. Human stays in space are becoming longer and longer as the space program shifts from the Space Shuttle to the International Space

Station. However, it has not been fully clarified what kind of influence human beings, who have evolved to adapt to the earth's environment, will have when they stay in the space environment. For this reason, the purpose of this lecture is to train human resources who will learn about the effects of staying in space on humans, and who will engage in the clarification of countermeasures in the future, in preparation for future manned space activities.

As laboratory tour, students from universities nationwide will be invited to visit laboratories for the purpose of observing space medicine research sites and learning about experiments, research, and development that are actually being carried out. In 2022, we visited 6 places (IHI Aerospace, Jikei University School of Medicine, Tokushima University, Kurume University, Fukuda Denshi Co., Ltd., Tokyo University of Science)(Fig.1).

Preparations were made to conduct a simulated microgravity exposure experiment (bedrest experiment) in the winter of 2023. Bedrest experiments are scheduled to be carried out at Gifu University of Medical Science. For preparation, we visited Gifu University of Medical Science and confirmed facilities and equipment for conducting bed rest experiments. In Kyoto University, we also purchased an electromyograph (FreeEMG1000), a motion

FIGURE 1
The scenes in the space medicine laboratory tours.

FIGURE 2
The Electromyograph (FreeEMG1000), and the motion analysis camera system (MICROCAM200-4).

analysis camera system (MICROCAM200-4), and an electrocardiograph (Bittium Faros180) for use in bed rest experiments, and prepared for measurements in the next year (Fig. 2).

Discussion

Since FY2022 was the first year of this commissioned project, preparations for building a space medicine education program were the main focus. However, since the Kyoto University Space Synthesis Research Unit already has experience in space medicine lectures and space medicine training, it seems that by using that foundation, it was possible to provide students with lectures and training smoothly to some extent. Regarding space medicine lectures, we were able to provide a fulfilling lecture. Until now, we have only had two experiences with space medicine training, the Jikei University School of Medicine and Gifu University of Medical Science, but this year, we have visited more places, including IHI, Tokushima University, Kurume University, and Fukuda Denshi Co., Ltd. We were able to set up five new locations for the company and the Tokyo University of Science. The problem was that it took a long time to adjust the schedule, so we were not able to provide sufficient information to the participating students in advance, and it was difficult to secure the preparation time. Regarding this point, we plan to make adjustments with sufficient margin in the next fiscal year. Preparations for the simulated microgravity exposure experiment (bedrest experiment) to be carried out next year went smoothly thanks to the efforts of Professor Tanaka of Gifu University of Medical Science. Gifu University of Medical Science has already secured the site and prepared the equipment to be used. At Kyoto University, preparations for the measurement equipment to be used have been completed, and we are very much looking forward to conducting the bedrest experiment next year. A unique aspect of this program is that it collaborates with space ethics commission fees from the perspective of bioethics. We were able to have a deep discussion with him about life and death in space. We are planning to hold joint workshops in the next fiscal year, and we would like to further discuss the importance of ethical perspectives and various fields not only for space medicine but also for manned space activities. As for outreach activities, we were able to participate in academic conferences in Japan and overseas, hold seminars, visit classes, and conduct international exchanges. With the cooperation of many researchers in Japan and overseas, we would like to continue holding attractive exchanges and events in the field of space medicine.

A bedrest experiment is scheduled to be conducted next year, so we would like to utilize this valuable experiment and practical training opportunity to provide content that can contribute to human resource development more than this year.

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μIMMUNE – Development of automated microfluidic immune monitoring for spaceflight: A first report

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Introduction

The human immune system is highly responsive to exogenous stress factors as revealed by studies on astronauts and overwintering crews in Antarctica (Crucian *et al.*, 2016, Mehta *et al.*, 2017, Buchheim *et al.,* 2020). Such stress factors associated with spaceflight are microgravity, isolation, confinement, disrupted circadian rhythm, changes in nutrition and the human microbiome, lack of exposure to natural sunlight, increased exposure to radiation, and the psychological pressure of high expectations, risk of failure and social constellations.

Consequentially, the incidence of symptoms (Crucian *et al.*, 2016) or reactivation of latent viruses can increase while in space (Mehta *et al.,* 2017). These observations are in line with altered cytokine shedding and correlate with a shift towards an immune response profile normally associated with Th2-mediated immunity (Mehta *et al.,* 2013).

However, these dysregulations have so far only been detected after completed missions. Therefore, there is a need for close-meshed, real-time monitoring of both innate and adaptive immunity, as these are not equally affected (Kaufmann *et al.,* 2012, Crucian *et al.,* 2018).

This is especially important regarding future missions of extreme length, where crew health maintenance is the major bottleneck restricting their implementation.

The µIMMUNE project sets out to develop an automated, real-time, microfluidics-based *ex vivo* assay for immune-relevant analytes for spaceflight or antarctic missions. This monitoring assay will be established on the PowerBlade platform: an antigen incubation chip for whole blood, generating the samples for subsequent fluorescence-based multiplex cytokine detection on a separate chip.

On the PowerBlade, microfluidic chips are installed on a centrifugal platform (figure 1) which features integrated and programmable pneumatic pumps

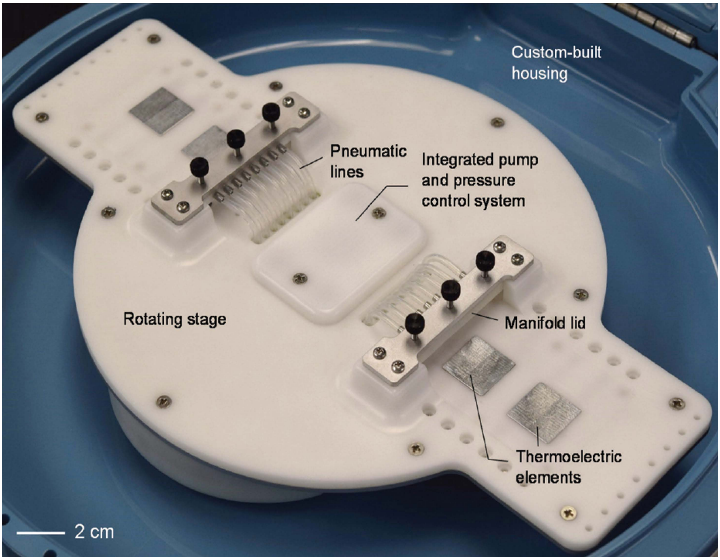

FIGURE 1
The PowerBlade platform with two cartridge spaces, each one with an eight-port manifold for pressurization or venting and two thermoelectric elements on a rotating stage (Geissler *et al.,* 2020).

for pressure control (Brassard *et al.,* 2019, Geissler *et al.,* 2020). This includes pressurizing or venting the chip and generating pressure differences to allow for flow switching, inward pumping or bubble-mediated mixing on top of centrifugation. With an unprecedented level of control and without the need for active components on the microfluidic chip itself, they are cost-effective and their design favors minimization of sample volume, hands-on working time and expert resources, also due to it's remote control feature.

Materials and Methods

This work is funded by German Aerospace Center (DLR) grant 50WB2222 and approved by the local Ethical Committee of LMU Hospital Munich (20-632).

Incubation cartridge manufacturing

Incubation chips were manufactured from Zeonor ZF14-188 polymer (Zeon specialty Materials), cut into blocks of 50x100x6 mm and with precisely drilled incubation chambers. These were sealed with 0.5 mm thick thermoplastic elastomer Mediprene OF400M sheets (Hexpol TPE) and another thin layer of Zeonor with punch holes (2 mm diameter). Manufacturing was performed in clean rooms (class 1000) and completed chips were exposed to UV radiation for 20 minutes.

Whole blood incubation

Whole blood incubation on chip and in corresponding cell culture setting was performed with heparinized whole blood from venous puncture, diluted 1:2 in unsupplemented RPMI-1640 media (Sigma-Aldrich #R7388), sealed airtight and incubated for six hours at 37°C in upright position before collection of plasma and cell residue, respectively. Cell residue was treated with TransFix (Cytomark #TFB-01-50), stained with Fixable Near-IR Dead Cell Stain (ThermoFisher #L10119) according to manufacturer's instructions with subsequent red blood cell lysis (FACS Lyse, BD #349202), two wash steps and measured by flow cytometry (Guava easyCyte 8HT, Merck). Analysis was done using guavaSoft3.3 InCyte Software (Merck) by gating for singlets based on forward scatter (FSC) area/width, lymphocytes based on FSC and side scatter (SSC) and live and dead cells using Near-IR signal.

Micropillar fabrication and functionalization

Micropillar scaffolds (Zeonor ZF14-188, Zeon specialty materials) were produced as previously described (Geissler *et al.,* 2022). Scaffolds were treated with ionized oxygen, incubated with 2 % (v/v) (3-aminopropyl) triethoxysilane (Sigma-Aldrich) for one hour, rinsed (PBS-T 0.05 % (v/v)) and dried with nitrogen airflow before incubation in 2 % (v/v) glutaraldehyde (Sigma-Aldrich) for one hour. After, 200 nL of respective capture antibodies (50 µg/mL) were added and incubated overnight in humid environment. Scaffolds were then rinsed with 1x Casein and covered in 1 % (v/v) ethanolamine (Sigma-Aldrich) for 5 minutes followed by another blocking step with 10x Casein (Sigma-Aldrich), several rinses and final drying with nitrogen.

Cytokine detection assays
Functionalized arrays were attached to a Mediprene sheet and a thin Zeonor sheet. Luer connectors in epoxy resin served as access points and were connected to tubing (inner diameter 1.02 mm, outer diameter 2.16 mm) and a syringe pump.

Recombinant IL-2 and IL-6 control samples at different concentrations (20 pg/mL to 200 ng/mL) were pushed unidirectionally through the flow chip (speeds: 15 µL/min to 200 µL/min), followed by washing with PBS-T 0.05 % (v/v) at 1000 µL/min, then biotinylated anti-IL-2/anti-IL-6 polyclonal goat IgG detection antibody (5 µg/mL each, BioTechne #BAF202 and #BAF206). After another wash, 20 µg/mL Streptadivin-Cy3 (Amersham) was pushed through the chip at 35 µL/min followed by one last wash.

The same procedure without flow chip and pump operation was performed for the dualplex cytokine assay with the following incubation times: recombinant interleukins for one hour at 37°C, detection antibodies for 30 minutes at 37°C and Streptavidin-Cy3 for 30 minutes.

Images were obtained by fluorescence microscopy using 550 nm excitation.

Results

Incubation on a simplified microfluidic chip does not impair cell viability
For whole blood incubation, test chips (figure 2) were designed to perform a previously established whole blood stimulation assay in drastically downscaled volumes. First experiments showed that cell viability was unaffected by the small reaction volumes as well as the chip material when compared to cell culture incubation (figure 3).

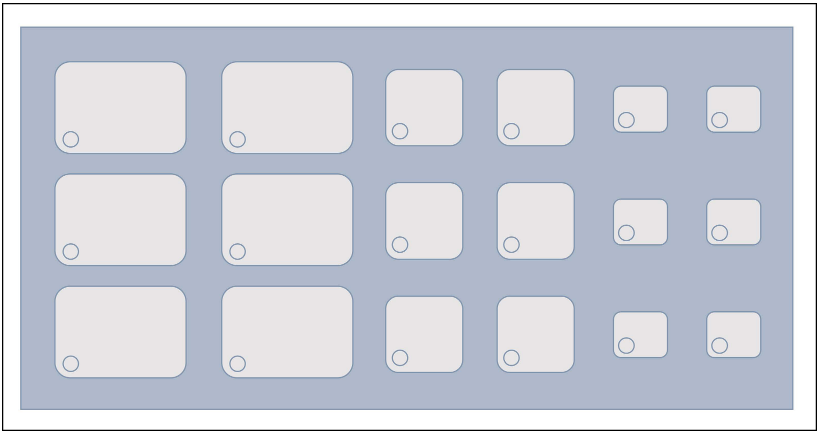

FIGURE 2
Simplified cartridge for whole blood incubation with varying chamber sizes.

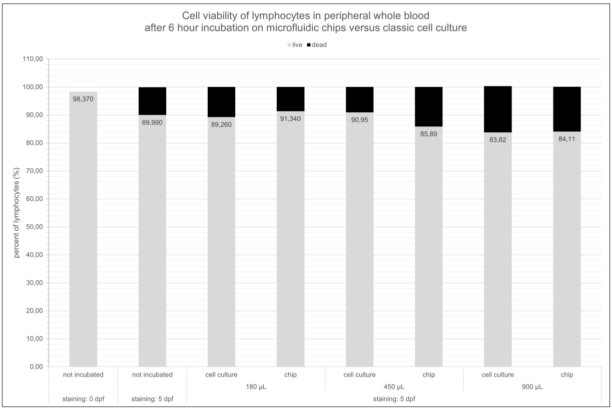

FIGURE 3
Cell viability of peripheral lymphocytes in whole blood incubated on chip and under cell culture conditions in different volumes. For reference, a fraction was not incubated but fixated and stained on 0 days post fixation (dpf). The same fixated sample was stained again on 5 dpf together with incubated samples. Shown are means of technical replicates.

Spatially indexed fluorescence-based cytokine detection forms basis for a single-color multiplex assay

For cytokine detection, a proof-of-concept experiment showed that spatially indexed multiplexing may indeed be suitable, as we did not observe any signal interference or cross-detection in the dualplex setting (figure 4A). In this setting, we used fluorescence-based detection using Strepavidin-Cy3, as our previous experiments using Streptavidin-Horseradish peroxidase (HRP) and unsoluble 3,3′,5,5′-tetramethylbenzidine membrane peroxidase substrate (TMB) performed less well.

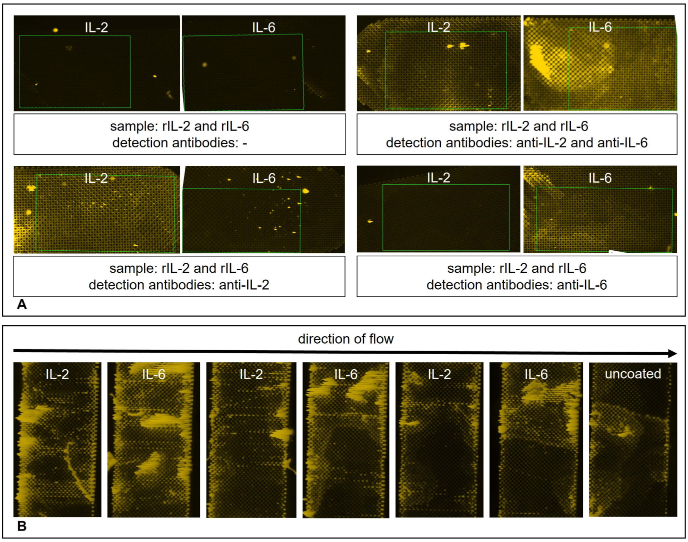

FIGURE 4

Fluorescence imaging of Cy3 signal on cytokine detecting micropillar arrays. Arrays were functionalized as labeled. **A** Images of arrays (green rectangles equal 1.1 mm^2) after incubation to test for cross-detection in a dualplex setting. Arrays were incubated with recombinant interleukins and detection antibodies as captioned. Not shown: all combinations involving interleukin single and blank controls, detection antibody single and blank controls and non-coated scaffolds. **B** Images of arrays on dualplex flow chip.

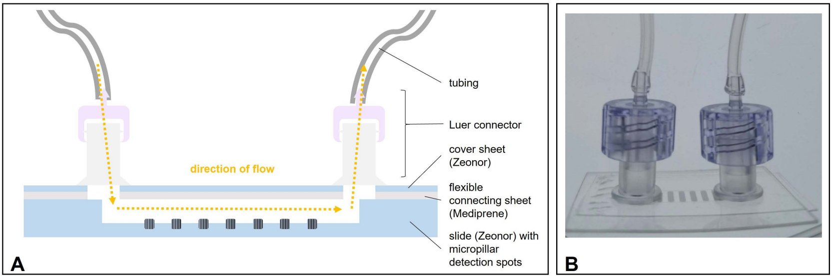

FIGURE 5
Microfluidic chip with cytokine detecting micropillar arrays. **A** Cross-sectional view of design. **B** Photograph (side view).

Additionally, we constructed manually operated chip elements (figure 5) that closely mimic the microfluidic flow that will eventually be controlled through centrifugation and pneumatic pumps on the PowerBlade.

Reliable quantification of fluorescence intensities was not possible, since some micropillars in the arrays were bent during flow chip assembly, thus artificially increasing signal although it seemed that overall fluorescence of each array decreased in the direction of flow (figure 4B).

Discussion

Since the downscaled volumes did not affect cell viability for short-duration incubation experiments, different stimulating antigens will be introduced next to investigate cellular activation behavior on the chip. The validating readout is comprised of cell surface marker stainings in addition to detailed cytokine profiling and compared to corresponding reference settings.

Simultaneously, the chip designs are continuously developed further and validated in the same way. Our new design contains similar incubation chambers but also a channel system to allow for a simulated microfluidic handling similar to the micropillar chip elements with syringes as a predecessor of automated chips running on the PowerBlade.

Regarding cytokine detection, we had to explore possibilities to multiplex with only single-color readout that will be available on the PowerBlade. Our experiments regarding spatial indexing look promising and upscaling towards eight-plex approach is ongoing. Since there is no tolerance for any cross-reactivity in this setting, extensive testing of every analyte combination is inevitable.

The flow chip elements (figure 5) are used for testing the microfluidic processing of this method in a semi-automated way and serve as a second validation after the aforementioned cross-reactivity tests. Our first dualplex flow chip experiments require repetition to optimize signal homogeneity within each array by adjusting the micropillar functionalization. Also, bidirectional flow is necessary to achieve more even detection between arrays.

Conclusion

Despite the laborious development of an automated immune monitoring system like µIMMUNE, its advantage of simple operation and real-time data will, once completed, greatly benefit the crew health monitoring in space or Antarctica by observing dynamics of immune dysregulation.

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Ageing and altered gravity; A cellular perspective

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Introduction

Astronauts and the elderly experience similar ageing symptoms. Osteoporosis, muscle atrophy, and a weakened immune system among other things. Although quite some review papers compare the signs of the higher-level systems in the body, like tissue and organs, relatively few analyses have been done on a cellular level. This literature study investigates the relationship between ageing and altered gravity from a cellular perspective. As a basis of comparison, five key papers describing the hallmarks of ageing were selected. From these literature studies we extracted the items for ageing at cellular level and used PubMed and Web of Science to search for gravity-related research performed on the particular items.

Material and Methods

Based on the works from López-Otín C. et al. (2013), Schmauck-Medina T. et al. (2022), Bajpai A. et al. (2021), Phillip J. M. et al. (2015), Starodubtseva M. (2011), we extracted 209 variables which alter during ageing. The observations were categorized in one of the eleven themes: DNA & epigenetics, Mitochondria, Nucleus, Immune system, Protein & metabolism, Lysosome & degradation, Cell cycle, Cytoskeleton, Extracellular Matrix (ECM), Cell mechanics and Cell signaling (see Figure 1).

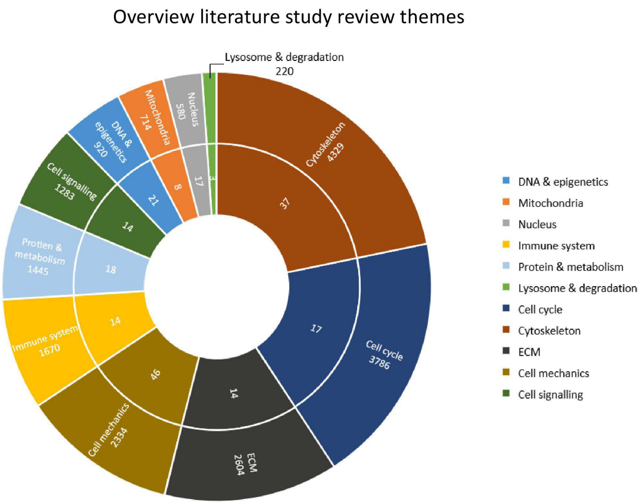

FIGURE 1
Overview of the themes identifies for this literature study. The inner circle shows the number of variables which alter during ageing per theme. The outer circle displays the themes and their relative size based on the number of references in gravity-related research on that theme in either PubMed of Web Of Science. ECM: Extracellular Matrix.

Each characteristic was evaluated for gravity-related research. The following search term was used in Web of Science and PubMed: (subject) AND ((Gravity) OR (hypergravity) OR (microgravity) OR (micro-gravity) OR (hyper-gravity) OR (unloading) OR (mechanical loading) OR (spaceflight)) NOT (radiation)). Radiation was excluded to obtain more refined results with a focus on altered gravity instead of radiation. Of course, any research performed in spaceflight could be affected as well by radiation. For each of the 209 variables the number of relevant gravity related peer reviewer papers were scored and ranked. Broad genetic screening was outside the scope of this literature review.

Results

A summary of our results is provided in Figure 2. It is shown that most of the identified characteristics of ageing in the cell have not been investigated yet in altered gravity. Nevertheless, in general, it seems that ageing and (simulated) microgravity have many similarities in cell behavior. However, there appears to be a surprisingly large number of opposite effects. Negatively correlated responses between ageing and (simulated) microgravity seem to appear especially in the themes of Cytoskeleton, Cell Signaling and to a lesser extent Extracellular Matrix and Cell Mechanics. Most similar effects were observed for Protein & Metabolism, Mitochondria, and Lysosome & Degradation. For many themes, it looks like that a lot of ageing related items have not been investigated in altered gravity, in particular, the themes Nucleus, DNA & Epigenetics and Cell Mechanics have a large gap in knowledge.

There are several methods to investigate altered gravity. Simulated microgravity: clinorotation, random positioning machine, bed rest, and hindlimb unloading were the most applied methods. Unexpectedly, despite the high cost of real microgravity experiments (spaceflight and parabolic flight), there appear to be more experiments in microgravity than in hypergravity.

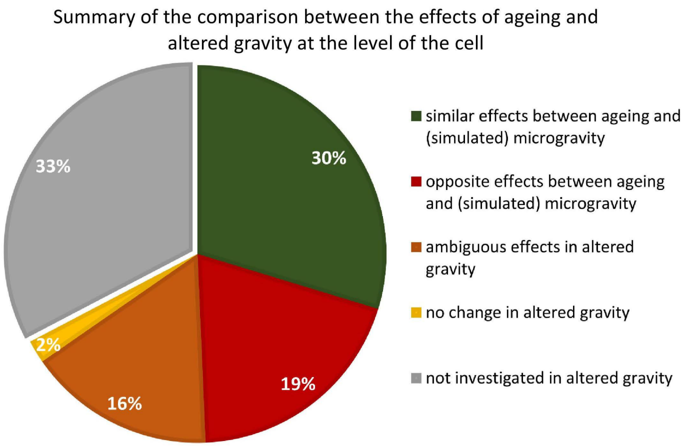

FIGURE 2
For all 206 identified characerities of ageing at the level of the cell, the comparison between the effects of ageing and altered gravity are summarised.

From our data collections we also established the following points of attention when researching the effects of altered gravity. Simulated microgravity does not per definition result in the same outcome as real microgravity. Our results seem to imply that for multiple items the effects for simulated and real microgravity were not always in line. Furthermore, we noticed that for some items a difference in behavior is seen between immortalized cell lines and primary cells/ *in vivo* studies.

Discussion

Ageing and (simulated) microgravity seem to have similar phenotypes for higher-system/organ level and to some extent also at a cellular level. We did find, however, a relatively large number of opposite responses as well. One of the main differences between ageing and microgravity is reversibility. While astronauts experience a lot of ageing phenotypes, after considerable revalidation, most effects will eventually be reversed. For ageing this is not the case. López-Otín et al. describe how ageing originates as damage in the primary hallmarks like genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis. A response to this damage are the antagonistic hallmarks which include deregulated nutrient sensing, mitochondrial disfunction and cellular senescence. Initially the antagonistic hallmarks will counteract the damage, but with time they become detrimental themselves (López-Otín et al. 2013). Finally, these hallmarks result in integrative hallmarks, like stem cell exhaustion and altered intracellular communication, that are responsible for the ageing phenotype. We suspect that altered gravity changes the signal translation of the mechanical environment. If altered gravity causes altered intracellular communication, this could result in a response of antagonistic hallmarks. Thereby creating a smaller, kind of vicious circle between antagonistic and integrative hallmarks. As the primary hallmarks are left outside this cycle, the loop will not persist after returning to Earth and restoring signal translation. As mentioned before, the themes Nucleus and Cell mechanics, which are key players in signal translation, are relatively poorly studied in altered gravity and should be investigated further.

Conclusion

Ageing and (simulated) microgravity both seem to create a vicious circle of ageing hallmarks at a cellular level. We suspect that the difference in reversibility lies in the origin of the cycle. We presume that altered intracellular communication is responsible for the ageing phenotype seen in astronauts, which upon return to Earth is restored. We recommend further research in the areas of signal translation of the mechanical environment in altered gravity. Furthermore, we occasionally noticed a difference in effects between simulated or real microgravity and between immortalized cell lines and primary cells/*in vivo*. We recommend using real microgravity as applied method and primary cells or *in vivo* setups as subject models for gravity-related research.

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Study of vascular hemodynamics in healthy men undergoing 21-day antiorthostatic hypokinesia

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Introduction

Among the actual problems of modern space medicine, an important place is occupied by the study of the principles of the circulatory system regulation and functioning under the influence of space flight (SF) factors on the human body. The key factors affecting vascular hemodynamics in the spacecraft are microgravity, overloads during the launch and descent of a manned spacecraft (up to 7 g) and mission duration (from several days to a record 437 days).

There is an assumption that conditions the transmural pressure decreases below the hydrostatic indifferent point and increases above it increases in microgravity, which, apparently, is reflected in the restructuring of the vascular hemodynamics regulation [Yegorov A.D., 2007].

Study of vascular hemodynamics in healthy volunteers in analogue experiments demonstrate the presence of quite pronounced changes in the vascular bed [Arbeille P. et. al., 1999; 2022]. But systematic investigations of the state of the vessels of the inferior vena cava system under conditions simulating the SF factors have practically not been carried out previously. It should be stressed that the venous system of the human lower extremities has a number of anatomical features: large absolute capacity of the venous bed; the presence of two independent, interconnected systems (interfacial and deep); complex multichannel structure of the bed in the joint area; the presence of various connecting vessels; the specific structure of the vein wall and the presence of reserve and backup mechanisms in ensuring the relative stability of venous blood return [Schwalb P.G., Ukhov J.I., 2009]. At the same time, a number of researchers recognize the existence of an interdependent continuous process of remodeling of the bed of the inferior vena cava system.

Thus, studying the state of the vessels of the inferior vena cava system will make it possible to assess both the individual reactions of the body and identify general physiological manifestations of adaptation, as well as to build a further strategy for the prevention of medical risks during SF, in particular, such as thrombosis in the superior and inferior cava systems.

Materials and Methods

General protocol

The experiment was carried out under HDT -6^0 conditions in the Unique Scientific Facility of the Institute of Biomedical Problems (Moscow, Russia). The study included 12 healthy, no bad habits men (age 29.8 $\pm$ 4.6 years, weight 75.2 $\pm$ 8.8 kg, height 177,8 $\pm$ 5.3 cm, BMI 23.8 $\pm$ 2,7 kg / m^2). The conducted studies were approved by the Bioethical Commission of the

Institute of Biomedical Problems of RAS (Protocol No. 599 of 06.10.2021, Protocol No. 621 of 08.08.2022) and fully complied with the principles of the 1964 Declaration of Helsinki. Each study participant voluntarily signed an informed consent after the potential risks, benefits and nature of the upcoming study were explained to him.

Each participant was staying in a specially equipped bed for 21 days. To carry out sanitary and hygienic procedures and also some research methods, it was necessary to transfer the test subjects on a special lifting mechanism to a horizontal position with support on their backs. For defecation, the test subjects were transferred to a sitting position for no more than 10 minutes per day. A constant air temperature (24°C - 25°C) and humidity (40% - 50%) were maintained in the room. During the experiment, fluid intake was recorded in the food diaries, the diet was standardized. The recommended daily caloric intake was 2315 ±117 kcal/day in accordance to WHO recommendations. The experimental conditions are described in detail in the article by A. Puchkova et al. (2023).

The candidates' selection criteria for this study were the absence of a history of venous system diseases (post-thrombotic disease, varicose veins disease), endocrine disorders, and the absence of taking antibiotics and hormonal drugs for 3-4 months before the launch of the experiment. All 12 volunteers met these criteria.

Procedure

The study of venous hemodynamics was carried out in the morning from 8 to 9 am (fasting), once before the beginning of HDT (BDC) – on the 6th, 13th, 20th day of HDT, and on the 7th day (R + 7) after the end of the experiment.

The "GE Logiq e" device was used: 220 V power supply, 2 sensors (3.5 and 7.5 MHz). All test subjects underwent ultrasound scanning of the neck vessels in B-mode, continuous wave Doppler and color Doppler imaging, with a linear sensor of 7.5 MHz. Before the study, a test subject was at rest for 5 minutes, in the supine position, the neck was extended. Scanning of the common carotid artery andinternal jugular vein (CCA, IJV) was performed with an anterior approach - in front of the sternocleidomastoid muscle in the projection of the upper edge of the thyroid gland. Ultrasound examination of the inferior vena cava (IVC), common iliac vein (CIV), internal iliac vein (IIV), external iliac vein (EIV) was performed in a horizontal position using a 3.5 MHz convex sensor in B-mode and color Doppler imaging mode, in typical "ultrasonic windows" [Lishov D.E. et. al., 2021]. To study the common femoral vein (CFV) and great saphenous vein (GSV), a 7.5 MHz linear sensor was used in B-mode and color Doppler mapping mode; the sensor was installed in the groin region in the projection of the sapheno-femoral junction. The study was performed by one certified ultrasound doctor.

Data Analysis

Statistical analysis of the data was done using the Statistica for Windows 13.5 software. The significance of differences was estimated using Wilcoxon's test.

Results

A statistically significant decrease in the diameter of the inferior vena cava (Figure 1) while the test subjects were staying under conditions of antiorthostasis, and subsequent recovery to background values in the aftreeffect period, as well as an increase in the medial-lateral diameter of the right internal jugular vein (Figure 2) in the aftreeffect period ($p<0,05$) were recorded.

Noteworthy is the statistically significant decrease in the diameter of the right common femoral vein (Figure 3) with a simultaneous decrease in the diameter of the right common femoral artery ($p <0.05$) (Figure 4).

Along with this, significant changes in the diameters of the aorta and common femoral artery were noted ($p < 0.05$) during the period the subjects were in HDT.

Discussion

Antiorthostatic hypokinesia (HDT) is one of the generally accepted experimental model that makes it possible to study the influence of SF

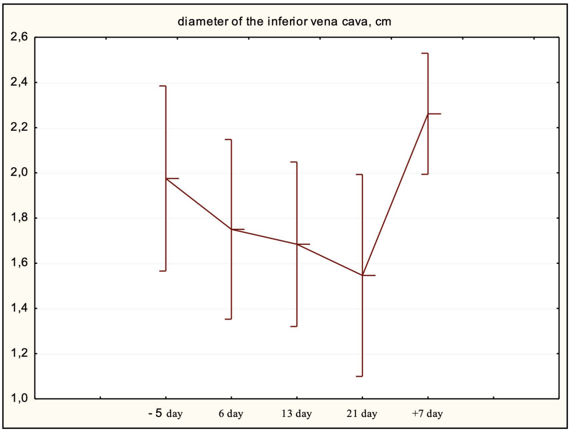

FIGURE 1
Dynamics of changes in the diameter of the inferior vena cava (significant differences between the background and 6th,13th, 21nd days of HDT, between the background and +7th day).

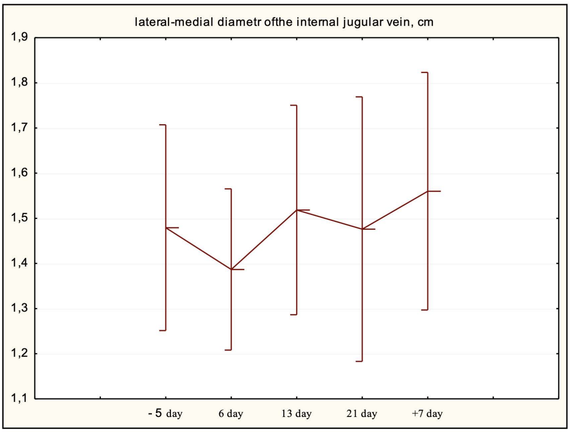

FIGURE 2
Dynamics of changes in the lateral-medial diameter of the right internal jugular vein (significant differences between 6 and +7 days).

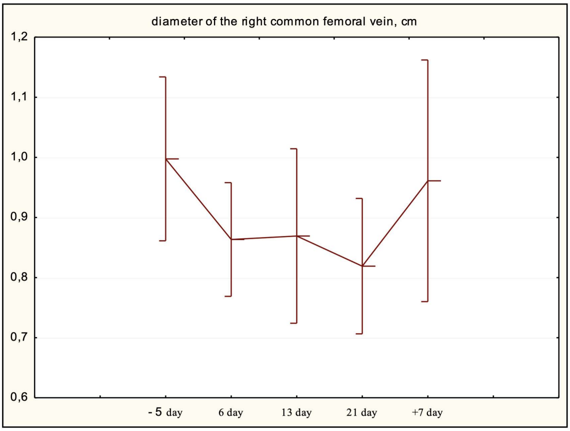

FIGURE 3
Dynamics of changes in the diameter of the right common femoral vein (significant differences between the background and 6th day, background and 13th day).

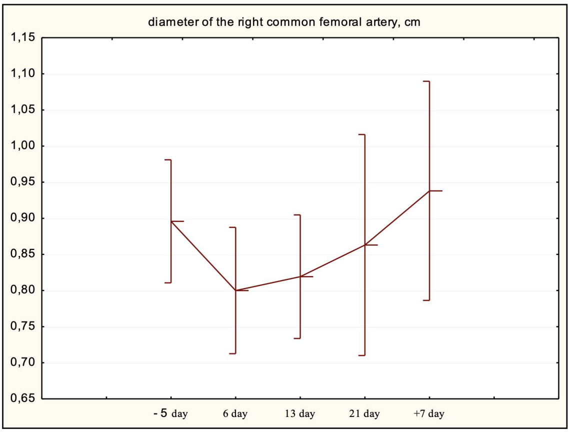

FIGURE 4
Dynamics of changes in the diameter of the right common femoral artery (significant differences between the background and 6th day).

factors (in particular, altered gravity) on the structural and functional restructuring of the cardiovascular system in terrestrial conditions.

The obtained US-angioscanning results indicate remodeling of blood flow from the lower extremities to the head, which in the early adaptation period (up to 7 days from the start of HDT) can provoke the appearance of headache symptoms in volunteers, and with prolonged stay in SF conditions lead to more serious changes in vascular parameters and blood flow.

Our hypothesis is also confirmed by studies conducted both in SF conditions [Marshall-Goebel K.et al., 2019] and in analogue experiments. In the work carried out by IBMP researchers the data on the state of the brachiocephalic vessels was obtained and a decrease in the speed and volume of blood flow in the external and internal carotid arteries, as well as a decrease in the cross-section of the jugular veins were revealed when test subjects were staying in a 60-day HDT at -6^0 [Tikhonov M.A. et al., 2003]. In the same experiment, an increase in the diameter of the inferior vena cava, common portal vein, superior mesenteric and splenic veins was noted, which was accompanied by increased pulsation of all epigastric vessels [Afonin B. et al., 1999]. Ultrasound signs of venous stasis in the splanchnic pool were revealed in men after 12-hour HDT at -15^0 [Afonin B. et al., 2007]. After 3 weeks, HDT showed a 20% reduction in leg artery wall thickness [Platts et al., 2009]. HDT at -6° for 5 weeks revealed “internal remodeling” of the femoral artery [Palombo et al. 2015].

Changes in vascular hemodynamics were also revealed in the experiments with “dry” immersion. In particular, the results of our study of the state of the vascular bed in women who were exposed to DI conditions for 3 days showed a statistically significant decrease in the diameters of the inferior vena cava, common, external, and internal iliac veins at different periods of examination ($p < 0.05$) [Vasilev I. et al., 2021], also a statistically significant increase in the diameter of the inferior vena cava and an increase in the medial-lateral diameter of the right internal jugular vein ($p < 0.05$) was revealed during the aftreeffect period in men who were under conditions of

7-day DI [Vasilev I. et al., 2022]. It was shown that the coefficient of signal return increased by 15% in a 4-day DI study [Arbeille P. et. al., 2022].

Thus, the results we presented are comparable with the data obtained in the experiments simulating the effect of SF factors and confirm the need to continue in-depth study of venous hemodynamics, especially the vessels of the inferior vena cava system, and to study the risks of occurrence and mechanisms of thrombus formation at different stages of space flight.

Conclusions

1. A statistically significant change in the diameter of the inferior vena cava, right common femoral vein and right common femoral artery was obtained;

2. Statistically significant changes in the lateral-medial size of the right internal jugular vein were obtained;

3. Changes in the hemodynamics of the vessels of the lower extremities in conditions of HDT are, apparently, of a compensatory-adaptive nature.

4. It is necessary to increase the number of observations in experiments simulating the action of SF factors for in-depth study of venous hemodynamics, especially the vessels of the inferior vena cava system, and to study the risks of occurrence and mechanisms of thrombus formation at different stages of space flight.

Acknowledgments
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A wearable-based system to reduce space motion sickness by multi-sensory pre-habituation

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Vollette, C., Bockisch, C., Straumann, D., Bertolini, G. A wearable-based system to reduce space motion sickness by multi-sensory pre-habituation.

Introduction

Motion sickness (MS) is a common disturbance occurring in healthy people exposed to specific real or illusory motion conditions. The most widely accepted hypothesis suggests a sustained conflict between expected and actual sensory inputs as the triggering factor (G. Bertolini et al. 2016). In space, these mismatches cannot be resolved into a stable self-motion perception as the brain cannot sense gravity. Accordingly, each transition between gravity levels implies space motion sickness SMS (M. Heer et al. 2006; HJ. Ortega et al. 2020) for roughly half of trained astronauts, significantly impairing missions and crew safety for days. Symptoms of motion sickness include vomiting and nausea, but also higher risk of disorientation, visual illusions and sopite syndrome. Although drugs diminish symptoms (e.g. meclizine, promethazine or scopolamine), they come with unwanted side-effects (sedation, drowsiness) (M. Heer et al. 2006; AP. Weerts et al. 2014) and risks related to intolerances, adaptation and addiction. An alternative to ameliorate MS symptoms are training programs employing centrifuges, rotating chairs and even rotating rooms that were proven effective in aircraft pilots, but not in astronauts (HJ. Ortega et al. 2020). The key problem is that how self-motion perception adapts to weightlessness is not yet established. Interestingly, astronauts with natural tendency to rely more on an ego-referenced frame (ideotropic vector) than on visual cues have been shown to have less SMS symptoms and a shorter adaptation time (DL. Harm et al. 1998). Pathological visual over-reliance may occur in patients after a transient vestibular insult and become chronic (Persistent postural-perceptual dizziness (PPPD) (S. Cousins et al. 2014)). A sudden exposure to weightlessness represents the equivalent of a strong vestibular insult: the vestibular sensors for gravity direction abruptly stop working. As for the PPPD patients, developing visual dependence is not uncommon in astronauts and has been related to higher and persistent SMS (DL. Harm et al. 1998). Multi-sensory cues to force reweighting of sensorial integration appear overall quite successful for vestibular patients (A. Viziano et al. 2019). Although visual dependence and maladaptation are also issues for astronaut, transfer of the know-how from these novel patient rehab in pre-flight habituation has yet to be evaluated.

The main aim is to develop a pre-rehabilitation lessening space motion sickness (SMS) by simultaneous manipulation of different sensory cues to create sensory conflict conditions that can be resolved when the subject adopts our desired reference frame. In practice, as astronauts with natural tendency to adopt an ego-referenced frame have been shown to suffer less SMS and adapt faster, the pre-rehabilitation should reinforce this reference frame against a visual-based one. A training paradigm successfully achieving this adapted state will have a double advantage: it will prevent overreliance on visual cues and promote a rapid switch to this learned strategy when gravity cues are absent (i.e. in orbit).

Material and Methods

The project is divided into three steps. The first aims to find the best combination of vestibular and non-vestibular stimuli (visual and haptic) for successfully manipulating the perceived “down” in a gravity-related illusion. Then, by combining the stimuli from the previous step in a training paradigm, we hope to induce a lasting reduction in the reliance on gravito-inertia as a cue for tilt. Finally, we will test the efficacy and retention of adaptation in parabolic flights (0-g analogue) and use Galvanic Vestibular Stimulation to make the entire equipment wearable.

To complete the first step, we use a motion simulator (Stewart platform) to expose participants to a “Hilltop” illusion. This illusion arises as lateral translations at low frequency (0.16 Hz in our study) and in darkness are interpreted as tilt-while-translating. The properties of the sensors for angular motion makes their estimate of tilt at low frequency unreliable, causing errors in the interpretation of estimated gravity direction.

The *Test* motion profile we used also included random angular tilts (-6° to 6°) to remove the participant expectation on verticality. Our prediction was that participants would report the random tilt, but overestimate them due to the illusion. The participant’s perception of gravity was measured during the *Test* stimulus using a continuous Haptic Vertical (HV) alignment as well as a Subjective Visual Vertical (SVV) test. In subsequent *Training* stimuli, the visual cue was manipulated with a Virtual Reality (VR) Headset providing verticality cues in contrast to gravitational reference (i.e. coherent with the illusion of an angular tilt). VR environment visually displayed a 1.5, 2 and 2.5 gain in the physical tilt during 3 trials of 3min each. Finally, perception of verticality was assessed again during a second assessment using the *Test* stimulus. Data before and after VR exposure were compared.

Results

In Figure 1, Hilltop illusion parameters assessment was done in 12 participants collecting both HV and SVV. Both measurements showed an error in perception of verticality attributable to this vestibular illusion.

After demonstrating that altered perception of verticality induced by low-frequency translation could be measured with our setup, Figure 2 shows how the adaptation induced by our visual-vestibular stimuli can increase the error in verticality perception. The results of the post-VR assessment with the *Test* stimulus showed a significant increase in the perception error using SVV measurement, but not with HV measurement.

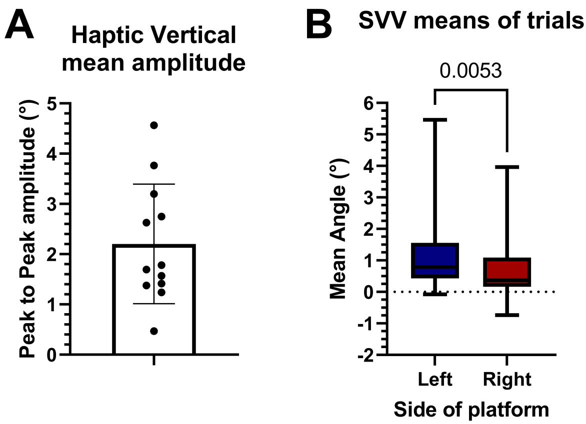

FIGURE 1

(A) Mean perceived angle with Haptic Vertical was 2,2°± 1,19°. (B) For Subjective Visual Vertical there was a significant difference of 0,51° (p=0,053) between perceived angle at left and right side of the platform.

A motion sickness assessment questionnaire (MSAQ) (Gianaros. P. G et al. (2001)) was performed after each session of the VR exposure experiment and showed overall low scores out of 100 in Figure 3.

Discussion

The first experiment allowed us to tune up motion parameters to induce a vestibular illusion that alters the perception of verticality, and hence of gravity direction, by 2,2° (as reported with HV) and 0,51°(as reported with SVV). Then, with the second experiment we obtained a rough increase of 10% of the amount of Gravito-Inertial Acceleration (GIA) reinterpreted as a tilt after less than 8 min of VR-vestibular training exposure according to our SVV measurements. SVV is already a robust and widely used clinical vestibular assessment method. However, HV did not show a significant increase. We believe that HV could be measuring a general report of the direction of

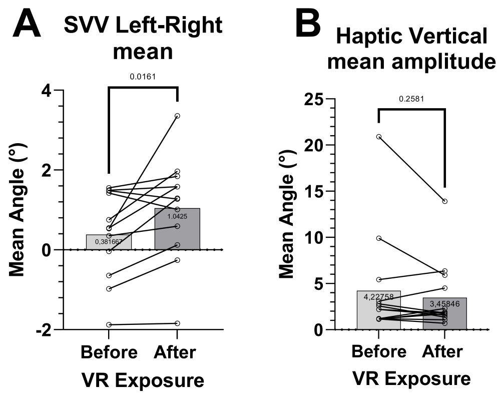

FIGURE 2
(A) Mean perceived angle using SVV was 0,381667° before VR exposure and 1,0425° after VR exposure. Angles were substracted right from left in order to suppress individual shifts bias. The increase in angle perception was significant (p=0,0161). (B) Mean perceived angle using HV was 4,228° before VR and 3,458° after VR. The difference was not significant (p=0,2581).

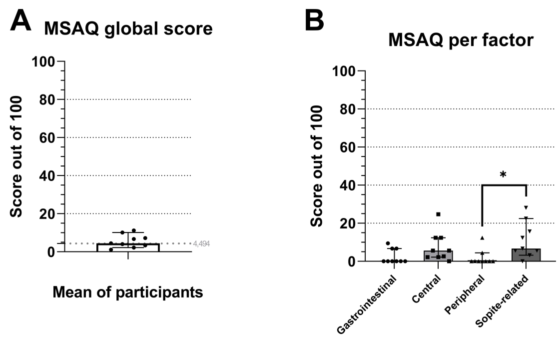

FIGURE 3
Motion Sickness Assessment Questionnaire scores out of 100: (A) per factor G= 0 ; C= 5,618 ; P= 0 ; SR= 6,742, (B) Global score = 4,49.

GIA (such as trying not to spill a glass), as well as it might be influenced by dexterity or motor abilities of the subject. Adding a haptic feedback to the visual cues could allow a better adaptation and be demonstrated with HV measurements.

Conclusion

In this study, the aim is to allow the brain to adapt to altered gravity levels by relying more on the ideotropic vector (internal axis) and less on visual cues. This will expectedly help alleviate SMS symptoms during missions and adapt rapidly to various G changes.

Using the Hilltop illusion allowed us to alter perception of verticality using a combination of optokinetic cues. Then, VR exposure to increased visual tilt showed a partial reinterpretation of the GIA to a perceived tilt. In other terms, visual cues entrained the brain to perceive a lateral acceleration partially as a head tilt, thus trusting visual signals more than vestibular and proprioceptive. This was crucial to allow us to move to the following step, adapting the brain to give more weight to vestibular and proprioceptive signals than to visual ones. A training paradigm successfully achieving this adapted state will have a double advantage: it will prevent overreliance on visual cues and promote a rapid switch to this learned strategy when gravity cues are absent (i.e. in orbit). Furthermore, developing a visuo-vestibular pre-rehabilitation that meets the needs of astronauts (i.e. healthy individual suffering MS when exposed to 'unnatural' conditions) can also positively impact society in the form of spin-off/transfer to habituation paradigms for current and future 'unnatural' motion stimuli, for instance autonomous driving, VR/AR and other emerging technologies.

Acknowledgments

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Direct comparison of tilt test and lower body negative pressure effects on human hemodynamics and baroreflex regulation after dry immersion

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Introduction
Head-up tilt test (HUT) and its spaceflight equivalent lower body negative pressure test (LBNP) are widely used in space medicine as cardiovascular system (CVS) stress tests. Both tests cause blood redistribution towards lower body but differ in cardiovascular outcomes due to the differences in regulatory mechanisms involved (Kitano et al., 2005; Tanaka et al., 2009). Dry immersion (DI) as model of gravitational unloading impairs CVS regulation (De Abreu et al., 2017), which may unequally affect the outcomes of the two tests.

The analysis of low frequency (LF) heart rate (HR) and mean arterial pressure (MAP) waves including α-coefficient estimation (Pagani et al., 1988) is commonly used to assess the cardiac baroreflex control. In this study, such analysis was used to assess the effects of HUT and LBNP in the same subjects before and after 7-day DI. In both tests, exposure duration was set to 3 min to address short-term hemodynamic responses mediated mostly by the autonomic nervous system (Goswami et al., 2019) and to minimize the risk of pre-syncope manifestations after DI.

Material and Methods
Nine healthy men (age 30.8$\pm$4.8 yrs., BMI 22.9$\pm$2.3 kg/m^2) were exposed to 7-day DI. HUT (65°, 3 min) and LBNP (“Chibis” suit, -35 mm Hg, 3 min) were performed before and on the first day after DI. ECG (NVX52, MCS, Russia), blood pressure and stroke volume (SV) (Finometer, Finapres Medical Systems, the Netherlands) were continuously recorded. Respiration rate was also recorded and fixed at the individual comfortable frequency higher or equal to 12 cycles/minute ($\geq$0.2 Hz), to exclude the overlap between respiratory and LF waves. Each test was repeated 5 times alternating with 3-min rest (horizontal position in HUT or atmospheric pressure in “Chibis” suit) (Figure 1.).

Data Analysis
HR and MAP were calculated for every cardiac cycle. Average amplitudes of HR and MAP waves in LF band (0.06-0.13 Hz) were calculated using

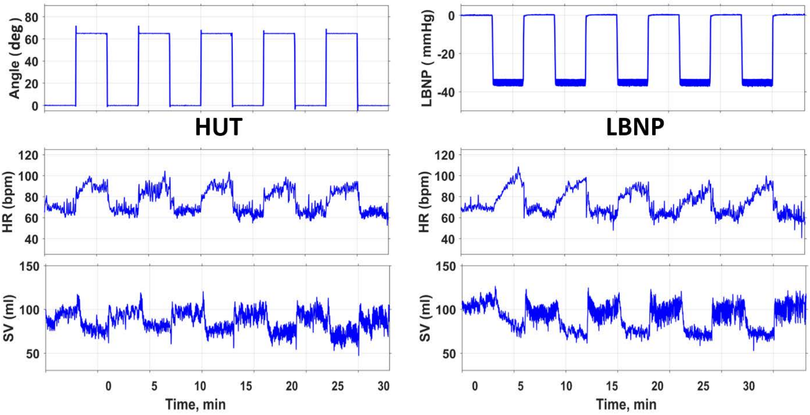

FIGURE 1
The protocols of head-up tilt test (left column) and LBNP test (right column). From top to bottom: tilt table angle or pressure level in “Chibis” LBNP suit (top), beat-to-beat values of HR (middle) and beat-to-beat values of SV (bottom) (Subject M.).

continuous wavelet transform. SV, MAP, HR, LF HR and LF MAP wave amplitudes were averaged over the last 70 sec of test and rest intervals in all repetitions. LF α-coefficient was calculated as the ratio of LF HR to LF MAP amplitudes.

HR, MAP and SV at rest (horizontal position in HUT or atmospheric pressure in “Chibis” suit) were averaged between tests. The differences between rest values of HR, MAP and SV before and after DI were assesed with paired Student’s t-test. Two-way and three-way ANOVA with Sidak’s multiple comparisons test were used to examine influence of DI on differencies between tests in hemodynamic responses and spectral parameters, respectively.

Results

Hemodynamics at rest state changed after DI exposure: SV decreased from 101±17 ml pre-DI to 81±15 ml post-DI ($p<0.01$), HR increased from 59±5

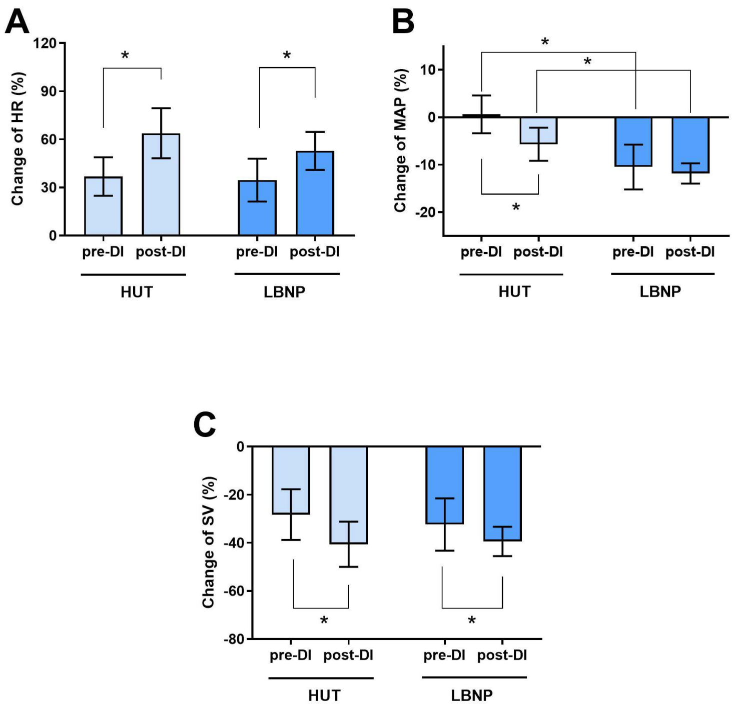

FIGURE 2
Responses of hemodynamic parameters to HUT and LBNP before and after DI. **A** – HR, **B** – MAP, **C** – SV. * $p<0.05$ (2-way ANOVA with Sidak's multiple comparison test).

bpm pre-DI to 67±10 bpm post-DI ($p=0.02$), while MAP increased from 88±6 mmHg pre-DI to 96±5 mmHg post-DI ($p<0.01$). Importantly, the degree of SV decrease did not differ between HUT and LBNP either before or after DI (figure 2).

Before DI, hemodynamics responses to HUT and LBNP were similar for HR and SV but differed for MAP (figure 2). While HUT led to almost none change of MAP, in LBNP it decreased by 10.5±5%. After DI, HR reaction increased similarly for both HUT and LBNP (figure 2A). DI increased reaction of SV in a similar manner in HUT and LBNP (figure 2C). However, DI changed response of MAP only in HUT, while MAP response remained the same in LBNP (figure 2B).

The spectra of HR and MAP waves are presented in figure 3. Two peaks were prominent in spectra: LF "baroreflex" (~0.1 Hz) and HF "respiratory" (~0.2 Hz) peaks. In this study, we focused on LF band (0.06-0.13 Hz).

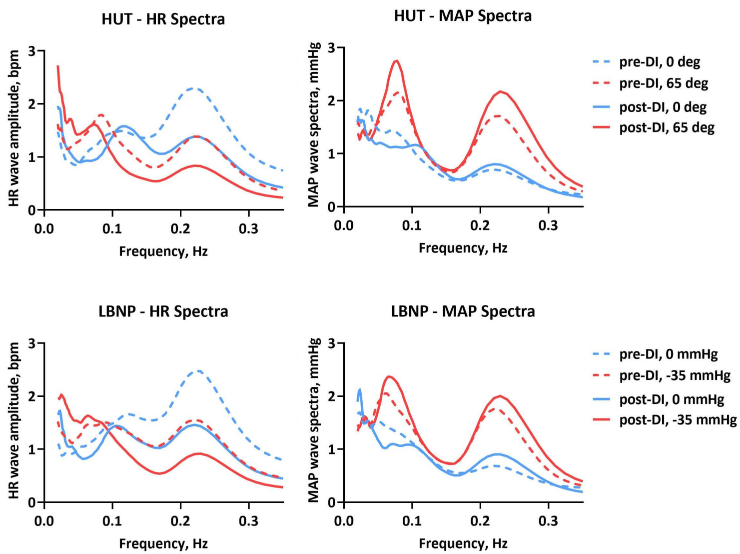

FIGURE 3
Wavelet spectra of HR (A, C) and MAP (B, D) in HUT (A, B) and LBNP tests (C, D) averaged over the group of subjects. Dashed lines represent spectra obtained before DI, solid lines represent post-DI data. Blue lines: rest (horizontal position in HUT and atmospheric pressure in "Chibis" LBNP suit); red lines: during orthostasis or LBNP.

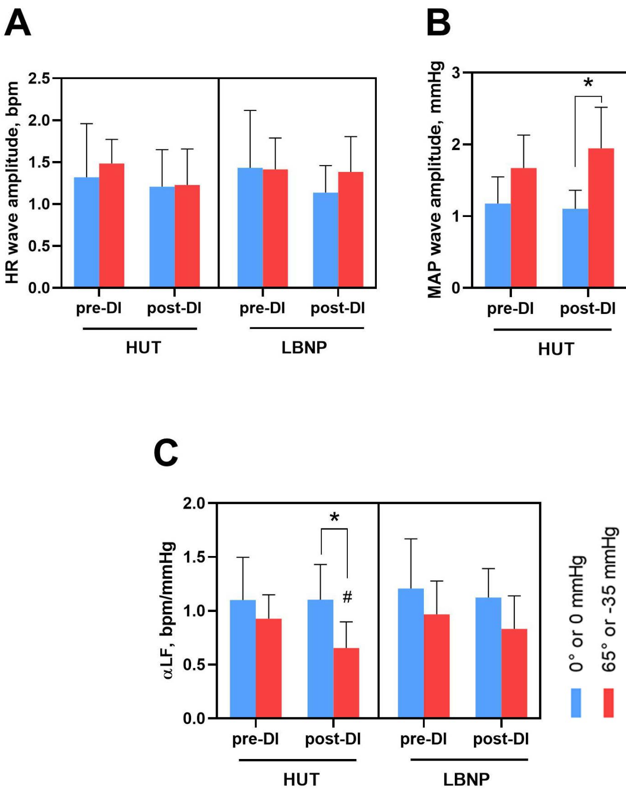

FIGURE 4

Spectral characteristics in rest (blue columns) and HUT/LBNP state (red columns) before and after DI. **A** – LF HR wave amplitude, **B** – LF MAP wave amplitude, **C** – LF α-coefficient; * $p<0.05$ during rest vs. HUT/LBNP state, # - $p<0.05$ the comparison between two tests in corresponding time and state (3-way ANOVA with Sidak's multiple comparison test).

Before DI, HUT and LBNP had no effect on the amplitude of LF HR waves (figure 4A). LF MAP wave amplitude tended to increase (no statistical significance was observed) (figure 4B). Correspondingly, α-coefficient in LF band (the measure of spontaneous cardiac baroreflex) did not demonstrate statistically significant changes (figure 4C).

After DI, there was still no change in LF HR wave amplitude either at rest or during HUT/LBNP (figure 4A). However, LF MAP wave amplitude increased significantly compared to the corresponding rest values in both tests ($p<0.05$), the increase was slightly more prominent in HUT (figure 3C, D and figure 4B). That led to statistically significant decrease in LF α-coefficient only in HUT ($p<0.05$). Moreover, after DI LF α-coefficient in HUT state was lower than in LBNP state ($p<0.05$).

Discussion

Similar SV reduction before and after DI makes 65° HUT and -35 mmHg LBNP comparable by the degree of central hypovolemia development. After DI, rest SV was reduced while MAP and HR increased and their reactions in both tests were more pronounced, which is associated with a decrease in blood volume under gravitational unloading (de Abreu et al., 2017). Similar changes of these hemodynamic parameters were observed earlier after short space flights (Levine et al., 2002). Before DI, the HR increased equally in two tests, at the same time MAP decreased in LBNP but did not change in HUT. Further, DI changed MAP response only in HUT, which may be due to the difference of mechanisms of hemodynamic changes in these two tests, as was discussed in our previous paper (Zhedyaev et al., 2023).

After DI, the changes in MAP LF oscillations at HUT were much more pronounced than before DI, which agrees with earlier findings in head-down bed rest (Tanaka et al., 2013). The same study demonstrated the increase in MSNA response to HUT after bed rest. In our study, the increase in MAP LF fluctuations after DI was slightly more pronounced after HUT than after LBNP, despite similar decrease in SV in these tests. This suggests that in HUT, a higher activation of the sympathetic nervous system and/or elevated vascular response may occur due to additional mechanisms that are less or not at all involved in LBNP. For example, HUT as compared to LBNP was shown to induce more significant unloading of sinocarotid baroreceptors (Smit et al., 2002), pronounced myogenic constriction of lower limb vasculature (Kitano et al., 2005) and activation of the vestibulosympathetic reflex (Tanaka et al., 2009).

Space flight and DI impair vagal baroreflex regulation of the heart rhythm (Eckberg et al., 2010; de Abreu et al., 2017). Thus, our data on the decrease of the α coefficient in HUT after DI are in accordance with the literature. Interestingly, after a 7-day DI, the response of the LF α did not change with LBNP. We believe that the differences in the effect of DI on the cardiac baroreflex in HUT and LBNP tests are associated with differences in the mechanisms activated by these two interventions. As discussed above, the reaction to HUT involves a greater number of regulatory mechanisms, the activity of which can be affected by DI (Smit et al., 2002; Tanaka et al., 2009).

Conclusion

For the first time, hemodynamic reactions to HUT and LBNP after DI were compared in our study. The obtained results may help to interpret in-flight LBNP and pre-/postflight HUT data. DI influence on MAP reaction and on LF α was evident only in HUT but not in LBNP. These observations suggest that DI induced changes in CVS control mechanisms which are involved mostly in the upright position. Therefore, short-term HUT may be more sensitive test then LBNP to assess microgravity-induced CVS deconditioning.

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