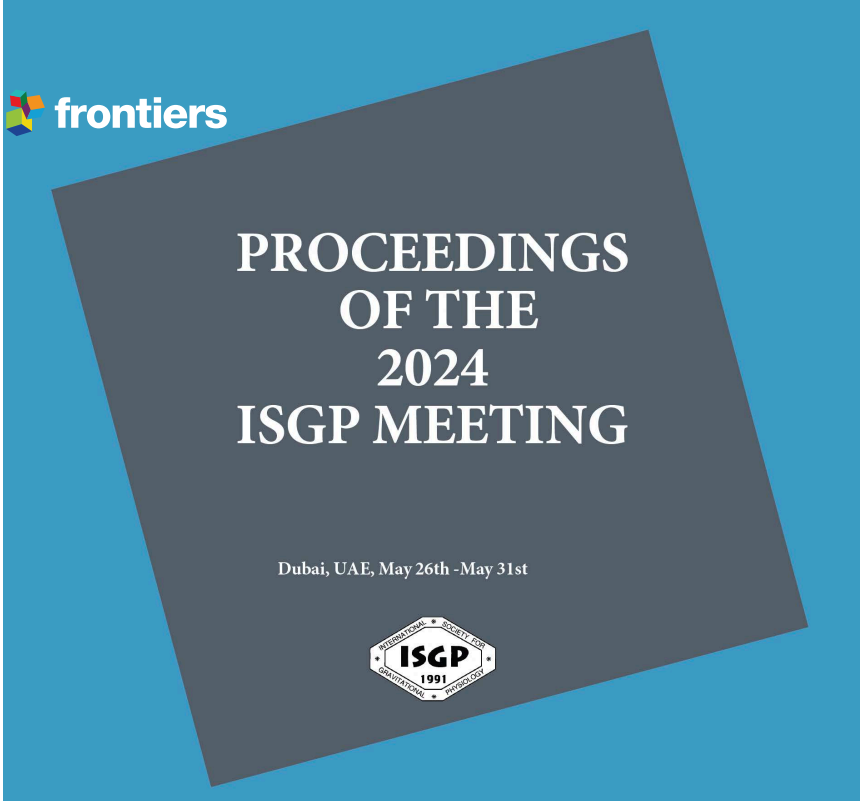

ISGP Annual Meeting, 2024

Dubai, UAE

INTERNATIONAL SOCIETY FOR GRAVITATIONAL PHYSIOLOGY
ISGP
1991

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Fatma Hussain Lootah for MBRSC
Marc-Antoine Custaud for ISGP. (2024)

May 26–31, 2024, Dubai, UAE

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Welcome to the ISGP Annual Meeting, 2024

ISGP, the International Society of Gravitational Physiology, hosts an annual meeting that serves as a platform for presenting original experimental research and reviewing current topics in the field. With gravity, life, and physiology at its core, ISGP covers a broad scientific spectrum. We were delighted to organize our 43rd annual meeting in 2024 in Dubai, UAE.

LIST OF ORGANIZERS

Fatma Hussain Lootah for MBRSC
Marc-Antoine Custaud for ISGP

Preface

43rd ISGP meeting Report - Dubai, UAE

The Mohammed Bin Rashid Space Centre (MBRSC) was honored to host the 43rd Annual International Society for Gravitational Physiology (ISGP) Meeting in Dubai, United Arab Emirates (UAE) - 2024.

More than 125 people attended the event, which was held at the Mohammed Bin Rashid University of Medicine and Health Sciences (MBRU). Those in attendance included UAE astronauts, scientists, young researchers, students, and representatives from international space agencies like the National Aeronautics and Space Administration (NASA), European Space Agency (ESA), German Aerospace Center (DLR) and French Space Agency (CNES).

Papers were presented on a variety of topics, including immunology, cardiovascular system, hypergravity, health, cellular function, and isolation studies were held during the four-day meeting.

We were excited to have the UAE astronaut Dr. Sultan AlNeyadi, head a plenary session where he discussed the importance of conducting biological and medical research on-board the International Space Station (ISS). It was a great opportunity for scientists to engage with an astronaut who has been to Space.

We had great participation from the UAE local science community as well, with a focus on Protein Crystal Growth studies on the ISS, with participation from Prof. David Sheehan and Dr. Saif Salah Alqassim.

We enjoyed hosting the young researchers from all around the world in our event "Careers and Gahwa" – *Gahwa the special arabic coffee.* The young researchers had the chance to engage with experts in different fields and simultaneously get exposed to the UAE's culture.

Finally, the Young Investigators Award was a memorable part of the meeting, where young researchers were given the stage to discuss their research in gravitational physiology. Out of the 38 submitted research abstracts, the top 12 presented their work at the meeting's plenary sessions.

To conclude, the Gala dinner was a highlight of the meeting, where we were able to engage with all the attendees and announce the winners of the Young Investigators Award.

Overall, ISGP 2024 was a special experience for MBRSC and the UAE's science community – we're grateful for the ISGP team for collaborating with us to host this meeting in 2024.

We're looking forward to the upcoming ISGP meetings!

Fatma Lootah
On behalf of the local organizing team


مركز محمد بن راشد للفضاء
MOHAMMED BIN RASHID SPACE CENTRE
DUBAI HEALTH
ISGP
1991
43rd Annual ISGP Meeting in Dubai
May 26th to 31st, 2024

Nello Pace Award 2024

Laurence Vico

In 1986, Laurence Vico attended her first ISGP meeting and immediately made an impact on our Society by her intellect, and kind personality. Many ISGP Board Members openly commented that they wish they could have collaborated with her. One of her co-authors felt that Professor Vico was a true delight with whom to collaborate.

Laurence Vico began her career studying the adaptation of the rodent skeleton either in space (Biocosmos and STS missions) or in preclinical space analogs, a model that she learned from Dr Emily Holton at NASA at Ames Research Center. Early in her career, Laurence Vico worked with Peter Rüegsegger in Zurich who built the first version of a peripheral quantitative computed tomographic device. This major imaging advance enabled measures of volumetric bone mineral density in separate cortical and trabecular compartments. Importantly, Dr. Vico and colleagues also documented incomplete recovery of bones in space even after six months back on Earth. Their Lancet publication of 2000 has been cited over 900 times and has been incredibly influential among space medicine researchers.

More basic research of Dr. Vico and colleagues has focused on the interactions between bone and the vascular system, articular-joint inflammation, skeletal muscle loss, and metabolic alterations. Dr. Vico has made major contributions over the last several decades as Research Director for INSERM at Lyon University, currently coordinating the work of 160 researchers in medicine, biology and engineering. In 2011 she was awarded the Chevalier of the Legion of Honor, one of the highest civilian honors given by the French government.

Save the date !

Next ISGP meeting in Sapporo May 18th – May 23rd 2025

For any information, please visit our web site:

www.ISGP-space.org


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JOIN US AT THE 44TH ISGP MEETING IN SAPPORO (18-23 MAY 2025) AND ATTEND THE DEDICATED EVENTS FOR YOUNG INVESTIGATORS!

Detailed Scientific Program ISGP 2024 in Dubai

مـــــركز محمد بن راشد للـــــفـــــضـــــاء
MOHAMMED BIN RASHID SPACE CENTRE

INTERNATIONAL SOCIETY FOR GRAVITATIONAL PHYSIOLOGY ISGP 1991

	Sunday, May 26th	Monday, May 27th	Tuesday, May 28th	Wednesday, May 29th	Thursday, May 30th	Friday, May 31st
09:00		Opening Ceremony (9:00 - 9h30) [Room: the Auditorium]	Plenary 2 Session dedicated to MBRSC (9:00 - 11:00) [Room: the Auditorium]	Plenary 3 Rodents in Altered Gravity: Advances in Space Biology Research (9:00 - 11:00) [Room: the Auditorium]	Morning Coffee break [Room: the Atrium]	Friday tour
09:30		Plenary 1 - CURRENT CONCEPTS Alomelu Sundaresan "The Lunar Challenge- Lunar dust and the human being in moon missions" Asa Berggren "Insects as food for space travel and planetary colonisations" [Room: the Auditorium]				
10:00					Plenary 4 LBNP as countermeasure (10:00 - 12:00) [Room: the Auditorium]	
10:30		Coffee break [Room: the Atrium]	Coffee break [Room: the Atrium]	Coffee break [Room: the Atrium]		
11:00		Young Investigators Session (1) 11:30 - 12:30 [Room: the Auditorium]	Young Investigators Session (2) 11:30 - 12:30 [Room: the Auditorium]	Young Investigators Session (3) 11:30 - 12:30 [Room: the Auditorium]		
11:30						
12:00		Lunch Break [Room: the Atrium]	Lunch Break [Room: the Atrium]	Lunch Break [Room: the Atrium]	Lunch Break [Room: the Atrium]	
12:30						
13:00		Lunch & Poster (13:00 - 14:00) [Room: the Atrium]	Lunch & Poster (13:00 - 14:00) [Room: the Atrium]	Institutional session (1) 14:00 - 15:00 [Room: the Auditorium]	Institutional session (2) 13:30 - 15:00 [Room: the Auditorium]	
13:30						
14:00						
14:30		Moon Exploration (14h00-15h30) [Room: the Auditorium] / Studies with gender differences (14h00-15h30) [Room: Case Method]	Muscle and movments (14h00-15h30) [Room: the Auditorium] / Medical issues for exploration (14h00-15h30) [Room: Case Method]	VIVALDI dry immersion study (15h00-16h30) [Room: Case Method] / Animal Models (15h00-16h30) [Room: the Auditorium]	Closing Ceremony (15:00 - 15:30) [Room: the Auditorium]	
15:00						
15:30						
16:00		Coffee break [Room: the Atrium]	Coffee break [Room: the Atrium]	Coffee break [Room: the Atrium]	ISGP General Assembly (16:00 - 17:30) [Room: Case Method]	
16:30						
17:00	Welcome of participants for registration	Space Exploration and extreme environements (16h30-18h00) [Room: the Auditorium] / Cells and plants (16h30-18h00) [Room: Case Method]	SIRIUS Isolation study (16h30-18h00) [Room: Case Method] / Cardiovascular system (16h30-18h00) [Room: the Auditorium]	Immunology and inflammation (17h00-18h00) [Room: Case Method] / Hypergravity (17h00-18h00) [Room: the Auditorium]		
17:30						
18:00						
18:30		Welcome Reception (18:30 - 21:30) [Room: the Atrium]	Young Investigator event : Ghawa & Career (18:30 - 20:30) [Room: the Atrium]			
19:00				Gala Dinner & Young Investigators Award Ceremony (19:30 - 22:00)		
19:30						

Program

Monday, May 27th

Opening Ceremony

Plenary 1 - Current Concepts

Alamelu Sundaresan

The Lunar Challenge- Lunar dust and the human being in moon missions

Asa Berggeen

& co-authors : Annette Bruun Jensen, David Copplestone, Roberto Guidetti, Martina Heer, Paola Pittia

Insects as food for space travel and planetary colonisations

Young Investigator session – 1

Ahmed Bakri

The impact of Microgravity on Experimental Periodontitis: An In Vivo Study

Damien Lanéelle

Orthostatic tolerance according to cerebral arterial pattern variations during hemodynamic stress combining lower body negative pressure and head-up-Tilt

Victorien Faivre-Rampant

Does gravity affect intrinsic cardiac function? Effects of different gravitational loads on the cardiac performance independent of the preload

D.A. Sidorenko

The ryanodine receptor stabilizer S107 prevented the increase in fatigue and the decrease in strength of rat soleus muscle after simulated gravitational unloading

Parallel Session (a) "Moon Exploration"

Salma Subhi

Chondrites: Understanding the Origins of the Solar System

Chiara Pucciariello

The REGOLIFE project: Bio-Engineering Lunar Regolith for Moon Crop Cultivation

Jay Bookbinder

SpinSat: a Novel Mission Architecture for Deep Space Radiation and Gravitational Studies

Shannon Marchal

Research into "Lunar Hay Fever" on Earth – Finding Answers in an in Vitro Airway Model?

Fawzan Mohamed Kareem Navaz

Utilizing bio-inspired hierarchical multi-shell structures (BHMSS) for radiation shielding in space exploration

Parallel Session (a) "Studies with gender differences"

Kunihiko Tanaka

Galvanic Vestibular Stimulation Decreases Parathyroid Hormone in Menopausal Women

Ivan Vasilev

Parameters Of Venous Hemodynamics In Female Volunteers During Their Stay In A 5-day "Dry" Immersion

Galina Vassilieva

Five-day "Dry" Immersion With Female Subjects ("Immersion-5F-LF"): Main Objectives And Results

Parallel Session (b) "Space exploration and extreme environment"

Yasmin Halawani

AstroBEAT: Cardiovascular Variability Analysis and Lunar Microgravity Twin

Monica Monici

Mechanisms of Adaptation to Extreme Environments The Exposome Signature Project

Elena Fomina
Methods for the prevention of monotony in interplanetary spaceflight

Leonardo Surdo
Crew-interactive AI-powered Health Applications via the ICE Cubes Media Set

Judith-Irina Buchheim
Support of a Crew Activity with the Crew Interactive Mobile Companion (CIMON)

Sandeep Sureh Babu
Potential of Bioprinting in Space Missions: Challenges on the way forward

Parallel Session (b) "Cells and plants"

Mahamed Ashiq
Hypergravity Confers Abiotic Stress Tolerance In Bread Wheat (Triticum aestivum L.)

Irina Ogneva
The Drosophila Melanogaster Oocytes Demonstrate The Mechanoreception Under Short-Term Modelling Micro- and Hypergravity

Devjoy Dev
The effect of short-term exposure to simulated microgravity on circadian clock gene expression in mouse embryonic fibroblasts

Mohamed Jamal
Oral tissues and neural crest derived stem cells as a model to study oral health in microgravity environment

Osman Patel
Impact of microgravity exposure on genes regulating cell turnover in rat mammary gland

Mauro Maccarrone
Simulated Microgravity Affects Specialized Pro-Resolving Mediators and Human Inflammatory Homeostasis in a Cell-Specific Manner

Tuesday, May 28th

Plenary 2 - Session dedicated to MBRSC

Fatma Lootah

Overview of Mohammed Bin Rashid Space Centre –

Astro. Sultan Al Neyadi

Overview of Astronaut Sultan Al Neyadi's Long Duration Mission to ISS –

Saif Al Qassim

Protein Crystal Growth / Presentation #1

David Sheehan

Protein Crystal Growth / Presentation #2

Young Investigator session – 2

Zeinab Ibrahim

Exploring Novel Therapeutics Targets Against Cardiovascular and Skeletal Muscle Deconditioning in Hindlimb Unloading Model

E. Yu. Gorbacheva

The Ovarian-Pituitary Axis Of Mice After Antiorthostatic Suspension During The Full Estrous Cycle

Ines Ebner

Changes in physical activity levels during 60-days of 6°head-down-tilt bed rest - a preliminary data analysis of the BRACE study

T.J. Pereira

Does an N95 mask improve Orthostatic Tolerance?

Parallel Session (a) "Muscle and movements"

Elena Tomilovskaya

Perspectives of electromyostimulation approaches for muscle strength and endurance maintenance under motor unloading conditions: from Space to Earth

Ivan Ponomarev

Effect of 7-day course of electromyostimulation on the contractile and viscoelastic properties of the muscles of the lower extremities under conditions of support unloading

Karolina Almeida Borges

Space Tourism- MyotonPRO experiment on Muscle Tone

Tatiana Shigueva

Effects of Electromyostimulation on Characteristics of Reflex Excitability of Calf Extensor Muscles Under Dry Immersion Conditions

Nelly Abu Sheli

Maximal Voluntary Muscle Force And Muscle Tone Of The Lower Extremities In Patients With Chronic Cerebrovascular Insufficiency And Deficit Of Physical Activity After A Course Of Modulated Electrical Myostimulation ("Russian Currents")

Anna Ganicheva

The Role Of Spaceflight Experience And Mission Duration In The Success Of Completing Model Tasks On The Planet Surface

Parallel Session (b) "Medical issues for exploration"

Monica Monici

Wound Healing and Tissue Regeneration in Space The SUTURE in SPACE Experiment

Elias A

Risk of Thromboembolism in Space: Current Evidence and Perspectives

Ilya Rukavishnikov

Analysis Of The Possibility Of Using Ground-Based Space Flight Models In Studying The Effects Of Stress, Accompanied By A Decrease In Motor Activity Of Various Duration, On Hemostasis Parameters And The State Of The Human Vascular Bed

Philippe Arbeille

Liver tissue changes during 6-month space flight measured by ultrasound RF signal processing

Parallel Session (a) "SIRIUS & Isolation studies"

Tatiana Agaptseva

Evaluation of individualized physical training protocols in experiments SIRIUS-21 and SIRIUS-23

Nandu Goswami
Effects of Prolonged Isolation on Human Health: From Ground-based Analogs to Spaceflight Environments

Asma Parveen
Effects of an 8-months isolation on Body Composition and Cardiopulmonary Exercise Testing

Carine Platat
Body composition and glucose homeostasis during a 8-month ground-based isolation study

Stefan Du Plessis
Effects of Isolation on Cardiovascular and Autonomic systems

Parallel Session (b) "Cardiovascular system"

Andrew Blaber
Altered Cardiorespiratory Interactions with Spaceflight: Preliminary Results from CARDIOBREATH

Carmen Possnig
Understanding mechanisms and unveiling countermeasures for the bed-rest- induced decrease in cerebral blood flow: Preliminary data

Adrien Robin
Gravitational dose-response curves for cardiovascular and ocular variables after 24h bedrest or drug-induced hypovolemia

Jacques-Olivier Fortrat
Self-organized criticality of Heart rate variability During Actual and Simulated Weightlessness: insights from Lower Body Negative Pressure

Olga Vinogradova
Synchronization Of Blood Pressure And Heart Rate Oscillations In Different Frequency Ranges As A Measure Of Disturbances In The Regulation Of Systemic Hemodynamics During Tilt Test

Wednesday, May 29th

Plenary 3 - Rodents in altered gravity: Advances in Space Biology Research

Alexander Andreev-Andrievskiy

Sex differences in vasopressin regulation of water-salt metabolism in hind-limb unloaded mice

Sara Tavella

Adaptation to 3g Hypergravity: A Multidisciplinary Tissue Sharing Program from a 27-Day Mouse Experiment

Daniela Santucci

Effect of 27 day-3g-exposure in C57BL/6J adult male mice: behavioural and neurobiological analysis

Young Investigator session – 3

Victoria Ly

Self-Generated Lower Body Negative Pressure Exercise, a Low Power Countermeasure for Deep-Space Missions

Zhiyao Ma

Exploring the Impact of Simulated Microgravity on Osteoarthritis Development: The Role of CD36 and Sex-Specific Responses in a Mouse Model

Constance Badalì

SpaceBike – Preliminary Insights into Neuromuscular Adaptation through Bed Rest

R. Yu. Zhedyaev

Direct Comparison of Head-Down Bed Rest and Dry Immersion Effects on Human Cardiac Baroreflex During Orthostatic Stress

Plenary session "Institutional session - 1"

During this session, a presentation of roadmaps and perspectives on life sciences in space will be presented by a panel of representatives from academic institutions and space agencies.

Parallel Session (a) "VIVALDI dry immersion study"

Rebecca Billette de Villemeur

Of The Dry Immersion Model For ESA: Description Of The VIVALDI I And II Studies

RK. Vergos

VIVALDI I And II: General Tolerance To 5 Days Of Dry Immersion In 38 Healthy Men And Women

Nastassia Navasiolava

Dry immersion effects on circadian rhythms and day-night variability of core temperature, heart rate, and blood pressure

Peter Fernandez

Exploring Bone Adaptation and Energy Metabolism Between Males and Females Under Dry Immersion Conditions

Adrien Robin

Venous functions and leg volume changes during the two ESA Vivaldi dry-immersion studies in men and women

Marc Kermorgant

Gender Related Differences On Dry Immersion-Induced Ophthalmological Changes

Parallel Session (b) "Immunology and inflammation"

Pauline Jacob

Hindlimb unloading, a physiological model of microgravity, modifies the murine bone marrow IgM repertoire in a similar manner as aging but less strongly

Mei ElGindi

Effects of Simulated Microgravity on Immune System Potency in 3D Micro-environment

Panel discussion on that topic

Parallel Session (a) "Animal models"

Theo Fovet

The NEBULA Project: Effect Of Pre-Flight Physical Training On Bone And Muscle In A Mouse Microgravity Analog Model

Jack J.W.A. van Loon

Fetal mouse long bones under continuous microgravity or in-flight periods of 1×g centrifugation as countermeasure

Timur Mirzoev

Spinal mechanisms triggering the spontaneous tonic activity of the postural soleus muscle under hindlimb unloading

Ameneh Ghadiri

Femurs of Mice Exposed to Hypergravity Show Deregulation of Genes Mainly Associated with ECM-receptor Interactions and Protein Digestion and Absorption

Angela Maria Rizzo

Hypergravity Exposure Induces Alterations Of Erythrocyte Membrane And Antioxidant Potential Of Mice Housed In The MDS Facility

Parallel Session (b) "Hypergravity"

Rebecca Billette de Villemeur

A 60-Day Bed Rest With Artificial Gravity And Cycling Exercise: The BRACE Study – Description Of The Study Method

Jan Millek

Does Artificial Gravity Tolerance Change Across seasons?

Maryam Almarzooqi

Comprehensive exploration of artificial gravity solutions for optimizing long-term space exploration missions

Alina Saveko

Effect of different short-radius centrifugation interval training modes on vertical stability

Thursday, May 30th

Plenary 4- LBNP as countermeasure

Nandu Goswami

Physiological effects of LBNP

Andrew Blaber

Role of the calf pooling in blood pressure regulation

Asrar Abdi

Effects of Menstrual Cycle on Hemodynamic and Autonomic Responses to Central Hypovolemia

Vishwajeet Shankhwar

Does Gender Influence Cardiovascular and Autonomic Responses to Central Hypovolemia?

Plenary session "Institutional session - 2"

Pierre Denise

SPACEMED Erasmus Mundus Joint MSc: The first European Master's program in Physiology and Medicine of Humans in Space and Extreme Environments

Angelique Van Ombergen

ESA's Human Exploration Enabling Science Activities: recent highlights, where are we going and how can you get involved?

Pauline Jacob

Gravitational Experimental Platform for Animal Models, a New Platform at ESA's Terrestrial Facilities to Study the Effects of Micro- and Hypergravity on Aquatic and Rodent Animal Models

Neil Melville

ESA's Parabolic Flight Activities: An overview of our campaigns, capabilities, and new application routes for Technological and Commercial proposals

Marisa Covington

Navigating the NASA IRB and human research multilateral review board (HRMRB): an ethics perspective

Cyndi Roman

ClinicalTrials.gov: Understanding the Clinical Trials Requirements at NASA

Closing ceremony and announcement of our next meeting in 2025

Posters Session

Posters presented during the session on Monday

1 - Tatiana Kostrominova

Role of Inositol-trisphosphate Receptors in the Regulation of Signaling Pathways During Unloading-induced Rat Soleus Muscle Atrophy

2 - Monica Christova

Activating Orthostatic Response with Motor Imagery: Potential Application in Returning Astronauts and Older Adults

3 - Amira Sayed Khan

Novel GPR120 agonist modulates systemic and neuroinflammation

4 - Aya Hesham

Space-Fit Far Infrared Suit for Back Pain Mitigation onboard the International Space Station (ISS)

5 - Alexandru Nistorescu

Assessing Achilles Tendon Mechanics With MusTone Device: A Myotonometric Approach To Understanding Tissue Dynamics

6 - Abdulrahman Alblooshi

Exploring the Therapeutic Potential of Gravitational Psychology in Disease Understanding

7 - Pauline Jacob

Long-duration head-down tilt bed rest confirms the relevance of the neutrophil to lymphocyte ratio and suggests coupling it with the platelet to lymphocyte ratio to monitor the immune health of astronauts

8 - Adel Elmoselhi

Effects of Isolation and Confinement on Vascular Health during Space Travel: Insights from a SIRIUS-21 Analog Mission

9 - Andreas Rössler

Effects of hemodynamic responses during stand test following 15 minutes of sinusoidal vibration of varying intensity

10 - Masahiro Terada

Performing the bedrest study for the space medicine educational programs

Posters presented during the session on Tuesday

11 - Devjoy Dev

Exploration of the biomechanical stress on the body while performing functional and operationally relevant movement patterns under variable gravitational stress

12 - Kristina Sharlo

Effects of Muscle Electrical Stimulation under 6-day Dry Immersion on Soleus Muscle Signaling

13 - Natalia Vilchinskaya

Time-course of alterations in the expression of mechanosensitive ion channels in rat soleus muscle under simulated microgravity

14 - Ameline Saouli

Effects of Simulated Microgravity on Sperm Function: An In Vitro Study Evaluating Sperm Quality and Function-Specific Genes

15 - Tiffany Stead

Examining Hypercoagulability in Females Exposed to Dry Immersion: a mechanism for Development of Venous Thromboembolism in Microgravity?

16 - Irina Bryndina

Sphingolipids as regulators of skeletal muscle phenotype at gravitational unloading

17 - Victoria Gulimova

X-Ray Phase Contrast Microtomography Investigation Of Thick-Toed Geckos Caudal Vertebrae After A Long-Term Space Flight Using Machine Learning

18 - Andrew Blaber

Exploring Cardio-postural Interactions in relation to Prolonged Space Missions

19 - Andréa Bertona

Evaluation of Short-Term Simulated Microgravity and Cognitive Task Effects on Central and Regional Hemodynamic Vascular Parameters during Progressive Head Down Tilt (HDT) Inclination

20 - Karolina Almeida Borges

Repetitive Movements and Ultra Long Flights as Predictors Influencing Musculoskeletal Disorders Among Commercial Airline Pilots: A Cross-Sectional Study

21- Sami Alghayath

Assessment of Hemodynamic and Autonomic Reponses to Changes in Posture in Diabetics in Dubai: A Prospective Cohort Study

EXTENDED ABSTRACTS

Insects as food for space travel and planetary colonisations

Author

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Roberto Guidetti – Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

Martina Heer – IU International University of Applied Sciences, Bad Reichenhall, Germany; Institute for Nutritional and Food Sciences, University of Bonn, Bonn, Germany

Paola Pittia – Faculty of Bioscience and Technology for Food Agriculture and Environment, University of Teramo, Teramo, Italy

Citation

Berggren, Å., Jensen, A.B., Copplestone, D., Guidetti, R., Heer, M., Pittia, P. Insects as food for space travel and planetary colonisations.

Introduction

Adequate energy intake is perhaps the most important aspect of astronaut nutrition for short-term functioning; however, for the maintenance of long-term body processes it is critical that essential nutrients are provided in the right balance to the foods' caloric density. Ideally, if enough food is consumed to meet energy needs, then other essential nutrients such as vitamins, minerals and amino acids, will be consumed in the required amounts (Smith, 2021). However, for space travelers, all food must currently be transported from Earth at a high cost of approximately 20 000 euros per

kilogram using the limited space available in spacecraft. Producing food on long travels, on the Moon, Mars or other planets would have several benefits, including reducing upload costs and providing psychological benefits to space travelers able to produce their own food. Sustainable food production systems that can provide enough food could be achieved through the use of hydroponics, aeroponics, or insect farming techniques to produce high yields in small spaces.

Growing insects for mass consumption on Earth is rapidly increasing (van Huis, 2013; Berggren et al., 2019), with five insect species, including House crickets (*Acheta domesticus*) and Mealworms (*Tenebrio molitor*), approved for human consumption by the European Commission (European Commission 2023). The main reasons for the current interest in insect mass-rearing is the insects' nutritional value (Rumpold and Schlüter, 2013; Makkar et al., 2014; Miech et al., 2016; Jantzen da Silva et al., 2020) and their resource efficiency when converting organic matter and water into protein (Nakagaki and DeFoliart, 1991; van Huis, 2013). This conversion efficiency has major implications for creating sustainable and circular food production systems on earth with insects as an integral part (Berggren et al., 2019). There are >2000 edible insect species recorded (Mitsuhashi, 2017), with different nutritional profiles (Finke, 2015).

Space environment and insect physiology

There is little knowledge on how changes in gravity and radiation that an insect would be exposed to in space affect growth and reproduction. Terrestrial studies have examined how radiation affects reproduction and physical processes for several different species (e.g., Coulin et al., 2014; Beresford et al., 2019; Fuciarelli and Rollo, 2021). But there are limited insect studies on effects of changes in gravity on physical processes and behaviour (Horn et al., 2002). For a few non-insect invertebrates (e.g., nematodes and tardigrades) studies have been conducted in space on survival and physical processes (Adachi et al., 2008; Rebecchi et al., 2009; Rizzo et al., 2015). The effects from radiation as well as changes in gravity have the potential

to manifest as changes in the individual's physiology as well as behaviour. Developmental patterns, longevity as well as reproduction may be negatively affected (e.g., Copplestone et al., 2008; Raines et al., 2020). These factors are all important for the long-term stability of reared populations (Carey, 2001). It is likely that the ability to cope with space-specific and extreme conditions varies between species, as well as the species' abilities to adapt to new environments. An increased understanding of the biology of insects that face extreme environments is crucial to be able to judge their potential as food for prolonged space travel and colonisations.

Future research

To be able to evaluate the potential of insects as a future food source for prolonged space travel, current knowledge in the areas of nutrition, insect biology and food quality need to be interdisciplinarily examined (Figure 1). Particularly focus should be on 1) nutritional value of insects for spaceflight needs, 2) quality and sensory properties of insect added foods in the space

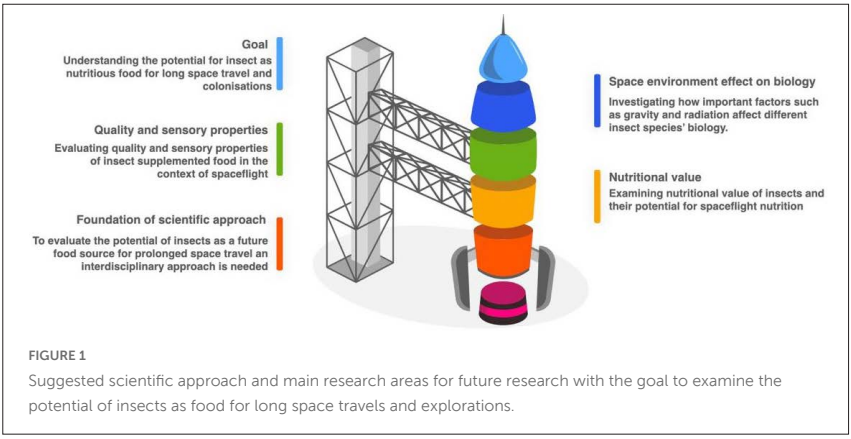

FIGURE 1
Suggested scientific approach and main research areas for future research with the goal to examine the potential of insects as food for long space travels and explorations.

conditions, and 3) the effect of space environmental factors on insect biology and production. For new knowledge also experimental studies in the areas have to be carried out and information acquired on insect species' physiological, behavioural, and reproductive responses to altered levels of gravity and radiation, and how this effect vital aspects of the food produced.

Conclusion

Currently edible insects are recognized as one of the most viable and sustainable food sources on earth. This indicates a potential to widening insects food production on earth, to insects as food for space missions and develop an ability to produce safe and highly nutritious space food. Examining insects and their potential as food will give very valuable insights into how they could play a part within a future food production system. Insects have a high nutrient content in terms of proteins, vitamins and minerals and are able to grow on waste products and with minimal water consumption. Currently knowledge is developing on how to successfully rear different insect species for food in terrestrial conditions. Adapting that knowledge to non-earth conditions in combination with understanding how this food source is best tailored for nutritious, safe and stable consumption would advance the field of food production systems necessary for creating viable long-term peopled missions in space.

Acknowledgements

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Vivaldi I and II: General tolerance to 5 days of dry immersion in 38 healthy men and women

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Citation

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Introduction

The dry immersion model (DI) provides a unique opportunity to study the physiological effects of the lack of a supporting structure for the body. However, there is yet insufficient data on DI (Navasiolava et al. 2011). To obtain standardization and validation, two DI studies were conducted on behalf of the European Space Agency (ESA). Vivaldi I (2021) was conducted in female subjects, then a similar set of measurements was carried out in male

subjects in the Vivaldi II study (2022) (Horeau et al. 2024; Robin et al. 2023; Kermorgant et al. 2024).

The question of tolerance to DI is crucial if further studies are to be conducted, and this work will highlight the general tolerance to the studies and the DI model in general.

Material and Methods

The main objective of the studies was to investigate the physiological effects of a 5-day dry immersion, and to create gender specific frames of reference. The study designs strictly followed the ESA DI standards for subject selection, general methodology, and DI core data collection. A few supplementary system-specific measurements were performed to reveal and understand the mechanisms of DI-induced changes.

Participants were hospitalized for 11 days, referred to as the ''dry immersion campaign'', consisting of 4 days of baseline data collection (BDC) in controlled ambulatory setting, 5 days of dry immersion (DI), and finally a 2-day period for recovery and data collection (R+). During DI, the subject was immersed in thermo-neutral water, with the skin protected from the water by a neutrally buoyant waterproof fabric. Only the head and arms were out of immersion. Subjects were taken out of immersion for hygienic purposes and certain restricted tests, during which a -6° head-down tilt was maintained as much as possible.

To assess tolerance to the DI model, parameters such as general discomfort, sleep, specific pains, and adverse events were monitored using specific twice-daily questionnaires and clinical monitoring.

Results

Over the two studies, 18 women and 20 men were included (Table 1). Two women were excluded from the study (one for technical issues and one for regulatory aspects unrelated to the study). A male participant dropped out of the study on the third day of DI, due to intense back pain. This subject's data is included for tolerance up until that point.

TABLE 1: Subjects' characteristics at baseline

	Vivaldi 1 (women)	Vivaldi 2 (men)
N	18	19
Dates	20/09/2021 - 10/12/2021	19/09/2022 – 24/11/2022
Age (years)	29 ± 5	28 ± 4
Height (cm)	164,8 ± 5,8	176,6 ± 4,2
Weight (kg)	59,3 ± 6,2	72,0 ± 6,7
BMI (kg/m2)	21,8 ± 1,8	23,1 ± 1,9
VO_2peak (ml/min/kg)	38,7 ± 6,4	45,3 ± 4,5
RMR (kcal/day)	1334 ± 107	1580 ± 152

Characteristics of the subjects at baseline for the Vivaldi 1 and Vivaldi 2 studies. Age, height, weight, Body Mass Index (BMI), maximum effort test results (VO_2peak) and Resting Metabolic Rate (RMR) are expressed in mean ± standard deviation. N is number of subjects who completed the study. Dates are the study dates from arrival of the first subject to the departure of the last subject.

General tolerance to the study was good overall, with an increase in discomfort during the DI phase (Figure1). All subjects but one reported some degree of pain at least once during the DI phase, especially lumbar and dorsal pain (N=37, 97%), with very high interindividual variability in terms of intensity and duration. Abdominal pain was also common during immersion, frequently associated with nausea or constipation, affecting 20 and 8 subjects respectively (53% and 21%). Headaches concerned 17 subjects (45%) throughout the whole campaign, but with mild intensity, and did not increase during immersion compared to BDC phase.

There was also a decrease in sleep quality during the first night of DI compared to the BDC period for all 38 subjects. This sometimes lasted throughout the DI period and only stopped at recovery. Urinary free cortisol increased significantly in the first days of dry immersion in both sexes,

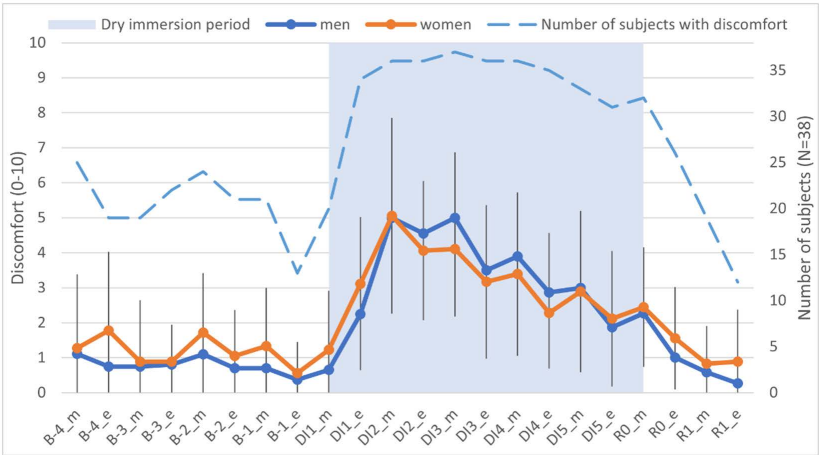

FIGURE 1
General discomfort (mean) during the study on a 0-10 scale. Survey was taken every day, morning (m) and evening (e) from BDC-4 (B-4) to R+1 (R1) for 19 men and 18 women.

In terms of feasibility, there was no major issue, but some practical constraints were highlighted concerning urination in women, skin humidity, and transmission of certain signals through the fabric and the water.

Conclusion

The general results for tolerance to DI reveal that it is a feasible and admissible model. The most common complaints were back pain, abdominal pain, headaches, and a decrease in sleep quality, with 97% of subjects experiencing some measure of discomfort. Intensity and duration are very individual-specific; pain was often moderate, but one of the 38 subjects dropped out due to back pain.

To study the physiological effects of microgravity, dry immersion is an ethically acceptable model. Moreover, the discomforts induced by dry immersion have some similarities to spaceflight, and the model could be used to test countermeasures that focus on sleep quality or pain.

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The authors would like to thank the European Space Agency (ESA) and the French Centre National d'Etudes Spatiales (CNES), as well as the ESA dry immersion expert group and the study participants.

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A 60-day bed rest with artificial gravity and cycling exercise: The BRACE study – description of the study method

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Introduction

Longer and farther space flights are planned, increasing the need to find effective countermeasures to maintain crew health and performance throughout their mission and upon their return to Earth. While several

countermeasures have been used and tested, they are currently not completely satisfactory in terms of time-constraint and efficacy, and with an important interindividual variability. It is now becoming clear that different methods will have to be combined, and that some may need to be individualized (Goswami et al. 2015; Jones, Petersen, and Howatson 2019).

The European Space Agency (ESA) and its bedrest expert group wished to study the effects of a combination of exercise and individualized artificial gravity (AG) using a short-arm human centrifuge (SAHC) on the physiological adaptations to microgravity. Combining the two countermeasures could synergize their effects, as well as reduce workload (Hargens, Bhattacharya, and Schneider 2013; Masatli et al. 2018; Frett et al. 2020; Diaz-Artiles, Heldt, and Young 2018)and Schneider 2013; Masatli et al. 2018; Frett et al. 2020; Diaz-Artiles, Heldt, and Young 2018.

Material and Methods

Bedrest Design

A long-term (60-day) head-down bedrest (HDBR) was planned on 24 healthy male subjects, divided into 3 groups: a control group, an exercise group using a supine bike ergometer, and a group combining simultaneous AG and exercise (Figure 1). The ESA bedrest standards were followed, and subjects were selected accordingly.

After a 14-day period of baseline data collection (BDC), the volunteers were placed in a strict -6° head-down bedrest for a period of 60 days. The only exceptions to this position are imagery sessions (DEXA, CT and MRI), a few brief tests (muscle biopsy) and the bike ergometry sessions, which were horizontal. This was followed by 14 days of recovery. The ESA bedrest standards were applied for the duration of the study, with a strict control of nutrition, sleep schedule, activity, and an absence of visits.

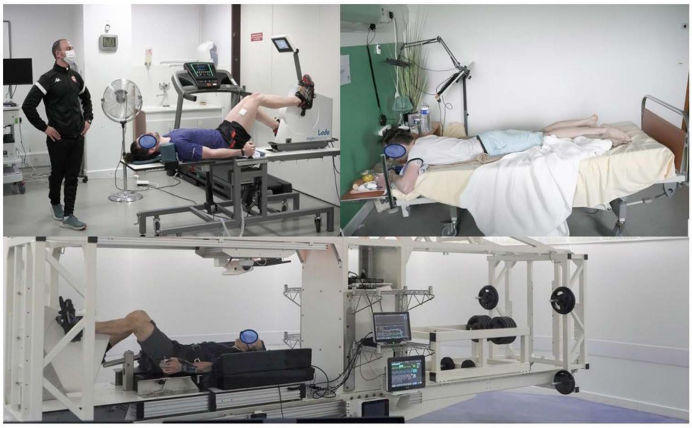

FIGURE 1
Groups in the BRACE study: bike only, control group and bike + artificial gravity.

Countermeasure protocol

While the control group had no exercise, both other groups had a 30-minute daily routine of exercise on a bike ergometer. Load was based on their individual VO_2max, as measured in BDC on a supine ergometer. There was an alternation of 2-minute intervals from 40%VO_2max to up to 80%VO_2max. In the AG + bike group, this same exercise was combined with simultaneous AG as the ergometer is fixed to the arm of the centrifuge. Acceleration depends on the individual's orthostatic threshold and pre-syncope level, as measured in BCD (Figure 2).

Measurements

A set of tests was performed to measure the effects of the bedrest and of the countermeasures on biological, physiological and psychological aspects, called the bedrest standard measurements (BSM). Through an ESA announcement of opportunity, fourteen different scientific teams' proposals

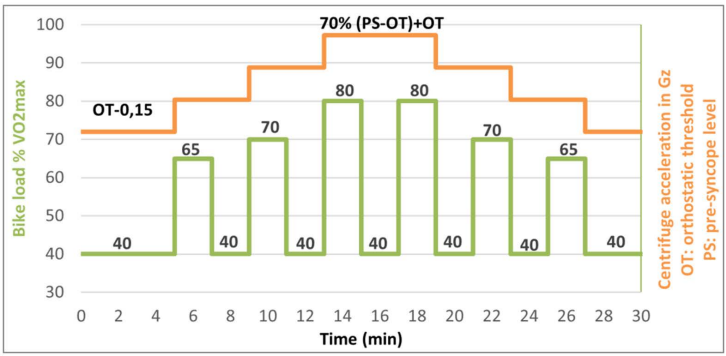

FIGURE 2
Daily countermeasure protocol for the bike + artificial gravity group
OT = Orthostatic Threshold (Gz)
PS = Presyncope level (Gz)
Bike load is expressed in %VO_2max

were implemented into the general study design. Great care was taken to avoid interferences between tests, interventions, and the general study design.

Through these measurements, a wide array of systems are being studied: cardiovascular, neurological (anatomical and functional), ophthalmology, vestibular system, muscle structure and performance, nutrition, metabolism and inflammation, bone and articulations, psychology, immunity; parallels between these systems will also be possible through an integrative approach.

Results

Out of 3193 applicants, 24 subjects were selected and participated in the study. The first campaign was carried out in Spring 2023 for the first 12 subjects, and the second campaign in the Spring 2024 for the last 12. Their characteristics at baseline are presented in Table 1.

TABLE 1: Subjects' characteristics at baseline

	Control	Bike	Bike + AG	Total
Age (years)	28,9 ± 6,6	29,6 ± 4,9	29,6 ± 5,8	29,4 ± 5,6
VO_2peak (ml/min/kg)	45,5 ± 4,8	45,0 ± 3,7	44,9 ± 5,5	45,1 ± 4,5
AG PS (Gz)	1,41 ± 0,22	1,47 ± 0,17	1,46 ± 0,22	1,45 ± 0,19
Height (cm)	172,6 ± 6,7	178,1 ± 4,3	177,4 ± 7,0	176,0 ± 6,4
BMI (kg/cm^2)	24,05 ± 1,65	23,99 ± 2,03	23,61 ± 2,00	23,88 ± 1,83

Characteristics (mean ± standard deviation) of subjects at baseline for the BRACE study (N=24)

AG PS = artificial gravity pre-syncope level

BMI = Body Mass Index

The 24 subjects have successfully completed the bedrest protocol and measurements as planned. Recovery follow-up visits were performed at one year for the 12 subjects of the first campaign, with the same visit planned in 2025 for the subjects of the second campaign. The 2-year follow-up visits are planned in 2025 for campaign 1 and 2026 for campaign 2 subjects.

Conclusion

The BRACE study will bring insight into the multi-system adaptations to long-term head-down bedrest, as a model to mimic the effects of microgravity. Through an integrative approach, it will assess the efficacy of an individualized protocol combining artificial gravity protocol with exercise. These results will pave the way for future research and contribute to the design of future in-flight countermeasures.

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Validation of the dry immersion model for ESA: Description of the Vivaldi I and II studies

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Introduction

Two ground-based models are used to simulate the effects of weightlessness on human integrative physiology: -6° head-down bedrest (HDBR) and dry immersion (DI). While the first model is now well-described and standardized, the latter has only been exported outside of Russia in 2015, when the first dry immersion study was carried out in Medes by CNES (French space Agency) (De Abreu et al. 2017).

To further pursue this line of research and test new countermeasure protocols, the model must be rigorously described, and a work of standardization had to be carried out. Indeed, having a consistent methodology in all future DI studies will help compare physiological effects and test countermeasures. The first step for ESA (European Space Agency) was therefore the validation and standardization of the model. Two studies were designed for this objective and to obtain a DI reference dataset: the VIVALDI I and II studies.

Methods

Designing the studies

The main goal of the ESA expert group was to validate the dry immersion model by gathering a comprehensive database on general physiological effects, as well as test a set of measurements in DI to see their feasibility and relevance. A battery of tests, called the Dry Immersion Core Data (DICD), was thus defined by the DI expert group based on the HDBR standard measurements, supplemented by additional tests to further investigate the model and to acquire a better understanding of the time course of the physiological changes. Through an ESA AO (announcement of opportunity), eight scientific proposals were selected to validate the model using DICD and for more system-specific effects.

Selection criteria were adapted from the ESA HDBR standards, namely physically active and healthy males and females between 20 and 40 years old. The rest of the studies' methodology was designed by adapting HDBR standards with up-to-date knowledge on DI. It was decided to carry out DI for a duration of 5 days, with 4 days of pre-DI baseline measurements and 2 days of recovery.

Studies description

In DI, the subject is immersed in thermo-neutral water, with the skin protected from the water by a neutrally buoyant waterproof fabric. The arms

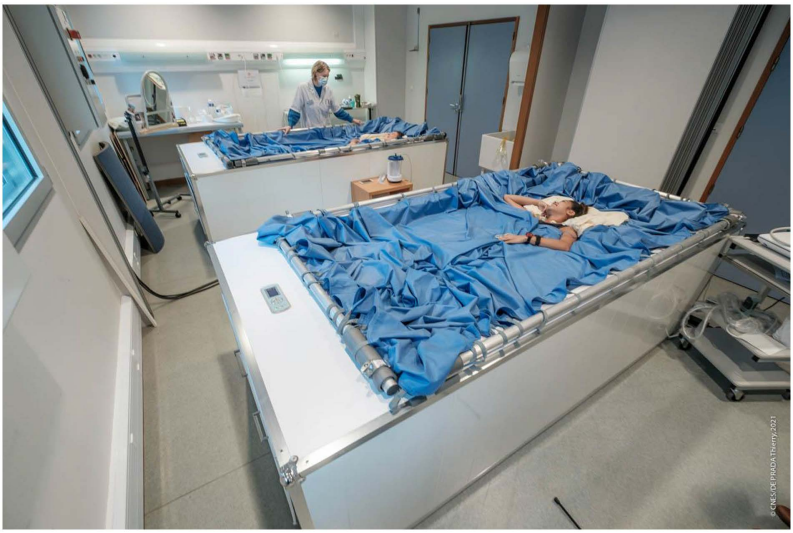

FIGURE 1
Subject in dry immersion during the VIVALDI I study © CNES/DE PRADA Thierry, 2021.

and head are out of immersion to permit a certain degree of movement, while the rest of the body is in a state akin to weightlessness (Figure 1). The duration of immersion was set at 5 days (120 hours). For hygienic purposes and a few restricted tests, the subjects could be taken out of the bath. During these limited times, they remained lying down with a -6° head-down tilt.

The first phase, VIVALDI I was carried out from 21st September to 16th December 2021 in 18 healthy women, and VIVALDI II took place a year later, from 20th September to 24th November 2022 in 20 healthy male subjects.

Tests and measures

From these two studies, physiological and biological data was gathered from 38 male and female subjects. It was of paramount importance for this

validation study that the different tests and measures did not interfere with each other, or with the model in general; an effort was made to limit out-of-bath time, so tests requiring this during the DI phase were limited. In total, data was collected that helped describe the effects of DI on general parameters, on cardiovascular musculoskeletal and immune systems, on nutrition and metabolism, and on neuro-vestibular, ophthalmological, psychological, and cognitive parameters.

Conclusion

These studies provide a comprehensive multi-system dataset on the effects of a strictly controlled DI on both men and women. They will help shape the future of ground-based physiological space research as they validated DI as a model to simulate human exposure to microgravity (Robin et al. 2023). Analysis of the two VIVALDI studies will also provide insight into the physiological comparability between sexes in adaptation to weightlessness.

Acknowledgements

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The role of spaceflight experience and mission duration in the success of completing model tasks on the planet surface

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Introduction

The prospects for long-term spaceflights pose the issue to describe the effect of prolonged stay in weightlessness on physical performance and task performance after returning to gravity conditions. It is important to identify changes in the physiological systems that ensure the success of human extravehicular activity on the planet's surface. Currently, flights to the International Space Station (ISS) are the best model for analyzing changes in gravity-dependent physiological systems after long-term missions (Fomina et al., 2022; Rusanov VB et al., 2022).

The assessment of the physical performance of cosmonauts in the early period of adaptation after spaceflight was previously carried out using the "Field Test", which included the following tasks: standing up from a sitting and a lying position, maintaining a vertical standing position, tandem walking, walking with obstacles, a task for coordinating hand movements and reproducing a given muscle effort (Tomilovskaya E. et al., 2014). Based on the analysis of the results of this test, we developed a battery of tests of on-planet activity "express test", including tasks for subjective assessment of the weight of loads, assessment of physical performance (push-ups and squats), a specific task for coordinating hand movements.

To countermeasure the effects of weightlessness, it is necessary to understand which factors, independent variables play a decisive role in the successful completion of extravehicular activity tasks on the planetary surface. The results of our study allow us to draw preliminary conclusions about the role of spaceflight experience and the duration of stay in weightlessness on the success of the "express test". The aim of the work was to determine the role of spaceflight experience and the duration of stay in weightlessness in the successful completion of model tasks.

Methods

Four cosmonauts took part in the study. Two of them had completed a six-month flight, and the other two had completed a one-year flight. In each of the two named crews, one of the cosmonauts had completed his first

FIGURE 1
The tasks of "express test".

flight, and the other already had spaceflight experience. In the early period of adaptation to gravitational conditions, on the 1st, 2nd, 3rd and 4th days after a six-month spaceflight and on the 3rd and 4th days after a one-year flight, the cosmonauts performed an "express test". On the 1st and 2nd days after a one-year flight, the test was not performed due to medical restrictions. The test included a hand movement control task, Romberg test, voluntary standing up from a supine and prone position, collecting loads along the perimeter of a designated area, estimating the weight of the loads, throwing weights to specified points, a dual task – tandem walking while counting by threes backwards from a specified number, and performing physical exercises – push-ups and squats – for 10 seconds (Figure 1). Heart rate (HR) was recorded throughout the test.

Results

The hand movement control test showed significantly higher target touching accuracy on the second day, compared to the first, after a six-month spaceflight. This confirms the hypothesis of rapid readaptation upon returning to gravity. No significant differences were found in the

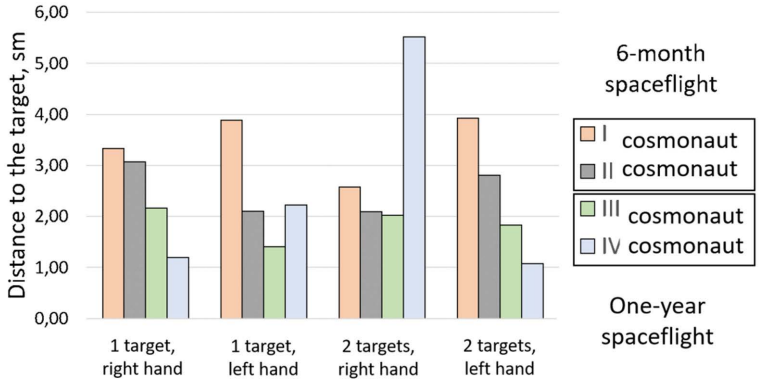

FIGURE 2
Results of the hand movement control test on the 3rd day after the spaceflight.

performance of this task between the 3rd and 4th days after six-month and one-year flights. An experienced cosmonaut who completed a six-month flight showed lower test performance accuracy (Figure 2). In general, in one crew, the success rate of the task was higher among the cosmonauts who had completed their first flight.

The cosmonauts after a six-month flight showed an improvement in the Romberg test performance from the first to the third day - an increase in the duration of maintaining balance, a decrease in swaying. At the same time, the average heart rate of the two cosmonauts during the test decreased (from 101 and 100 beats per minute on the first day - to 78 and 84 beats per minute on the third). In the cosmonauts who had completed a year-long flight and had different flight experience, no differences in maintaining posture on the 3rd and 4th days were found.

The strategies for getting up from the prone and supine positions after a six-month flight had some peculiarities on the 1st and 2nd days after the flight - the cosmonauts turned on their sides and leaned on both hands. On

the 3rd and 4th days, the cosmonauts used fewer points of support, that is, they leaned on one hand. After a one-year flight, on the 3rd day, the strategy was similar to the 2nd day after a six-month flight, but by the 4th day this difference was reduced. Thus, after a long flight, the time for readaptation and restoration of the usual strategies for getting up increases. When performing a dual task, the ratio of the number of named numbers to the time of the tandem passage was lower for the cosmonauts who completed a one-year flight, that is, the success was lower than after a six-month flight (Figure 3). Therefore, the duration of weightlessness affected the success of performing a dual task. In addition, our results show a positive effect of space flight experience, since the success was higher after a repeat flight.

Subjective assessment of the load weight was more accurate after a six-month flight than after a one-year flight. However, flight experience did not affect the results of this task (Figure 4).

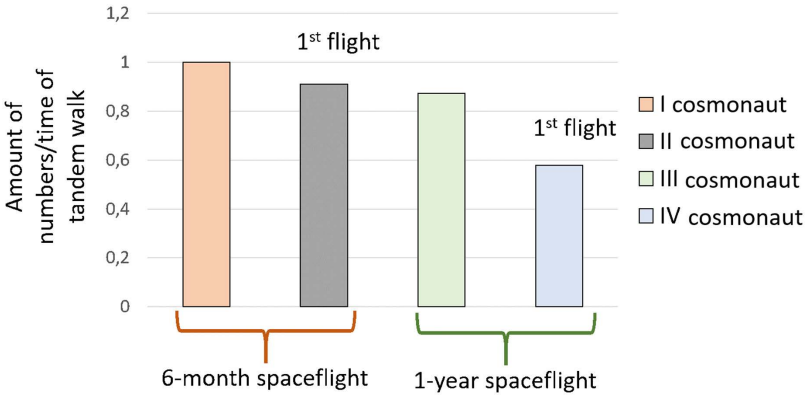

FIGURE 3
Results of the dual task performed by cosmonauts on the 3rd day after the spaceflight.

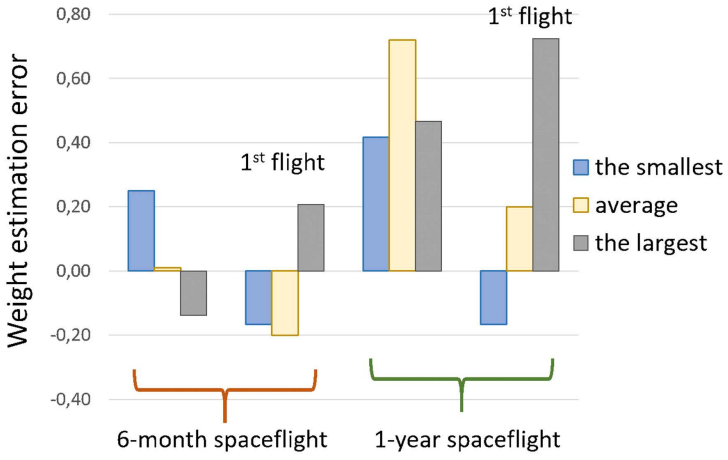

FIGURE 4
Subjective assessment of the load weight by cosmonauts on the 3rd day after the spaceflight.

Among the cosmonauts who completed a six-month flight, there was a tendency for push-ups and squats to improve from the 1st to the 3rd day after the flight (a decrease in the heart rate/number of exercises ratio). The best result was shown by the cosmonaut after the repeat flight. After the one-year flight, the crew completed this task only on the 4th day due to medical restrictions, and no differences were found between the cosmonauts of this crew.

Discussion

The results obtained may indicate a rapid readaptation of the cosmonauts upon returning to gravity conditions. The success of most tasks increases by the 3rd day after the space flight and remains at this level on the 4th day. Thus, the accuracy of touching the target in the hand movement control task improved by the 3rd day for both sequential and simultaneous touches. Improvement by the 3rd day was also observed in other tasks – in performing the Romberg test, the dual task, rising from a supine and prone position.

When studying the relationship between the duration of the mission, flight experience and the success of the model tasks, differences can be noted between the crews and between the cosmonauts within the same crew. Between the crews after missions of different durations, differences were noted in the performance of almost all tasks. Thus, after a year-long flight, in the tasks of rising from a prone position on the 3rd day, the verticalization strategy was similar to the strategy on the 2nd day after a six-month flight (with a large number of support points), although by the 4th day this difference was reduced. Cosmonauts who returned from a six-month flight performed the dual task better on the 3rd day than cosmonauts after a one-year flight. Even in the task of subjective assessment of the load weight, for which no regular dynamics were revealed from the first to the third day, differences can be traced between the crews of missions of different durations. Thus, after a longer space flight, the rate of readaptation is lower.

Flight experience is likely to be important mainly when performing a dual task, since no patterns or connections with this factor in other tasks have been noted. However, given the high probability of multitasking conditions occurring during extravehicular activities, this factor must be taken into account for successful mission planning.

Conclusion

The results of the study indicate that the flight duration factor has a greater impact on the performance of such model tasks as the Romberg test, rising from a supine and prone position, dual task, subjective assessment of the weight of loads, in the early period of adaptation after spaceflight than the experience of repeated astronaut flights. However, experience was important for the successful performance of the dual task. Evaluation of the dynamics of recovery after the flight shows an improvement in the results of the "express test" by the third day and its stabilization thereafter.

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The ovarian-pituitary axis of mice after antiorthostatic suspension during the full estrous cycle

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Citation

Gorbacheva, E.Y., Biryukov, N.S., Ogneva, I.V. The ovarian-pituitary axis of mice after antiorthostatic suspension during the full estrous cycle.

Introduction

The spaceflight affects almost every system inside human body. Despite the history of space exploration lasts more than six decades, a state of a women reproductive system is usually lays beyond scientific interest. There are a few studies but lack of attention resulted in their fragmental and controversal character making them inapplicable for spaceflight influence estimation (Mishra B., Luderer U., 2019; Kikina A.Y. et al., 2024). At the same time women life quality and healthy aging are inseparably bound with state of reproductive system and during and after spaceflight this question should be considered as key-problem to provide spaceflight goals successful achievement. There are a few latest ground-based experiments simulating spaceflight effects

– "dry" immersion (Gorbacheva E.Y. et al., 2023; Robin A. et al., 2023). Our previous results showed the microgravity affects reproductive system in a surprising manner, resulting in, for example, intensive follicle growth. In this work we tried to broaden and to clarify previous results (Kikina A.Y. et al., 2024; Gorbacheva E.Y. et al., 2023).

Material and Methods

The experiment was carried out on the ovaries and pituitary glands of 12-week old BALB/c mice (n = 14). The effects of microgravity were reproduced by antiorthostatic suspension during 96 hours (estrous cycle). The animals received standard vivarium food and water *ad libitum*, the day/night regime was 12/12 hours, all animals were kept under standard conditions. Two groups were formed: control group C (n = 7, m = 27.9$\pm$0.6 g) and hindlimb suspension group HS (n = 7, m = 27.1$\pm$0.9 g). The animals were euthanized using «Foran» inhalation anesthesia (Abbott, USA) and the ovaries were isolated, weighed and immediately frozen in liquid nitrogen for subsequent immunohistochemistry staining (IHC), protein and mRNA isolation. All procedures with animals were approved by the Commission on Biomedical Ethics of the SSC RF Institute of Biomedical Problems RAS (Protocol No. 521 dated September 25, 2019).

IHC analysis was performed according to a standard protocol with primary antibodies to the luteinizing hormone receptor (LHR) (#PA5-21271, Thermo Fisher Scientific, USA), goat-anti-rabbit FITC for fluorescent staining was used as secondary antibodies (#SAB3700884-2MG, Sigma-Aldrich, USA).

Total protein content was isolated from frozen ovaries and pituitary glands, using Laemmli buffer with a protease inhibitor cocktail (Calbiochem, USA) and after measuring concentration Western-blotting was performed with specific primary antibodies (LHR and LH – #PA5-21271 and # PA5-106875, respectively, Thermo Fisher Scientific, USA; beta-actin, alpha-actinin-1, alpha-actinin-4, acetylated alpha-tubulin – #sc-81178, #sc-17829, #sc-

393495, #sc-23950, respectively, Santa Cruz Biothecnology, Inc., USA; alpha-tubulin – #ab52866, Abcam, UK) and secondary antibodies to detect mouse and rabbit IgG (Cell Signaling Technology, Danvers, Massachusetts, USA, #7076S and #7074S, respectively), with Super Signal™ West Femto Maximum Sensitivity Substrate (Thermo Scientific, Waltham, MA, USA) for detection.

RNeasy Micro Kit (Qiagen, Germany) was used for total mRNA isolation according to the instructions. For reverse transcription 500 ng RNA and $d(T)_{15}$ as a primer were used. Real-time PCR was performed using primers for Lhcgr forward/reverse (5'...3') – *ctgttcacccaagacactcca/caggtagagccccatgcaaa*, for H3f3a – *cctcggtgtcagccatcttt/gccatggtaaggacacctcc*. The expression of target genes was normalized to histone H3 (H3f3a) and quantified by the 2-ΔΔCT method (Livak K.J. et al., 2001).

Statistical processing of the data was carried out in ANOVA using a post hoc t-test with a significance level of $p < 0.05$ to assess the significance of the differences determined between groups. Data are presented as mean ± standard error of the mean (M ± SE). All the methods were carried out in accordance with the relevant guidelines and regulations.

Results

After 96-hour antiorthostatic suspension, the weight of the mice' ovaries of the control and experimental groups did not differ (9.6 ± 0.8 mg vs. 9.0 ± 0.6 mg). The thickness of the granulosa cell layer in the antral follicles of the ovaries of mice increased by 54% ($p < 0.05$) (Figure 1A, 1B). Moreover, the relative fluorescence intensity of luteinizing hormone receptor-specific antibodies increases after suspension by 71% ($p < 0.1$) (Figure 1C, 1D).

Indeed, the data for determining the protein content show that in the experimental HS group LHR content was 66% higher ($p < 0.05$) than in the

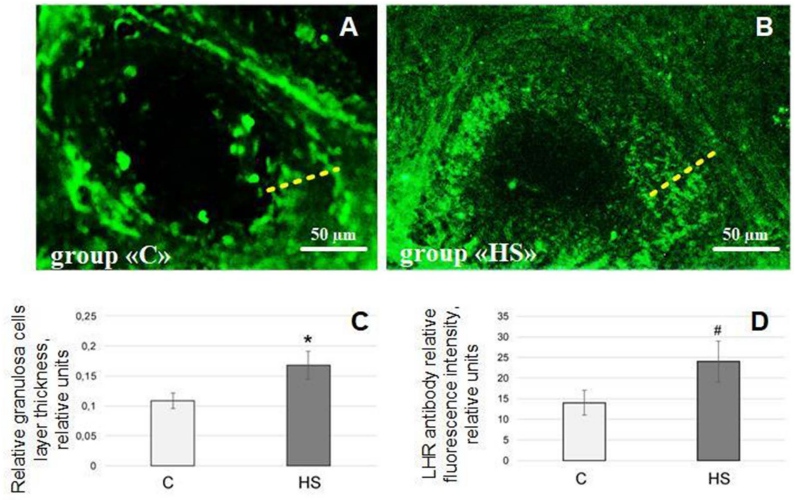

FIGURE 1
Distribution of luteinizing hormone receptor in mouse ovarian follicles after 96-hour antiorthostatic suspension. (**A**) – the representative image of the ovarian section of the control group C, stained with antibodies to the luteinizing hormone receptor (LHR). (**B**) – the representative image of the ovarian section of the HS antiorthostatic suspension group with a similar color. The yellow dotted line indicates the layer of granulosa cells. The scale line corresponds to 50 microns. (**C**) – the relative thickness of the granulosa cell layer. (**D**) – the relative fluorescence intensity of the LHR. (**C**) – control group, HS – antiorthostatic suspension group. * - p<0.05 relative to the control group C. # - p<0.1 relative to the control group C.

control (Figure 2A), the relative content of the corresponding mRNA was also 2.1 times higher ($p < 0.05$) (Figure 2B).

After 96 hours of antiorthostatic suspension of female mice pituitary gland mass decreased by 32% ($p < 0.05$), but the luteinizing hormone (LH) content did not change (Figure 3).

In the pituitary gland the relative content of beta-actin, did not change, while the content of tubulin and its acetylated form, decreased by 40% ($p<0.05$)

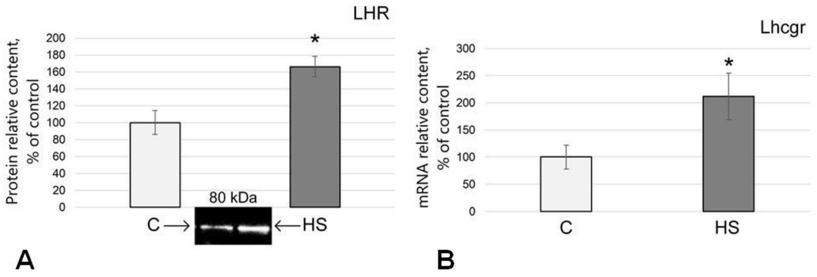

FIGURE 2

Relative content of luteinizing hormone receptor protein and mRNA in mouse ovaries after 96-hour antiorthostatic suspension. (**A**) – the relative content of the LH receptor in ovarian tissues with a typical Western blot. (**B**) – the relative content of the mRNA of the gene encoding the luteinizing hormone receptor (Lhcgr) in ovarian tissues. (**C**) – control group, HS – antiorthostatic suspension group. * - p<0.05 relative to the control group C.

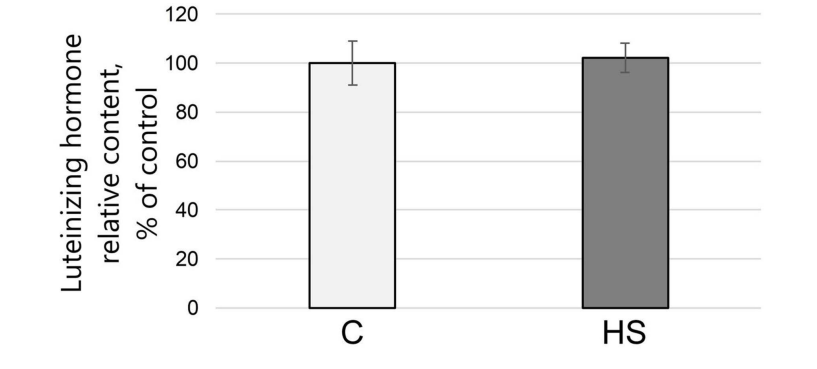

FIGURE 3

Relative content of luteinizing hormone in the pituitary gland of mice. C – control group, HS – antiorthostatic suspension group.

and 44% (p<0.05), respectively (Figure 4). The content of alpha-actinin-1 and alpha-actinin-4 was higher by 64% (p<0.05) and 49% (p<0.05), respectively, compared with the control group.

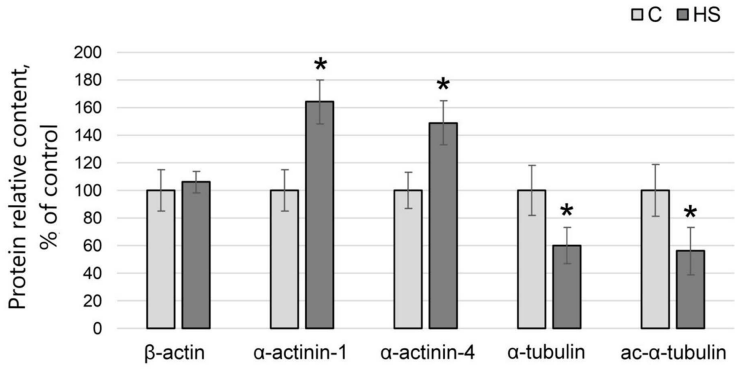

FIGURE 4
Relative content of cytoskeletal proteins in pituitary tissues after 96-hour antiorthostatic suspension. β-actin is the main protein of microfilaments, α-actinin-1 and -4 are isoforms of actin–binding protein, α-tubulin is the main protein of microtubules, ac-α-tubulin is the acetylated form of alpha-tubulin (a marker of stable microtubules). C – control group, HS – antiorthostatic suspension group. * - $p<0.05$ relative to the control group C.

Discussion

In mammals, a growing oocyte receives nutrients primarily from granulosa cells, an increase in the number of which is a good prognostic criterion for its quality (Fan W. et al., 2023). We previously observed in human blood a dramatic decrease of LH content after subjects being exposed to real or simulated microgravity by «dry» immersion (Gorbacheva E.Y. et al., 2023; Kikina A.Y. et al., 2024). We proposed this effect to be dependent on LH-receptor (LHR) content and/or on amount of granulosa cells. Results of the current study shows that there are changes in ovary-pituitary axis in mice after 96-hours of antiorthostatic suspension (this duration corresponds with complete estrous cycle). Particularly, we observed the increase of granulosa cells layer thickness. Since the normalization was carried out for the area of the granulosa cell layer, it can be assumed that the content of LH receptors on these cells also increases, that may lead to LH content reduction in mice' blood if LH production remains stable. The obtained data allows us to claim

the significant increase in both LH-receptor content and its mRNA (*lhcgr*) in ovaries, proving the ability of granulosa cells to capture LH from the blood more effectively and supplying follicle more intensively in comparison with control level. As a consequence it may lead to the healthy follicle growth, even under microgravity conditions.

We didn't succeed in measuring directly LH content in the blood during this experiment, but instead we measured protein and mRNA content directly in the pituitary gland and its did not change. But, on the other hand, we observed changes of cytoskeleton proteins in pituitary cells, which could influence an excretion process (alpha-actinins) as well as an intracellular transport (tubulin) along with significant pituitary gland mass reduction previously described by other authors (Tou J. et al., 2004). This may cause a reduction in LH production into the blood.

Conclusion

We observed alterations in mice ovary-pituitary gland axis after 96-hours hindlimb suspension, which results in significant increase in LH-receptor content in ovaries. The obtained results are in good correspondence with the effect in women subjected to 5-days "dry" immersion and may indicate involving granulosa cells into protective pathways aimed at keeping follicles intact under microgravity conditions.

Acknowledgements

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Machine learning-assisted micro-CT study of gecko vertebrae post-spaceflight

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Citation

Gulimova V., Bukreeva I., Krivonosov Y., Buzmakov A., Junemann O., Asadchikov V., Saveliev S. Machine learning-assisted micro-CT study of gecko vertebrae post-spaceflight.

Introduction

Thick-toed geckos (TG) are a unique model for studying the influence of space flight factors (SFF). They proved high adaptability to SFF in 12 -30-day

orbital experiments (Barabanov et al., 2018; 2019) TGs ability to climb vertical surfaces and attach to objects by their subdigital pads helps them to preserve normal behavior in weightlessness (G_0), which maintains the functional state of their bones and muscles (Gulimova et al., 2019). TGs skeletal organization is similar to that of other vertebrates, and their mineral metabolism is closer to mammals than to lower vertebrates. TGs retain throughout their lives the notochord, an elastic rod that serves as the main axial skeleton during embryonic development (Jonasson et al., 2012). The notochord is formed by vacuolated cells and chondrifies in the middle of the vertebra, forming notochordal cartilage or septum (NCS), which can mineralize over time in adult TG (Witten et al., 2022). We examined NCS calcification to identify the effect of G_0 on TGs' mineral metabolism. The small amount of calcified tissue in the NCS allows the detection of even small changes in the mineral balance, whereas in bone these changes may not be obvious due to the high degree of mineralization. This new approach could be useful for studying bone remodeling in space. To assess the three-dimensional internal structure of the proximal caudal vertebrae of TG, we used X-ray phase contrast microtomography (XPCT). The visualization of the entire vertebra at the cellular level has been achieved by us via XPCT without the need for destructive sample preparation. XPCT provides information about the phase shift caused by objects, which allows for increased image contrast and high-resolution 3D images of both bone and soft tissue (Stefanutti et al., 2018; Massimi et al., 2018). The main problem in segmenting the structure of biological objects is the reliable determination of tissue boundaries. With traditional light microscopy, this process is carried out manually based on visual cues. Manual segmentation of a large number of images of biological objects - is labor-intensive and requires painstaking work of high-level specialists. At present, it is possible to use "supervised" machine learning, where an algorithm is trained to identify the morphological features of an object from several labeled images, and is then able to segment other images of similar objects. This is particularly helpful for XPCT, where it is necessary to process thousands of sections in which interstructural boundaries appear in the form of differences in the reconstructed electron density, expressed in grayscale.

Material and Methods

To study adaptations to the factors of a 30-day space flight using XPCT, the proximal caudal vertebrae (C1-C5) of adult females of TG (*Chondrodactylus turneri* Gray, 1864) were used. Three females from the ground-based delayed synchronous control (DSC) group and the same number from the flight group were investigated. The flight experiment (approved by the decision of the Commission on Biomedical Ethics of the State Scientific Center Institute for Biomedical Problems of the Russian Academy of Sciences dated April 4 2013, protocol No. 319) took place on the biosatellite "Bion-M1" April 19 - May 19, 2013. DSC was carried out on July 27 – August 26, 2013 on the basis of the Institute of Biomedical Problems, Russian Academy of Sciences, under conditions similar to those in flight. The samples were examined

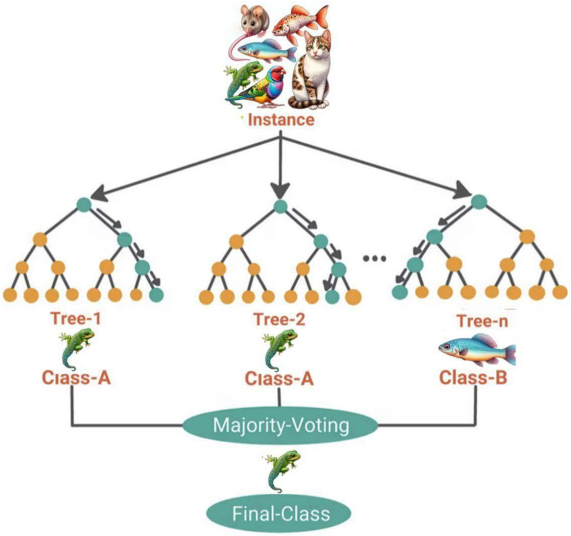

FIGURE 1
Ilastik – segmentation using artificial intelligence: decision tree method, Random forest. The decision is made based on a combination of characteristics such as color intensity, image brightness, border structure, and image texture.

using XPCT at the ID17 beamline of ESRF synchrotron (Grenoble, France), as well as traditional histological methods. Detailed information about animals and experimental conditions are described in (Barabanov et al., 2015). The logistics and methods of sample preparation, the XPCT experiment and data processing, and the statistical analysis and results of the histological study are described in detail in (Bukreeva et al., 2023). For image segmentation, we used specialized software Ilastik, which implements the supervised learning method - "random forest" (Figure 1) and is built on the process of interactive image marking (Berg et al., 2019; Breiman, 2001). Further segmentation of the cortical and trabecular bone tissue of the vertebrae was performed using morphological operations (dilation, erosion, closing, etc.) described in (Gonzalez et al., 2004).

Results

Using XPCT data, a quantitative assessment of the morphometric parameters of TG's spine and notochord (Figure 2) was carried out to identify possible changes caused by G_0. It has been shown that mineralization of NCS is not limited to the autotomy plane of the caudal vertebrae, but can also be found in the proximal part of the tail, where autotomy doesn't happen. These data are consistent with our histological results.

In the main bone parameters, such as bone volume fraction (BV/TV), cortical bone volume fraction (Ct.BV/TV) and trabecular bone volume fraction (Tb.BV/Sc.V), there were no significant differences between the DSC and the flight group revealed ($p>0.2$). According to the averaged trabecular parameters, including the thickness of the trabeculae (Tb.Th), the distance between the trabeculae (Tb.Sp) and the number of trabeculae (Tb.N), after the experiment, no significant differences were also found between flight and DSC. Mineralized cartilage volume (Sept.MCV) and mineralized cartilage volume fraction (Sept.MCV/TV) of NCS in the notochordal canal of the vertebra showed a significant decrease (– 73.19%, $p < 0.003$ and – 77.83%, $p < 0.001$; respectively) in flight group compared to DSC. In contrast, a statistically significant increase (+35.73%, $p = 0.0343$) in bone volume (BV) was found for intercentra after spaceflight. Bone volume fraction (BV/TV)

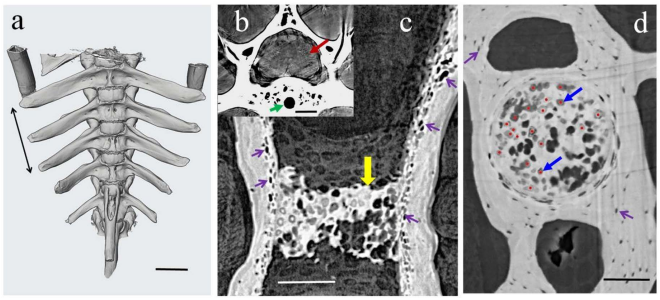

FIGURE 2
XPCT images of the proximal caudal part of the gecko's body: (**a**) –3D image of the proximal caudal vertebrae studied in our research (double sided arrow); (**b**) – gecko's spinal cord (gray color) surrounded by vertebra (white color), red and green arrows show vertebral and notochordal canals, respectively; (**c**) – longitudinal section of the vertebra with notochordal canal and notochord with mineralized notochordal cartilage (yellow arrow), where the violet arrows show osteocyte lacunae in the vertebral bone; (**d**) – the transverse section of the vertebra at mid-vertebral region corresponding to reduced diameters of the notochord with mineralized notochordal cartilage, where the blue arrows indicates chondrocytes, the violet arrows show osteocyte lacunae in the vertebral bone and red dots show chondrocyte nuclei. Pixel size: (**a,b**) – 3.3 μm^2, (**c,d**) – 0.7 μm^2. Scale bars: (**a**) – 1.5 mm, (**b**) – 500 μm, (**c,d**) – 100 μm.

parameters were not calculated for intercentra, because they are composed almost entirely of cortical bone.

After manually labelling about 5% of the entire data set, the machine learning algorithms were able to label the remaining layers fairly accurately, taking into account the texture of the images, their morphology and brightness characteristics (Figure 3).

Discussion

New findings confirm our previous observations about the resistance of the TG skeleton to bone loss in G_0. On the other hand, for the first time a statistically significant decrease in the mineralization of notochordal cartilage

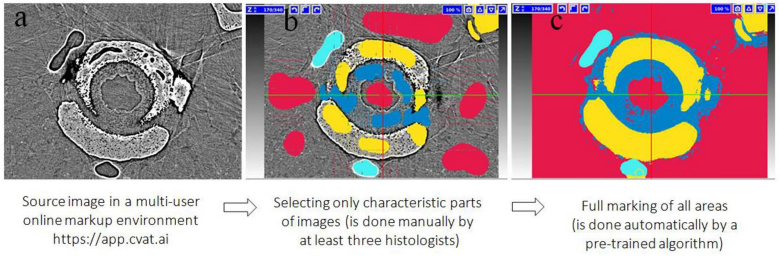

FIGURE 3
Training the algorithm to distinguish tissue types in the Ilastik program. At the first stage, the initial image (**a**) is manually marked by several histologists (at least 3) on a small number of images (**b**). In this case, only some characteristic areas for each tissue are highlighted. Then, based on the manual marking, the algorithm itself completely marks all the tissues in the area of interest (**c**). In this marking, the colors mean: yellow – bone, blue – connective tissue, turquoise – cavities, red – other tissues.

and an increase in intercentral bone volume were shown in TG after 30 days in space. These facts require further research. Based on our data, we can conclude that XPCT is an effective method that provides the necessary sensitivity, spatial resolution and field of view for studying the morphological structure of bones and soft tissues at the cellular level. Machine learning methods have been shown to be effective in segmenting phase-contrast images, in both two-dimensional slices and volume processing modes.

Interactive machine learning methods for image segmentation with supervision offer a more efficient approach to image annotation and analysis, significantly reducing the associated workload. The primary challenge for researchers lies in accurately performing the initial annotation and selecting the appropriate number of segmentation classes and classification features. By addressing these key tasks, the effectiveness of the segmentation process is greatly enhanced. Consequently, these methods streamline the workflow, allowing researchers to focus on more complex analysis and interpretation tasks.

Conclusion

Understanding of the mechanisms of bone loss, muscle atrophy and other health problems that occur in astronauts in prolonged space flights may be significantly improved due to the studying the bones and notochord of geckos under G_0. The results of machine learning, after refinement of algorithm, may be used in biology and medicine to reliably determine the boundaries of tissues in high-resolution tomographic analysis, as well as to speed up and standardize the analysis of large volumes of data.

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Neural crest-derived stem cells as a model to study oral health in microgravity environment

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Introduction

Microgravity, experienced during spaceflights or aboard the International Space Station (ISS), is known to disrupt human organs' normal function, often leading to degenerative conditions. These include significant bone and muscle mass losses, reduced cardiovascular capacity, compromised

immune function, and delayed wound healing(Blaber et al., 2014, Blaber et al., 2015, Lang et al., 2017). Beyond these systemic effects, evidence shows that microgravity similarly affects oral tissues. This may account for the occurrences of barodontalgia (pressure- induced dental pain), periodontitis, alveolar bone (mandibular bone) loss, and facial pain reported in microgravity environments (Rai et al., 2012, Rai et al., 2011b, Orsini et al., 2017, Stevens et al., 2020).

Although strict pre-flight dental screening and oral hygiene protocols are enforced for all astronauts, dental emergencies might still occur in space. Hidden caries, undetected failures of previous dental treatments such as root canals, or accidental trauma leading to tooth or facial bone fractures can result in dental emergencies. Furthermore, the extended duration of space missions increases the possibility of new dental diseases developing mid-flight/mission. Research has shown that extended exposure to microgravity can reduce saliva production and changes in oral bacteria, elevating the risk of caries and other oral health diseases over time (Rai et al., 2011a, Rai and Kaur, 2013, Orsini et al., 2017, Stevens et al., 2020).

Therefore, innovative dental care solutions must be developed to mitigate the risks of dental emergencies during extended spaceflights. These solutions should be designed specifically for the microgravity environment and tailored to astronauts' needs. A deeper understanding of how microgravity affects oral tissues' biological and physiological functions is critical to achieve this. Unfortunately, replicating microgravity conditions on Earth remains a significant challenge, and conducting clinical dental studies on space missions is not practical now. This project aims to overcome these challenges by proposing cranial neural crest-derived mesenchymal stem cells (NC-MSCs)(Jamal et al., 2018b) as a model to study oral and dental tissue development, normal function, and disease processes in a simulated microgravity (SMG) environment. In this project, we have investigated the effect of SMG on the genotype and differentiation function of NC-MSC, and in this abstract, we present our preliminary results.

Material and Methods

Derivation and culture of NC-MSCs

A two-stage directed differentiation protocol was used to derive NC-MSCs from human induced pluripotent stem cells (hiPSCs)(Jamal et al., 2018b). In the first stage, neural crest (NC) cells were derived by incubating hiPSC in a media containing a small molecule inhibitor of TGF- β signaling (SB431542) and a small molecule activator of Wnt signaling (CHIR99021) until a single $HNK1^{+}p75^{bright+}$ population was established. In the second stage, the derived NC cells were incubated in MSC media (containing alpha-MEM2 (Gibco), 10% FBS (Gibco), 1 mM l- alanyl-l-glutamine and 0.1 mM b-mercaptoethanol (Invitrogen)) to give rise to NC-MSCs. NC- MSCs will be maintained in MSC media and passaged every 4-5 days in 1:4 (vol./vol.) ratio. The MSC identity of cells is confirmed by their expression of known MSC markers (CD73, CD90, CD105, CD13) and their tri-lineage differentiation ability using established published protocols(Jamal et al., 2015, Jamal et al., 2018a, Jamal et al., 2018b).

Optimization for the Microgravity Simulator (MGS) experiments

To determine the optimal seeding density of NC-MSCs, we seeded the cells at six different densities: $5x10^3$, $1x10^4$, $2x10^4$, $3x10^4$, $4x10^4$, and $5x10^4$ cells/well. We monitored their viability using the presto blue viability assay over 14 days, ensuring that the cells continued to proliferate during the duration of the experiment. The MGS used is Random Position Machine, AIRBUS, NL.

Global gene expression profile

Passage 3-4 of NC-MSCs were seeded in 4-well culture plates at a density that was determined during the optimization stage. Cells were maintained in normal earth or SMG environments. Total RNA was isolated at days 7 and 14 and processed for next-generation sequencing.

Osteogenic differentiation assay

NC-MSC were seeded in 4-well culture plates at a density that was determined during the optimization stage and allowed to reach 80% confluence within 7 days. At the starting day of differentiation, the MSC media was replaced with osteogenic differentiation media (StemPro Osteogenic differentiation kit, Life Technologies) and kept in either normal or SMG environments. Total RNA was isolated at days 7 and 14 and processed for next-generation sequencing (NGS).

NGS and data analysis

Purified total RNA with a high integrity number were used as a template for preparing libraries to perform RNA-Seq (Illumina Hi-Seq platform). Data acquired were filtered to various statistical workflows through high-performance computing platforms (HPC-Dalma, NYU Abu Dhabi) with known computational workflows consisting of DESeq2 or Tuxedo suite. These will highlight unique genomic programming signature governed by differential gene expression patterns that can be utilized in gene ontology analysis.

Results

Cell viability assay over 14 days indicates a consistent increase in proliferation in the groups with the initial cell concentration of $5x10^3$ and $1x10^4$, while it decreases in groups with initial concentration of $3x10^4$, $4x10^4$, and $5x10^4$ / well (Figure 1). The seeding density $1x10^4$ is the highest seeding density that could maintain its viability and proliferation for the duration of the experiment (Figure 1). Principal Component Analysis (PCA) of global genome changes shows that cells signify variation based primarily on differentiating conditions (at 64%) followed by gravity conditions (at 17%) (Figure 2).

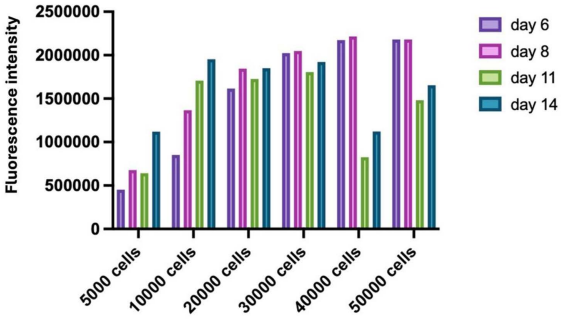

FIGURE 1
Cell viability assay.
Cell viability assay over 14 days using the different seeding densities with the viability assessed at different time points. Results indicate increased proliferation in the groups with the initial cell concentration of $5x10^3$ and $1x10^4$. The seeding density $1x10^4$ is the highest seeding density that could maintain its viability and proliferation for the duration of the experiment.

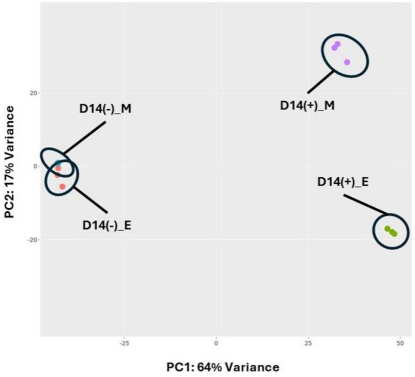

FIGURE 2
Principal Component Analysis
Principal Component Analysis (PCA) of global genome changes of NC-MSC at day 14 that were maintained at normal earth gravity, either differentiated (D14(+)_E) or undifferentiated (D14(-)_E) and or maintained in simulated microgravity environment either differentiated (D14(+)_M) or undifferentiated (D14(-)_M). PCA signifies variation based primarily on differentiating conditions (at 64%) followed by gravity conditions (at 17%). All samples are based on triplicates (n=3), thereby displaying tight clustering among sample conditions and corresponding biological replicas.

Discussion

Microgravity has been shown to influence bone structure, with multiple studies suggesting its impact on bone development and homeostasis through various biological pathways (Vico et al., 2000, Man et al., 2022). In the present study, we aimed to develop an in-vitro model to investigate the effects of SMG on oral tissues, utilizing stem cells as a model system. Our preliminary data demonstrate the successful differentiation of hiPSCs into mesenchymal stem cells through a neural crest intermediate, following our previously established protocols (Jamal et al., 2018b). Additionally, we identified that specific cell seeding densities enabled sustained cell viability over a 14-day period. Upon differentiation of NC-MSC into osteogenic lineages, our initial findings revealed genome-wide alterations, suggesting that microgravity may modulate gene expression profiles. These changes appeared to result not only from cellular differentiation but also from the microgravity environment itself. Ongoing analyses aim to identify differentially expressed genes, affected pathways, and potential genotype alterations in undifferentiated cells under normal and simulated microgravity conditions. Furthermore, we plan to validate the expression of targeted genes identified through NGS analysis.

Conclusion

Simulated microgravity appears to influence a genome-wide alteration in NC-MSC that has undergone osteogenic differentiation. Further analysis is required to determine the differentially expressed genes, affected pathways, and potential genotype alterations in undifferentiated cells under normal and simulated microgravity conditions.

Acknowledgements

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Role of inositol-trisphosphate receptors in the regulation of signaling pathways during unloading-induced rat soleus muscle atrophy

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Kostrominova, T., Zaripova, K., Belova, S., Sharlo, K., Nemirovskaya, T. Role of inositol-trisphosphate receptors in the regulation of signaling pathways during unloading-induced rat soleus muscle atrophy.

Introduction

Muscle unloading results in the increased accumulation of calcium ions in skeletal muscle fibers (Ingalls et al., 1999) (Shenkman and Nemirovskaya, 2008). Calcium is a secondary messenger playing an important role in the activation of calcium-dependent signaling cascades and transcription

factors. Inositol 1,4,5-trisphosphate receptors (IP3R) regulate intracellular calcium metabolism. IP3R are found in significant quantities in muscle fibers in the sarcoplasmic reticulum, nuclei, and mitochondria. IP3R are essential for normal skeletal muscle activity, exerting their effects by regulating gene expression, energy metabolism, and mitochondrial activity (Valladares et al., 2018). The throughput of IP3Rs is regulated by Ca2+ and IP3, and depending on the concentration of IP3, IP3Rs can induce different intensities of calcium signaling (Foskett et al., 2007). The current study tested a hypothesis that IP3R activation regulates soleus muscle unloading-induced atrophy by different mechanisms, including the effect on gene expression via an increase in nuclear calcium ion concentrations (Carafoli and Krebs, 2016). 2-APB is a membrane-permeable IP3R functional antagonist. If our hypothesis is correct, then the inhibition of IP3R would result in the decrease of muscle atrophic processes.

Material and Methods

Male Wistar rats were randomly assigned into one of 8 groups (8 rats in a group): control rats with placebo for 3 days (3C), rats treated with 2-APB for 3 days (3CA; 10mg/kg of body weight), 3 days of unloading/hindlimb suspension with placebo (3HS), 3 days of unloading/hindlimb suspension treated with 2-APB (3HSA; 10mg/kg of body weight), control rats with placebo for 7 days (7C), rats treated with 2-APB for 7 days (7CA; 10mg/kg of body weight), 7 days of unloading/hindlimb suspension with placebo (7HS), 7 days of unloading/hindlimb suspension treated with 2-APB (7HSA; 10mg/kg of body weight). The experiments reported in this study were carried on at the IBP, RAS, Russia and complied with the ARRIVE guidelines and rules of biomedical ethics. Animal experiments were reviewed and approved by the Committee on Bioethics, RAS (protocol 627; 12/06/2022).

Results and Discussion

This study for the first time showed that relative soleus muscle weight was improved by 2-APB treatment for 3 days but not 7 days of unloading. At the same time, 2-APB treatment for 7 days of unloading significantly improved the cross-sectional area of both slow and fast muscle fibers (Figure 1). The

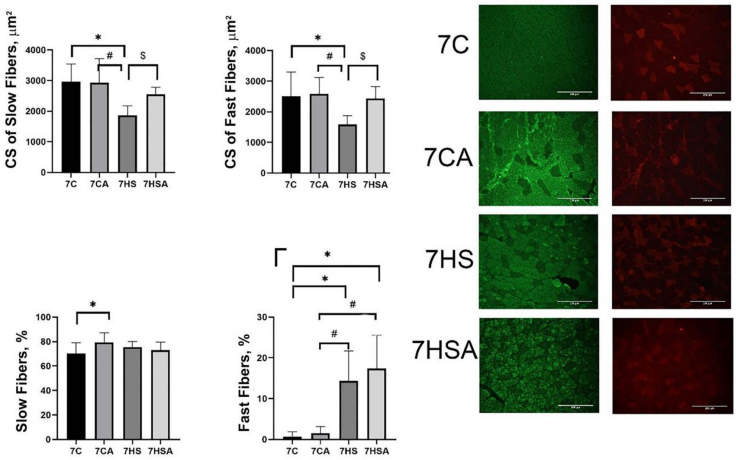

FIGURE 1
Evaluation of soleus muscle cross sections and fiber-type composition in control rats (7C), control rats treated with 2-APB (7CA), rats with 7 days of unloading (7HS), and 7 days of HS with 2-APB (7HSA). *indicates a significant difference from the control, and # indicates a significant difference from the 7HS group, $p < 0.05$.

discrepancy between fiber cross-sectional area and muscle mass may be due to the non-fibrous muscle components such as extracellular matrix and fluid (water, blood, lymph). In particular, it is known that during muscle unloading, muscle blood flow and, accordingly, blood supply to the soleus muscle decreases (Kimura et al., 2012), which could affect muscle mass. The percentage of fast muscle fibers similarly increased in both the 7HS and 7HSA groups (Figure 1). This correlated well with the increased mRNA expression of MyHC IId/x and IIb in the 7HS and 7HSA groups (not shown).

The degree of muscle atrophy during unloading is regulated by the balance between protein synthesis and protein degradation. eEF2 is an important regulator of protein synthesis in skeletal muscle. Similar to previous observations (Shenkman et al., 2015) (Tyganov et al., 2019) eEF2 phosphorylation was increased in the 3HS group (Figure 2). Phosphorylation

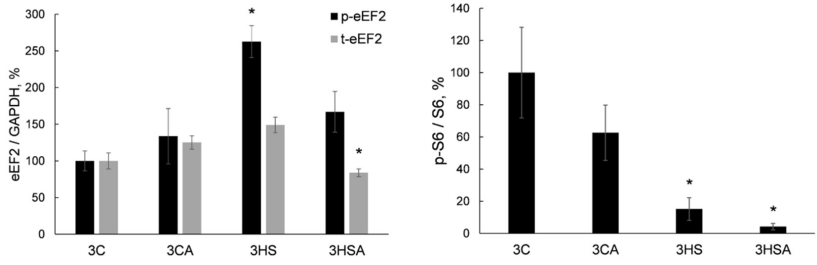

FIGURE 2

Evaluation of phospho- and total eEF2 and phospho-S6 content in control rats (3C), control rats treated with 2-APB (3CA), rats with 3 days of unloading (3HS), and 3 days of HS with 2-APB (3HSA). *indicates a significant difference from the control, and # indicates a significant difference from the 3HS group, $p < 0.05$.

of eEF2 by eEF2K kinase prevents its translocation into the nucleus, blocking elongation and protein synthesis on the ribosome. Treatment with 2-APB diminished total and phosphorylated eEF2 content in the 3HSA group when compared with the 3HS group (Figure 2) suggesting improved protein synthesis. The rate of muscle protein synthesis is also regulated by the content and phosphorylation of S6 ribosomal protein (Cao et al., 2019). The content of phospho-S6 ribosomal protein was decreased in both the 3HS and 3HSA groups. Previously, a decrease in the content of S6 protein was found during the development of atrophic muscle processes (Kawano et al., 2007).

Muscle unloading is associated with protein degradation and the activation of the ubiquitin-proteasomal system, including increased expression of E3-ubiquitin ligases MuRF1 and MAFbx (Bodine and Baehr, 2014). Treatment with 2-APB did not affect the unloading-induced upregulation of MuRF1 and MAFbx mRNA expression in 3HSA and 7HSA groups when compared with 3HS and 7HS groups, accordingly (Figure 3). In addition to the ubiquitin-proteasomal system degradation of muscle proteins during unloading is also regulated by the lysosomal system. ULK1 is a serine-proline kinase involved in autophagy (Rahman et al., 2024). It is known that IP3R is an activator of ULK1

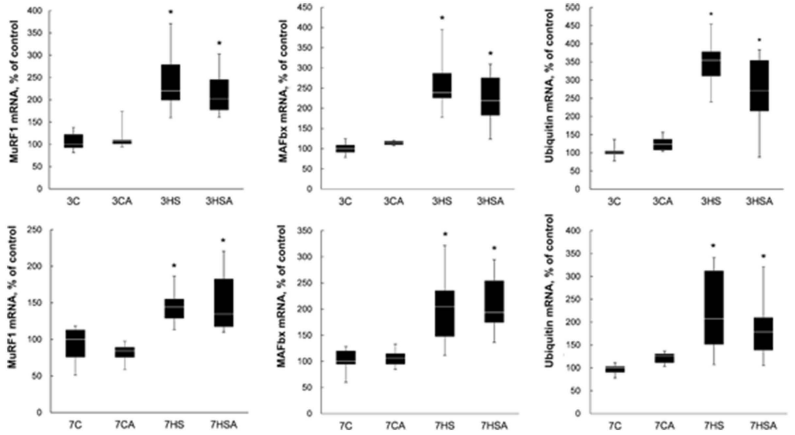

FIGURE 3
Evaluation of MuRF1, MAFbx, and ubiquitin mRNA expression in control rats (3C and 7C), control rats treated with 2-APB (3CA and 7CA), rats with 3 or 7 days of unloading (3HS and 7HS), and 3 or 7 days of HS with 2-APB (3HSA and 7HSA). *indicates a significant difference from the control, and # indicates a significant difference from the HS group, $p < 0.05$.

expression (Kania et al., 2017). ULK1 mRNA was equally increased in 3HS and 3HSA groups, but it was significantly decreased in the 7HSA group when compared with the 7HSA group (Figure 4). Similar results were observed for the mRNA expression of IL6 (Figure 4). IL6 is a key player in muscle atrophy. Blocking IL6 receptors prevents the development of muscle atrophy (Yakabe et al., 2018), and the introduction of IL6 into the muscle, on the contrary, leads to its atrophy (Sun et al., 2021). This suggests that 2-APB regulates muscle atrophy during unloading via ULK1 and IL6 signaling rather than E3-ubiquitin ligases MuRF1 and MAFbx.

Intracellular calcium is one of the regulators of CaMKII beta activity (Witczak et al., 2008) (Raney and Turcotte, 2008). Increased calcium concentration leads to the CaMKII beta phosphorylation on Thr-286 (Rostas and Skelding, 2023). The content of phosphorylated CaMKII beta in muscle nuclei was significantly increased in the 3HS group compared with the control, while in

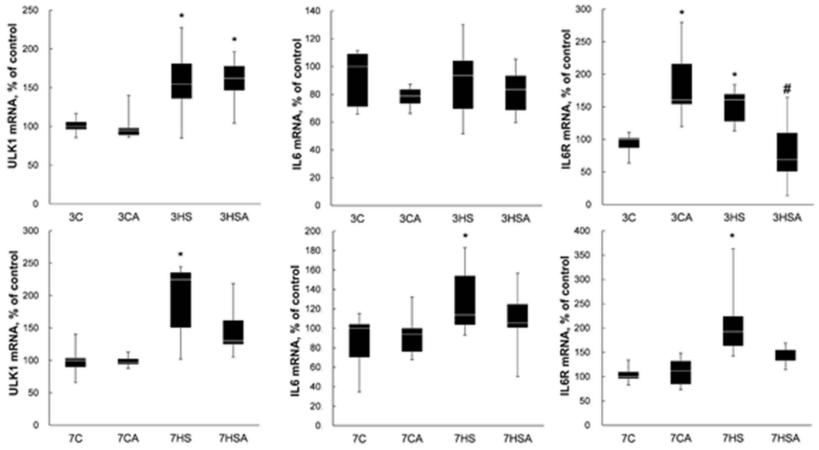

FIGURE 4
Evaluation of ULK1, IL6, and IL6R mRNA expression in control rats (3C and 7C), control rats treated with 2-APB (3CA and 7CA), rats with 3 or 7 days of unloading (3HS and 7HS), and 3 or 7 days of HS with 2-APB (3HSA and 7HSA). * indicates a significant difference from the control, and # indicates a significant difference from the HS group, $p < 0.05$.

the 3HSA group, it was similar to the control values. According to previous publications (Rose et al., 2006), the CaMKII phosphorylation can be used as a marker of increased calcium concentration. Calcineurin (CaN) and IP3R are well-known calcium sensors (Tu et al., 2016). The content of CaN and IP3R was increased in the 3HS group, but it was not different from the control in the 3HSA group (Figure 5). The results of our study suggest that the use of the IP3R inhibitor 2-APB during unloading leads to a decrease in the calcium signal in muscle fibers.

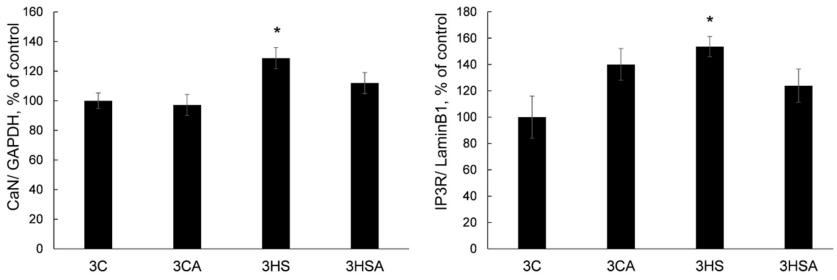

FIGURE 5

Evaluation of CaN and IP3R in control rats (3C), control rats treated with 2-APB (3CA), rats with 3 days of unloading (3HS), and 3 days of HS with 2-APB (3HSA). *indicates a significant difference from the control, and # indicates a significant difference from the 3HS group, $p < 0.05$.

Conclusion

It can be suggested that maintenance of muscle mass during unloading after treatment with 2-APB is associated with hindering of the decrease of protein synthesis. At 7 days of unloading, it was also affected by the downregulation of ULK1 and IL6 expression in the unloaded soleus muscle of rats treated with 2-APB. At the same time, MuRF1 and MAFbx mRNA expression was not significantly affected by the 2-APB treatment.

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Simulated microgravity affects pro-resolving properties of primary human monocytes

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Introduction

Space-related stressors such as microgravity lead to cellular and molecular alterations of the immune and inflammatory homeostasis that have been linked to disorders suffered by astronauts during their missions (Hughes-Hulford M., 2011). Most of the research over the past 30 years has consistently documented that innate and adaptive immune cells represent a target of microgravity, which leads to their defective or dysfunctional activation, as well as to an altered ability to produce soluble mediators - e.g., cytokines/chemokines and bioactive lipids - that altogether control tissue homeostasis (Lv H. et al., 2023). Bioactive lipids include a vast array of endogenous molecules that drive induction, intensity and outcome of inflammatory events. However, none of the studies published so far has addressed a recently characterized class of lipid mediators called "specialized pro-resolving mediators" (SPMs), which orchestrate the resolution of inflammation (Fava et al., 2024). The latter is the active control and confinement of the inflammatory torrent, which is mostly driven by other lipid signals called eicosanoids. SPMs are emerging as crucial players in those processes that prevent acute inflammation to degenerate into a chronic event. SPMs, along with their metabolic and signaling machinery, are being increasingly linked to many inflammatory disorders (Leuti A. et al., 2020), thus it seems of the outmost importance to fully interrogate their engagement in Space-related disorders, also with the perspective of developing therapeutic countermeasures. In the present work we investigated the effect of rotary cell culture system (RCCS)-simulated microgravity in primary human peripheral blood mononuclear cells (PBMCs) and found that monocytes displayed rearranged gene and protein expression of pivotal resolution-related receptors and enzymes, as well as reduced levels of RvD1 (a prominent SPM). This represents the first evidence of an effect elicited by microgravity on the resolution of inflammation in human immune cells.

Material and Methods

All materials and methods are reported in Leuti and Fava et al., 2024.

Results

RCCS-simulated microgravity rearranges gene and protein expression of resolution- associated elements in monocytes and causes RvD1 reduced biosynthesis

We sought to understand whether simulated weightlessness elicited a change in the expression of pivotal genes and proteins associated to the main SPM-binding receptors (i.e., GPR32, ALX/FPR2, GPR18, ChemR23, GPR101 and LGR6) or their main metabolic enzymes (i.e., 5-, 12- and 15-LOX and 15-PGDH) in immune cells isolated from healthy donors.

Thus, we cultured peripheral blood mononuclear cells (PBMCs) from healthy donors for 24h in RCCS or at 1 x g. Cells exposed to RCCS-simulated microgravity displayed a significant increase in the gene expression of GPR32, ALX/FPR2, GPR18 and ChemR23 in respect to 1g controls (Figure 1B), as well as a significant increase in the gene expression of 5-LOX (figure 1C). Protein products of these genes were then further investigated by means of polychromatic flow cytometry to obtain cell-specific data: monocytes were the immune cell type that was mostly affected by RCCS-simulated microgravity. In particular, monocytes displayed a significantly increased surface expression of SPM receptors GPR32 and GPR18, which engage RvD1 and RvD2, respectively, and decreased intracellular levels of 5-LOX, which is pivotally involved in the metabolism of several DHA-derived SPMs, as compared to 1g control samples (Figure 1D). By contrast, CD4 lymphocytes expressed lower levels of all the aforementioned proteins in respect to monocytes (both a 1g and after RCCS exposure), and simulated microgravity did not exert any significant effect on any of the SPM elements that were tested (Figure 1E).

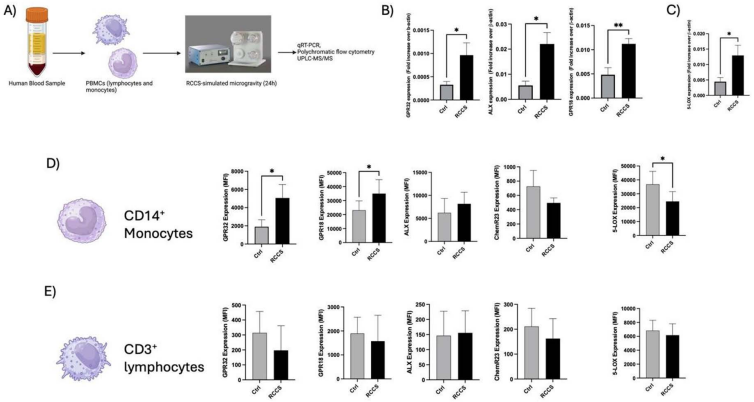

FIGURE 1

Gene and protein expression of the main SPM-related receptors and enzymes in PBMCs, monocytes and lymphocytes under 1 x g and RCCS-simulated microgravity: (**A**) Experimental setup. (**B**) qRT-PCR of SPM receptors and 5-LOX in PBMCs. (**C**) protein expression of SPM-receptors and 5-LOX in monocytes and lymphocytes by polychromatic flow cytometry. Data are shown as mean ± SEM of 6–9 independent experiments, each in duplicate. * $p < 0.05$; ** $p < 0.01$ (paired Student's t-test).

RCCS-simulated microgravity affects SPM biosynthesis in LPS-stimulated PBMCs

Given the enhanced expression of the GPR32 and GPR18 observed in monocytes kept for 24h in RCCS, we sought to understand whether microgravity might affect the biosynthesis and metabolism SPMs related to the main ligands of these two receptors – resolving (Rv) D1 and RvD2, respectively. Thus, using HPLC-MS/MS, we assayed whether PBMCs undergo a change in the amount of RvD1 and RvD2 following 24h in RCCS (Figure 2A). LPS stimulation was used to specifically activate monocytes, and led to a significant ~20% increase in the amount of RvD1 produced by cells at 1 x g, which returned to those of vehicle-treated samples following the exposure to RCCS-simulated microgravity (Figure 2B). This suggests that microgravity can indeed impair the biosynthesis of pro-resolving lipids. This is in consistent with the observed downregulation of 5-LOX in monocytes that experienced weightlessness.

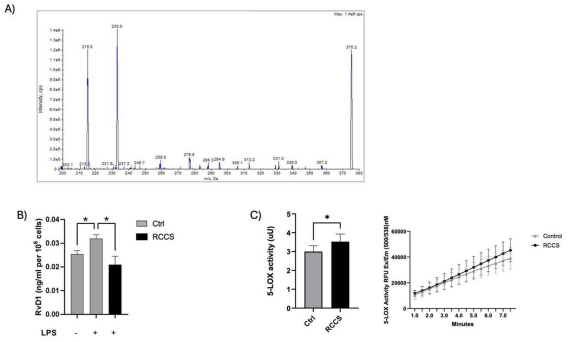

FIGURE 2
SPM metabolism in LPS-stimulated PBMCs under RCCS-simulated microgravity: (**A**) representative tandem mass spectrum of RvD1. (**B**) Quantitation of RvD1 in PBMCs that were exposed to RCCS-simulated microgravity for 24 h before being stimulated with LPS 100 ng/mL. (**C**) 5-LOX activity. Data are expressed as mean ± SEM of four independent experiments. * $p < 0.05$ (paired one-way ANOVA and Bonferroni's post hoc test).

Instead, we failed to detect any amount of RvD2 in all our samples.

Affinity essays also revealed that 5-LOX undergoes a slight enhancement under microgravity (Figure 2C).

Discussion

Microgravity is involved in immune alterations, which might be related to ineffective resolution. Here we demonstrate that RCCS-simulated weightlessness causes a monocyte- specific rearrangement of the SPM receptors at both gene (GPR32, GPR18 and FPR2/ALX) and protein (GPR32 and GPR18) level, suggesting that microgravity might indeed act on the monocyte responsiveness to pro-resolving stimuli. Furthermore, 5-LOX, which is involved in the biosynthesis of many SPMs, including those binding GPR32 (i.e., RvD1) and GPR18 (RvD2), is downregulated after RCCS incubation. Incidentally, we have also demonstrated in past years that LOX

enzymes are also pivotally involved in immune responses to microgravity: indeed 5- LOX is responsible for PBMC reduced proliferation and apoptosis following both authentic and simulated microgravity (Battista et al., 2012), while 1-LOX works like a "gravity sensor" as demonstrated by its enhanced substrate affinity (i.e. Km reduction) following parabolic flight (Maccarrone et al., 2001). The downregulation of 5-LOX might contribute to the reduced levels of RvD1 that we observed in cell supernatants from PBMCs that underwent RCCS; of note, the observed enhanced expression of 5-LOX gene and of its catalytic activity might represent an insufficient attempt to compensate for SPM production.

Conclusion

To date only a few studies have investigated the role of bioactive lipids in microgravity, with most of them focusing on endocannabinoids and eicosanoids. Thus, the possible role of SPMs in space biology remains as yet completely neglected. The present data represent the first attempt to understand the role of microgravity on pro-resolving lipids, and show that monocytes – one of the most important immune cells involved in the resolution process – display altered SPM signalling and metabolism during simulated microgravity. This investigation might represent a starting point for further studies on the resolution machinery, that might both help understanding the pathogenesis of space-related disorders and develop countermeasures that exploit or enhance the function of pro-resolving compounds in immune cells.

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Self-generated lower body negative pressure exercise: A low power countermeasure for deep-space missions

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Introduction

In space, astronauts experience conditions to which they are not adapted. On Earth, gravity-dependent hydrostatic forces within blood vessels maintain the body's blood pressure gradient, i.e., pressure is greater in the feet than at head and heart levels. During spaceflight, the absence of gravity eliminates all hydrostatic blood pressure gradients, resulting in a

redistribution of body fluids towards the head (Hargens and Richardson, 2009). Microgravity-induced headward fluid shifts are associated with mild but chronically-increased intracranial pressure (ICP), which may contribute to the pathogenesis of Spaceflight Associated Neuro-Ocular Syndrome (SANS) (Wojcik et al., 2020). Lower body negative pressure (LBNP) may effectively counteract headward fluid shifts and SANS (Marshall-Goebel et al., 2019). This study investigates self-generating LBNP as a low-volume, low mass, low-powered countermeasure against SANS. We hypothesize that self-generating LBNP reduces headward fluid shifts similar to traditional LBNP in a model of simulated microgravity.

Materials and Methods

SELF device

The self-generating lower body negative pressure (SELF) device is a collapsible, cylindrical chamber that encloses the user's legs and abdomen (Figure 1, A and B). The SELF device expands and contracts longitudinally, but not radially. It is similar to the device that Watenpaugh and colleagues developed in 1999 (Watenpaugh et al., 1999). Both ends of the device consist of a rigid circular plate. An opening in the top plate with a neoprene skirt and belt provide a waist seal. Valves on the top plate allow the user to manually control airflow into the device. A vest attached to the top plate is worn to evenly distribute mechanical load across the user's shoulders.

Traditional LBNP chamber

The traditional LBNP chamber is a rigid, hemicylindrical chamber that encloses the user's legs and abdomen. A neoprene skirt, belt, and shoulder straps maintain a seal around the waist. A vacuum cleaner attached to the chamber generates LBNP.

Methods

Eleven healthy subjects completed four trial conditions: 10-minutes seated upright posture, 10-minutes supine posture, 15-minutes traditional LBNP, and 15-minutes SELF LBNP. Heart rate, blood pressure, and cross-sectional area (CSA) data of left and right internal jugular veins (IJV) were collected. Measurements of IJV CSA served as an indicator of ICP since IJV CSA and ICP are positively correlated (Arbeille et al., 2015). Baseline measurements were collected in seated upright posture and subjects served as their own internal control. Traditional and SELF LBNP conditions were completed at 25mmHg in supine posture, as supine posture served as the microgravity analog. The order of conditions was semi-randomly assigned for each subject such that

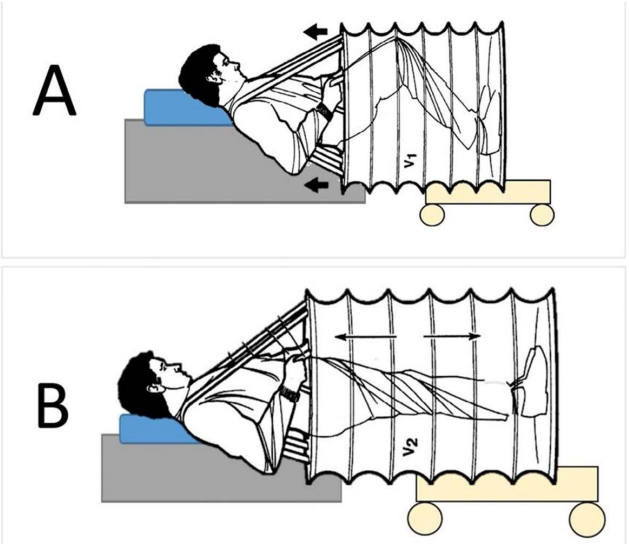

FIGURE 1
SELF Device. A raised platform supports the user's upper body while a moveable platform underneath the bottom plate allows the chamber to expand and contract.
A. Squat maneuver with open valves.
B. Leg extension with closed valves. Expansion of the sealed chamber generates negative pressure.

two LBNP conditions were not consecutive. Subjects completed all trials during a 1.5-hour, single day study.

All trial conditions were performed at rest, with the exception of SELF LBNP. During the SELF LBNP condition, subjects performed moderate exercise to generate negative pressure: subjects performed a squat with the SELF device's valves open (Figure 1A), closed the valves, then extended their legs to generate LBNP (Figure 1B). Small leaks in the device eventually caused the chamber to lose pressure. Upon reaching ~22mmHg, subjects reopened the valves to equilibrate the pressure inside the device and returned their legs to a flexed position. This procedure was repeated for the duration of the trial. Subjects practiced these motions to ensure they could generate 25mmHg.

Subjects' heart rate and blood pressure were continuously recorded throughout the experiment using the Finometer system (Finapres, Enschede, Netherlands). Cross-sectional images of the IJV were obtained via ultrasonography. For consistency and reliability, images of the IJV were taken in triplicate just caudal to the bifurcation of the common carotid artery. Two independent sonographers analyzed measurements of IJV CSA using image computing software (3D Slicer). For each set of images, sonographers identified and analyzed the smallest IJV CSA immediately before a carotid pulse. A MANOVA was used to determine if there were significant differences between time points within a single LBNP trial. This study was further analyzed using a non-parametric Friedman's Test. Wilcoxon signed-rank tests compared conditions. Bonferroni corrections were applied to address multiple comparisons. P-values less than 0.05 were considered significant.

Results

Mean values for heart rate and blood pressure during SELF LBNP were significantly higher than baseline measurements ($p<0.01$ for both variables). There were no significant differences in heart rate nor blood pressure between other experimental conditions. IJV CSA did not significantly differ over the course of each experimental trial. However, there were significant differences between left and right IJV CSA. Therefore, each side

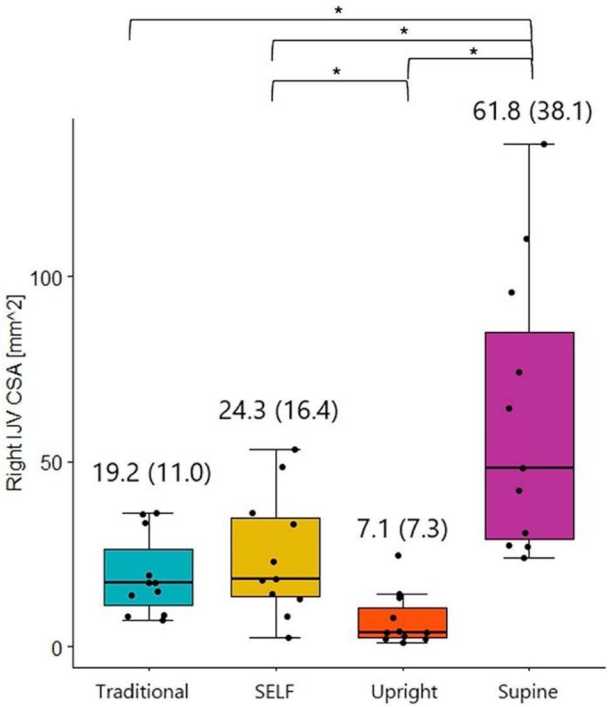

FIGURE 2
Right IJV CSA [mean (sd)] across different conditions.

was analyzed separately. Right IJV CSA during SELF LBNP was significantly smaller relative to supine posture (p=0.005, Figure 2). Right IJV CSA during SELF LBNP also differed significantly when compared to upright posture (p=0.002). Left IJV CSA values during SELF LBNP did not significantly differ from supine posture (p=0.365), but did differ when compared with upright posture (p=0.032). Both left and right IJV CSA during SELF LBNP did not significantly differ from traditional LBNP values (left p=0.465, right p=0.577). However, traditional LBNP significantly reduced IJV CSA on both sides (left p=0.001, right p=0.005) when compared to supine posture.

Discussion

Our findings indicate that SELF LBNP may reduce IJV CSA, and therefore, headward fluid shifts, as effectively as traditional LBNP. Our data support our hypothesis that SELF and traditional LBNP have similar effects on IJV CSA. Further, our results indicate that both SELF and traditional LBNP significantly reduce right IJV CSA during supine posture, but not to upright levels. Moreover, we expect significant increases in heart rate and blood pressure during SELF LBNP due to the moderate exercise involved.

The SELF LBNP device has some notable limitations. The neoprene skirt is not always leak-free. Additionally, short subjects do not expand the chamber as much as tall subjects do in order to generate 25mmHg LBNP. Therefore, our current device requires modification to accommodate more subjects.

A SELF LBNP device is a viable option for long-duration spaceflight. Compared to traditional LBNP, the SELF device has lower volume, lower mass, is collapsible, and requires little or no electrical power. Our results warrant further investigation of SELF LBNP as a countermeasure against headward fluid shifts and SANS.

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Research into “lunar hay fever” on Earth – finding answers in an *in vitro* airway model

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Introduction

Lunar exploration has captivated human interest throughout history. The concept of utilizing the moon as a crucial steppingstone towards further human space exploration has currently gained increasing support, partially in light of the potential for economic gain for space resource utilization and exploitation. A significant challenge for sustained human presence on the moon revolves around lunar dust. At the latest after the Apollo missions, it was clear that lunar regolith dust could be a significant nuisance during space missions. Astronauts' suits accumulated substantial dust deposits that could not be easily brushed off. Astronaut Harrison Schmitt reported the Apollo 17 Lunar Module as temporarily "full of dust", affecting the astronauts' breathing environment (Taylor *et al.,* 2012). In the past, some Apollo astronauts have had adverse reactions to lunar dust. In 1972, Harrison Schmitt suffered a brief sneezing attack, red eyes, an itchy throat and congested sinuses in response to lunar dust. Some further Apollo astronauts also reported allergy-like symptoms ("lunar hay fever") after tracking dust into their vehicles (Turci *et al.,* 2015). This issue, and its potential impact on biological systems, is closely tied to the unique properties and behaviors (Miranda *et al*., 2023).

Material and Methods

A tissue engineering approach (Maurer *et al*., 2023) was used to test the extraterrestrial interaction of lunar regolith dust particles on the human airway system. A special 3D model with properties of a native bronchial epithelium was generated using an implemented SISser (small intestine submucosa without serosa) matrix colonized with primary bronchial epithelial cells and donor-matched bronchial fibroblasts. One-week submerged conditions (proliferation phase) were followed by a three-week air-liquid-interface to stimulate maturation of the epithelial cells towards a functional pseudostratified epithelium. Completion of the epithelial layer was assessed

by transepithelial electrical resistance (TEER) measurement. Afterwards, the model was exposed to a common lunar dust simulant (JSC-1, particle size <20 µm) for up to 72h.

Results

First observations showed that the airway mucosa model responded consistently pro-inflammatory to the lunar dust exposure as shown by transcriptional and protein data. Epithelial integrity was impaired. In addition, methods of dust removal by the airway epithelium through mucus production and ciliary beat were affected. A major advantage is that we were able to use a single model to investigate the effects of regolith dust at different levels of cell biology (functional tests, histology, gene expression and secretome).

Discussion and Conclusion

The established airway model also proves to be a suitable model to investigate the interrelations of regolith particles and the host system. Future milestones for the airway model include the expansion of the co-culture with immune components and the adaption of the system for experiments in simulated and real microgravity environments (Figure 1).

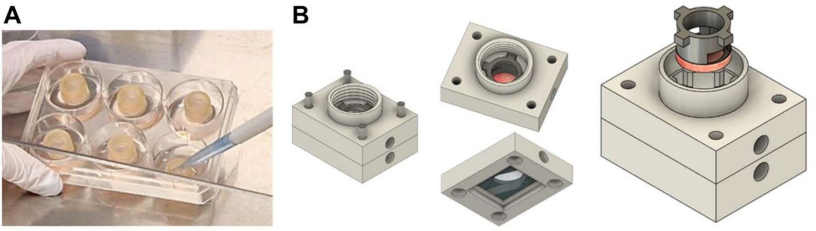

FIGURE 1
Artificial airway mucosa model. In this project we bring medicine, biology and technology together. (A) Starting with the crowns for producing the airway model, (B) the team is also working on the appropriate hardware for microgravity and hypergravity experiments.

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Mechanisms of adaptation to extreme environments the exposome signature project

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Background

The EXPOSOME SIGNATURE (or NEPTUNE-SDM/PP) project studies the adaptation mechanisms of the human organism to extreme environments, in particular space and deep sea. The project focuses on issues related to the health and well-being of the crew, which are considered enabling for human exploration of deep space.

Based on the studies done so far, long missions beyond the Low Earth Orbit (LEO) could predispose astronauts to a higher risk of chronic disease onset, because spaceflight adaptation mechanisms alter physiological functions, tissue homeostasis, metabolic processes, the interrelation between immune response and microbiome, the production of endotoxins and pro-inflammatory molecules [Lane et al.,1999; Strollo et al., 1999; Demontis et al., 2017; Strollo et al., 2018; Afshinnekooet al., 2020]. Chronic low-grade inflammation (LGI) is connected to these alterations and it is also a distinctive feature of major chronic diseases on Earth.

In addition to microgravity and cosmic rays, the space exposome also includes other stressors, such as confinement, isolation and psychophysical stress, which represent important risk factors in future space exploration missions beyond LEO. In fact, not only these stressors can induce alterations of the psychological profile, anxiety and depression, but they could also be contributing to the on-set of above-mentioned pathophysiological changes [Pagel J. I. and Choukèr A., 2016].

The exposome signature project: Overview and objectives

Analyzing biological samples (blood, saliva, urine, skin and hair) collected from astronauts before, during and after short- and long-duration space missions, the EXPOSOME SIGNATURE project aims to elucidate the changes induced by spaceflight in the neuroendocrine, metabolic, redox, immune and inflammatory profile of the subjects, as well as in the composition and activation of their microbiome and virome. The comparison between the effects caused by short and long exposure to the space environment helps to distinguish and better understand the acute pathophysiological alterations from those that appear later or persist even after returning to Earth. Parallel studies are conducted on submariners of the Italian Navy involved in operational underwater missions of duration comparable to that of the space missions. The comparison between the data collected from astronauts and those obtained from submariners allows us to pursue one of the main objectives of this project, that is to distinguish the effects induced by microgravity and cosmic rays, stress factors peculiar to the space environment, from those caused mainly by confinement, isolation and psychophysical stress, common to life on the International Space Station (ISS) and on board a submarine. Indeed, the submarine can be considered the best terrestrial analogue of the ISS for studying the effects produced by confinement, isolation, and psychophysical stress. Except for microgravity and cosmic radiation, other conditions on board the ISS and submarines, such as confinement, isolation, demanding tasks, artificial light, dietary changes, disturbances of circadian rhythm and sleep, are very similar.

Establishing cause-effect relationships between the pathophysiological changes observed during spaceflight and the different factors contributing to the space exposome is complex, but necessary to develop effective countermeasures, diagnostic markers, and reliable risk models.

By monitoring many parameters representing processes and functions crucial for the homeostasis of the organism, the EXPOSOME SIGNATURE project is expected to collect a wealth of information on: i) the mechanisms of adaptation to spaceflight and to the conditions of isolation-confinement; ii) the pathophysiological alterations in short- and long-term missions (namely, acute and chronic effects); iii) the recovery processes after returning to Earth. Data processing using machine learning techniques makes it possible to evaluate the interrelationships among the multiple functional alterations and generate models capable of predicting the long-term effects of the exposure to the space environment and consequent possible health risks. This approach allows us to have a more holistic knowledge of the many concomitant pathophysiological alterations induced by space flight in the human organism and their possible implications in the onset of acute and chronic diseases.

Further objectives of the project are: i) selection of the best biomarkers to monitor astronaut's health during and after space missions, to reveal the onset and progression of diseases, and to evaluate the effectiveness of countermeasures; ii) identification of possible targets for tools and strategies preventing or treating alterations induced by the space exposome; iii) development of predictive risk models for short- and long-term exposure to extreme environments. These predictive tools could allow for better targeting protocols and therapies to be adopted in extreme, isolated environments. The logo of the EXPOSOME SIGNATURE project is showed in Figure 1.

The results we present in this paper were obtained by assessing samples collected from submariners of the Italian Navy before, during and after some submarine missions of different length. These are preliminary analyses, as many of the tests planned in the isolation and confinement studies here described, are still ongoing.

FIGURE 1
The Exposome Signature project logo.

Materials and Methods

Sampling procedures

Blood sampling

Blood draw was performed by a sterile cubital venipuncture after an overnight fast of at least 8 hours, with no food or drink consumed, and before breakfast.

Saliva sampling

Saliva sampling was performed after the same 8-hour fast and before breakfast. Subject did not brush teeth, drink water, smoke, or chew gum for 30 min before sampling. Two sampling procedures were required per session: in the first, the subject produces saliva in the mouth (2-3 ml) and drools into a polypropylene centrifuge tube; in the second, the subject chews a swab and spit it with the absorbed saliva into a tube.

C-reactive protein evaluation

C-reactive protein (CRP) levels were quantified using an ELISA kit (R&D Systems, DY1707), requiring 100 µL of sample. The assay involved incubation with a murine anti-human CRP capture antibody, followed by sample or standard addition, a biotin-linked detection antibody, and streptavidin-conjugated horseradish peroxidase. Absorbance was measured at 450 nm. The detection range for CRP was 15.6–1,000 pg/mL, based on a recombinant standard.

Protein carbonylation

Protein carbonylation, a marker of oxidative stress, was quantified using the Protein Carbonyl ELISA Kit (ab238536). The assay required 100 µL of sample, which was incubated with dinitrophenylhydrazine to convert carbonyl groups into detectable derivatives. An anti-DNP antibody was then added, followed by a secondary antibody conjugated to horseradish peroxidase. Absorbance was measured at 540 nm, with a detection range of 0.375–7.5 nmol/mg.

Malondialdehyde evaluation

MDA was quantified by HPLC (Jasco, Japan) with a UV detector, following the method of Karatas et al. [2002]. MDA standards were prepared by diluting 10 µL of TEP (1,1,3,3 tetraethoxypropane) in 10 mL of 0.1M HCl, heating in a boiling water bath for 5 minutes, and cooling on ice to generate a hydrolyzed acetal. A 40 µM MDA stock solution was then made by diluting 1 mL of the hydrolyzed acetal in 100 mL of water. For sample preparation, 100 µg of cell proteins were mixed with 40 µL of 0.1M HClO4, centrifuged at 4500g, and the supernatants were used for HPLC analysis. This procedure allows protein precipitation and release of bound MDA to the amino groups of proteins, and other unbound amino compounds. The mobile phase was KH_2PO_4 (6.8g KH_2PO_4 in 100ml distilled water)/ /methanol/acetonitrile (72/18/11, v/v); the flow rate was 1mL/min. Chromatograms were monitored at 254 nm.

Results

Spaceflight induces several effects, such as immune dysfunction, muscle atrophy, bone loss, vascular dysfunction, and metabolic alterations, which share molecular mechanisms with chronic diseases, including oxidative stress, mitochondrial dysfunction, autophagy, and impaired DNA repair. These processes activate inflammation, either directly or indirectly. Post-flight, astronauts exhibit elevated cytokine levels, indicating a systemic pro-inflammatory state [Buchheim et al, 2019]. Similarly, space analogues on Earth have shown that I-C conditions activate the autonomic nervous system, promoting pro-inflammatory gene transcription, thus exacerbating inflammation [Scatà et al, 2023]. Consequently, monitoring systemic inflammation in astronauts is crucial to prevent increased low-grade inflammation and reduce the risk of chronic disease onset during extended space missions.

CRP is a marker of inflammation, released in response to interleukin-6 by macrophages and T cells during various inflammatory conditions. In submariners involved in long missions, CRP levels in saliva showed a significant increase post-mission, suggesting that extended mission duration may lead to heightened inflammatory responses due to prolonged exposure to stressors. Protein carbonylation is a major marker of oxidative stress, representing one of the most damaging irreversible protein modifications. Analysis of protein carbonylation levels in saliva samples showed a significant increase in protein carbonylation during long missions, with a further increase at the end of the mission. This suggests that extended mission duration leads to elevated oxidative stress, likely due to prolonged exposure to environmental and physiological challenges. To confirm the findings on oxidative stress from protein carbonylation, the levels of malondialdehyde (MDA) — a marker of lipid peroxidation — were measured in plasma samples of submariners using HPLC. While there was a non-significant increase in MDA levels during the mission, a significant rise was observed in the post-mission phase, further indicating heightened oxidative stress following long-duration missions.

Conclusions

These preliminary results provide some interesting insights, suggesting that:

- On average, inflammation levels are higher during and immediately after the mission compared to pre-mission levels.
- In most cases, the mission conditions significantly alter the oxidative phenotype, resulting in increased oxidative stress.
- Submariners serve as a valuable model for studying the effects of isolation and confinement in extreme environments, offering insights relevant to space missions.

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Wound healing and tissue regeneration in space the suture in space experiment

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Introduction

The wound healing (WH) process is essential for the integrity and survival of the organism; therefore, it is strictly regulated by many biochemical and biophysical factors and highly conserved throughout evolution. On Earth, the process has been studied in depth, nevertheless there are still scientific problems that have not been fully resolved, such as the role of biophysical factors in the regulation of tissue repair/regeneration mechanisms. Defects in WH lead to pathological conditions, ranging from chronic ulcers to fibrosis. They affect a large number of patients, with serious impact on their quality of life and high costs for national healthcare systems. In Space, there have been relatively few studies on WH, tissue repair and regeneration mechanisms.

In vitro studies have highlighted changes in the behavior of cell populations involved in the healing process, such as fibroblasts, endothelial cells and keratinocytes [Pietsch et al., 2011; Morbidelli et al., 2005; Monici et al., 2011]. Studies on animal models, albeit with contradictory results, report alterations in the three phases of WH, namely inflammation, proliferation and remodeling, generally resulting in a delay of the process [Davidson et al., 1999; Campbell et al., 2005]. There are no studies on humans, other than anecdotal observations. However, the pathophysiological alterations induced by spaceflight could affect the organism's resilience to injury. Therefore, a better understanding of WH in Space is needed to implement procedures and tools to manage emergency surgery, trauma, serious burns and wounds that may happen in future manned space exploration missions beyond Earth's orbit, at a distance incompatible with medical evacuation to Earth. Furthermore, these studies are a unique opportunity to understand healing mechanisms not yet fully known on Earth, for example the regulatory mechanisms involving mechanical stress.

The Suture in Space (SiS) experiment, selected by the European Space Agency (ESA-AO-ILSRA-2014) and funded by the Italian Space Agency (C. ASI N. 2018-14-U.0) aimed to study the behaviour and healing of ex vivo sutured wound models (SWMs) in human tissues exposed to unloading conditions. In particular, the objectives of the experiment were to investigate: i) the effects of gravitational forces on the behavior of sutured wounds and the WH process; ii) the adaptation of suturing materials and techniques to the space environment, characterized by microgravity (μg) conditions, thus developing strategies to enhance WH in space; and iii) how to improve suturing techniques on Earth to promote WH avoiding the formation of fibrotic scars.

The Suture in Space (SiS) experiment was launched on 26th November 2022 with SpX-26 (Cargo Dragon 2), Expedition 68, and it was conducted on board the International Space Station (ISS) from 28th November to 7th December 2022, with the collaboration of the JAXA astronaut Koichi Wakata. The logo of the SiS experiment is showed in Figure 1.

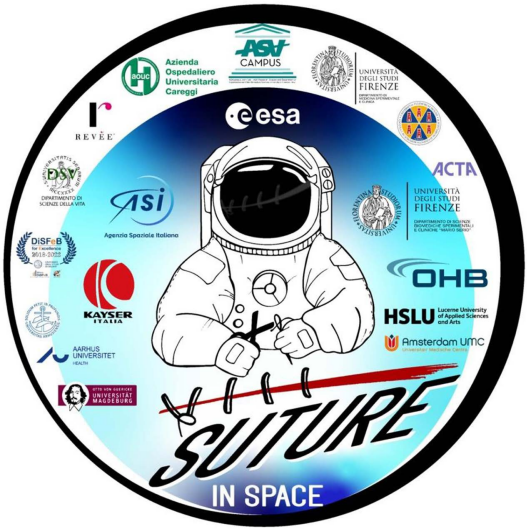

FIGURE 1
Suture in Space experiment logo.

The SWMs were prepared from human skin and blood vessel biopsies derived from plastic, vascular and cardiovascular surgery in healthy subjects and collected under informed consent and approval by Local Ethic Committee.

The experiment preparation required intense research activity in order to: a) standardize procedures for sample collection, SWM preparation, tissue culturing and monitoring, postflight sample analysis; b) define the requirements for hardware (HW) development. To ensure tissue viability throughout the experiment (4 weeks), a novel tissue culture technique was developed, based on enriched culture media and a device capable of modeling physiological tensile strength in tissues. It allowed the tensile strength to be monitored throughout the experiment, thus studying the suture tightness.

Materials and Methods

Experiment design and preparation of sutured wound healing models

A detailed description of the SiS experiment design was previously reported [Monici et al., 2021]. Briefly, the human biopsies were collected at Careggi Hospital in Florence, Italy, stitched to specifically developed frames to model tissue strength, cultured in transport containers with modified RPMI-based culture medium at T= 4±2°C, and transferred at the launch site. Here, the SWMs were prepared by performing, and then suturing, linear incisions on the skin samples, or end-to-end anastomoses on blood vessels. The SWMs were integrated in the HW, whose culture chamber was filled with DMEM-based culture medium modified by adding serelaxin (recombinant human H2 relaxin hormone) (60ng/mL) and metal-nonoateZn(PipNONO)Cl (28ng/mL). The tensile strength in the sutured tissues was monitored throughout the experiment by the load cell integrated within the HW. During experiment's handover and upload to the ISS, samples were kept in NASA Double Cold Bags (DCBs) at about 24°C. Eight SWMs, four skin and four blood vessel samples were transferred on board the ISS, photographed by the astronaut and placed in the Biolab facility. The HW of the experiment was developed by OHB and Kayser Italia and described by Monici et al. [2021].

After 4 days of incubation at 32°C, two HWs, each containing one skin and one vessel, were removed from the Biolab, frozen, and maintained in the cold stowage facility at -80°C. After 9 days, the same procedure was applied to the remaining two HWs, each containing one skin and one vessel. Experiment timeline and temperature profile were monitored throughout the experiment. During the transfer from the ISS to Earth, samples were maintained at about -20°C.

A ground-control experiment was performed reproducing the experimental conditions of the in-flight experiment, except for microgravity.

Histology and electron microscopy

Tissue samples were processed using two distinct protocols for histological analyses. For conventional histology, samples were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Sections (5 µm thick) were cut and stained with hematoxylin-eosin, picrosirius red, or aldehyde fuchsin to assess general tissue structure, collagen, and elastic fibers. For electron microscopy, samples were fixed in Karnovsky fixative, post-fixed in osmium tetroxide, dehydrated, and embedded in epoxy resin. Light microscopy images were acquired using a Nikon Eclipse E200 microscope, and electron microscopy was performed using a JEM 1010 microscope.

Immunofluorescence staining

Immunofluorescence was conducted on 5 µm paraffin-embedded sections to identify cellular markers, including those for mast cells, blood vessels, fibroblasts, and ECM components. Antigen retrieval preceded overnight incubation with primary antibodies, followed by a 2-hour incubation with fluorescent secondary antibodies. Microscopy was performed using Olympus, Leica, and Nikon microscopes. Negative controls were run by omitting the primary antibodies.

Western blotting

Western blot analysis was performed on frozen tissue samples. Protein extraction was achieved via homogenization in lysis buffer, followed by centrifugation. Proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were incubated with primary and peroxidase-conjugated secondary antibodies, and protein bands were visualized using a Chemi-Doc™ Touch instrument. β-actin was used for normalization.

Proteomic analysis

Proteins were extracted, quantified by BCA assay, and analyzed by SDS-PAGE. Tryptic digests were subjected to mass spectrometry (nLC-nESI-HRMS/MS) for proteomic profiling. Label-free quantification was used to identify and quantify proteins using MaxQuant software.

Gene expression profile

Three analyses were conducted: (1) broad-spectrum analysis of genes involved in apoptosis, ECM remodeling, and cell differentiation; (2) targeted gene expression profiling using PCR arrays for cell motility and signaling pathways; and (3) analysis of key apoptotic genes. RNA was extracted, reverse transcribed into cDNA, and analyzed using real-time PCR, with results normalized to housekeeping genes.

TUNEL assay and propidium iodide staining

The TUNEL assay was performed according to the manufacturer'sprotocol (Abcam, ab206386) and analyzed with a Leica DM 2000 microscope. Palatine tonsil cross-sections were taken as positive controls using an objective with a calibrated magnification of 100×, 200× and 400×. For propidium iodide (PI) staining, skin tissue specimens were fixed with paraformaldehyde and embedded in paraffin. 5-µm paraformaldehyde-fixed skin sections were prepared, stained with PI solution and examined using a Zeiss LSM 800 confocal microscope.

Results

After retrieval, SWMs were assessed for suture tightness, morphology and ultrastructure, endothelial function, proteomic profile, expression profile of genes involved in WH, markers of fibroblast activation, extracellular matrix (ECM) turnover, apoptosis and necrosis.

Both in ISS samples and controls, the sutures were preserved as well as tissue morphology with well-preserved epidermal, dermal, and vascular structures. However, significant alterations were observed in the ISS samples, particularly affecting the ECM turnover, structure and composition, as demonstrated by changes in collagen and elastic fibers. These findings suggest that microgravity impacts tissue repair mechanisms and ECM remodeling, potentially influencing the mechanical properties of healing tissues. Ultrastructural analysis further revealed cellular alterations in ISS samples despite overall preservation. A decrease in cell proliferation and activation was observed in ISS samples, particularly concerning keratinocytes and fibroblasts, compared to ground controls. Specifically, in ISS samples, an impressive decrease in the TGFβ expression was observed, in agreement with a decrease in expression of the keratinocyte and fibroblast activation marker Hsp-47.

In addition to the alterations observed in the ECM by histology, proteomics and gene expression data revealed significant changes in ISS samples with regard to ECM turnover, quantitative alterations in ECM components, alterations in the spazial distribution of ECM components, such as collagen III, cytoskeleton organization, oxidative stress response, cell migration and apoptosis. Concerning the latter, TUNEL assay showed a moderate increase in apoptosis in ISS samples, which only affected endothelial cells and not fibroblasts.

Conclusions

The results obtained from the SiS experiment can provide interesting insights concerning the role of gravity and mechanical factors in tissue repair and to design and develop strategies for wound diagnostics, management and therapy in Space and on Earth. The tissue culture technique developed for the SiS experiment might be usefully applied in Space and on Earth in the field of tissue regeneration and engineering, as well as in preliminary pharmacological studies.

Acknowledgements
The authors thank ESA (SiS-ESA-AO-ILSRA-2014 and MAP-WHISPER Project C.N. 4000130928/20/NL/PG/pt) and ASI (SiS C.N. 2018-14-U.0) for their support. The authors thank astronaut Koichi Wakata for his collaboration to the experiment on board the ISS.

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Exposure to hypergravity induces changes in the erythrocyte membrane and antioxidant potential of mice housed in the MDS facility

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Citation

Murgia, G., Zava, S., Colombo, I., Ferranti, F., Corsetto, P.A., Rizzo, A.M. Exposure to hypergravity induces changes in the erythrocyte membrane and antioxidant potential of mice housed in the MDS facility.

Introduction

All living organisms have evolved and adapted to live under Earth's gravitational force. Understanding how various effectors, such as gravitational force, affect organisms is crucial for maintaining safe human

space exploration. Altered levels of gravity affect the physiological function of multiple tissues, cells, and organs in living organisms (Demontis et al., 2017).

Lipid membrane composition is altered by several unfavorable space conditions, including hypoxia, hypothermia, and microgravity, which increase susceptibility to oxidative stress. Indeed, previous research—also conducted in our lab—suggested that microgravity alters plasma membrane permeability and erythrocyte cellular metabolism, changing the levels of phospholipids and cholesterol. Furthermore, hypergravity impacts physiological processes in tissues and organs; assessing the effects of hypergravity is a crucial first step to fully understand how the body reacts to changes in gravity.

The purpose of this study was to examine in vivo how hypergravity affected the lipid phenotype and antioxidant potential of mouse erythrocytes.

Methods

Animals were housed in the Italian Space Agency's Mice Drawer System (MDS), a facility designed to house rodents on the ISS and adapted by Thales Alenia Space to the Large Diameter Centrifuge (LDC-ESA), to expose mice to a 3xg environment (Cancedda et al., 2012; van Loon et al., 2009). Vivarium animals and MDS-like cage animals were compared as controls.

After 27 days of 3xg exposure, a tissue sharing protocol was performed among international researchers to analyze all tissue specimens. We purified and analyzed the red blood cells from whole blood.

The membrane lipid phenotype was assessed by gas-chromatography and liquid-chromatography. Antioxidant enzyme assays were performed on hemolyzed fractions to examine the effect on oxidative homeostasis (Rizzo et al., 2012).

Results

Our findings demonstrate a direct impact of the elevated gravity level, as mice exposed to a modified gravity showed a change in the fatty acid

composition of 3xg mice in comparison to control mice. Furthermore, the membrane cholesterol was significantly increased.

The ratio of inflammatory to anti-inflammatory eicosanoid precursors was measured to assess the impact of hypergravity conditions on the animal's metabolic and inflammatory processes. The arachidonic acid/eicosapentaenoic acid ratio, a well-known marker of inflammatory status, showed a slight decline. These findings could be due to a process of metabolic compensation during long-term exposure that leads to a resolution of inflammation.

The peroxidability index (PI), which gauges how sensitive fatty acids are to peroxidation, was measured to assess the effect of fatty acid composition on the possible degree of oxidative stress; under 3xg conditions, PI was noticeably elevated.

Lastly, we examined the amount of glutathione present in the red blood cells as well as the endogenous antioxidant activity of scavenging enzymes in the hemolysate. In comparison to control mice, 3xg mice exhibited a marked increase in glutathione peroxidase enzyme activity.

Conclusions

This study shows that the antioxidant system and lipid composition of erythrocytes are altered by hypergravity. Our results will be correlated with other tissue and metabolic data obtained by other researchers in the team to have an integrated vision of hypergravity effects on animal physiology. Additional research will be required to determine potential preventive measures to guarantee a sufficient degree of crew safety and health during extended space flights.

Acknowledgements

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Impact of microgravity exposure on genes regulating cell turnover in rat mammary gland

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Introduction

For millions of years, earth's gravitational force has impacted the life forms on earth, and influenced their physical, biochemical and molecular subtleties (Adamopoulos et al., 2021). On the earth, human systems, such as, musculoskeletal and cardiovascular have evolved in structure and function to counteract the constant gravitational acceleration (Garroffolo and Pesce 2019; Murphy et al., 2018). Similarly, the digestive system has developed to function effectively under the terrestrial environment to facilitate breakdown of food and provide the energy needed for growth, development, and reproduction (Yang et al., 2020). Equally, the endocrine system that regulates a plethora of body's function, including homeostasis, has evolved to work optimally under earth's force of gravity (Masini et al., 2012). However, exposure to microgravity causes significant alterations in the output of diverse human systems from musculoskeletal to endocrine (Masini et al., 2012; Patel et al., 2020; Adamopoulos et al., 2021). For example, exposure to the space ecosystem perturbs the nervous and immune systems leading to asthenia, cyclothymic and biorhythm disorders and increases disease susceptibility (Yang et al., 2020; Masini et al., 2012).

The mammary gland is a dynamic organ that undergoes significant reprogramming and functional remodeling during pregnancy to support milk production (Inman et al., 2015; Fu et al., 2020). These dynamic structural changes that include proliferation of epithelial cells and profound ductal branching facilitates the formation of the basic milk synthesizing and secretory unit called the alveolus. Accordingly, the distinct anatomical and functional evolution of the organ, coupled with the greater ease of access due to its anatomical position has provided scientist an ideal model to study normal and pathological development of an organ during environmental perturbations (Inman et al., 2015; Fu et al., 2020). A coordinated balance between proliferation and apoptosis is vital for the development, differentiation, functioning and morphology of the mammary gland (Fu et al., 2020). Dysregulation of proliferation and/or apoptosis are widely implicated in diverse pathological conditions of the mammary gland, including cancer (Inman et al., 2015). Previous studies have shown how changes in gravity-load affected the endocrine, metabolic, and reproductive systems (Plaut et al., 1999; Casey et al., 2015; Patel et al., 2008). However, there is limited data on the effect of microgravity exposure on genes associated with cellular turnover in a dynamic organ, like the mammary gland. Therefore, the focus of this investigation was to use a microarray-based approach to analyze the expression profile of genes associated with cellular turnover in the mammary gland of pregnant rats exposed to microgravity.

Materials and Methods

Animals and treatment conditions

The research protocol was reviewed and approved by the NASA Animal Care and Use Committee prior to experimentation. Briefly, time-bred pregnant Sprague-Dawley rats (n=4) were flown aboard the space shuttle (STS- 70) from days 11 to 20 of pregnancy, and samples were collected surgically within an hour of the shuttle landing following induction of anesthesia with halothane (Casey 2015). The control group of pregnant (n=4) rats were exposed to matching environmental conditions, such as light and temperature, present on the shuttle as described previously (Casey 2015).

RNA preparation for microarrays & functional analysis

Manufacturer's recommended protocols for RNA amplification and biotinylating were followed (NuGEN, San Carlos, CA). Thereafter, samples were hybridized to the Rat 230 2.0 GeneChip® array (Affymetrix, Santa Clara, CA) (Casey 2015). The data normalization and transformation were done using multichip analysis approach (Casey 2015). Thereafter, the resultant raw data files were imported into R (Bioconductor) for analysis with LIMMA. DAVID was utilized for functional annotation and enrichment analyses (Casey 2015).

Results

The functional clustering of differentially expressed genes revealed an enrichment of genes associated with proliferation (Enrichment score 5.96, $P < 0.01$), and apoptosis (Enrichment score 4.63, $P < 0.01$). The highly up- and

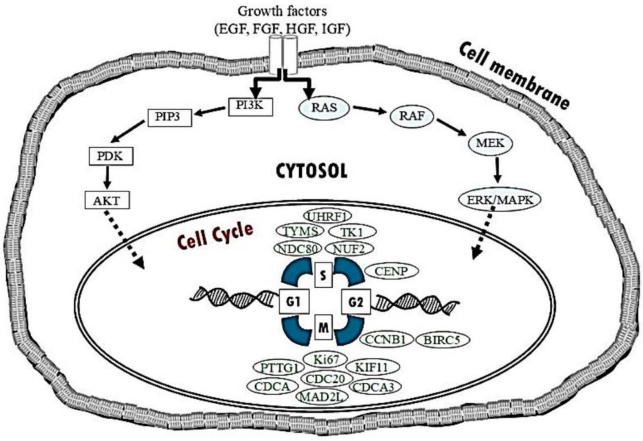

FIGURE 1
An illustration representing proliferation-related downregulated genes at the subcellular level in microgravity-exposed periparturient rat mammary gland compared to ground-based controls.

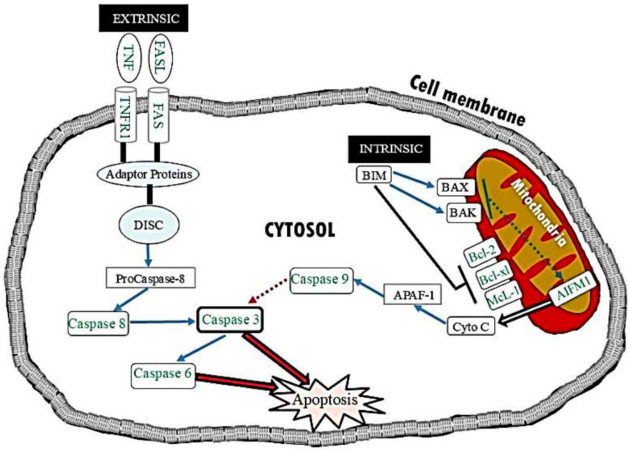

FIGURE 2
An illustration demonstrating apoptosis-related downregulated genes at the subcellular level in microgravity-exposed periparturient rat mammary gland compared to ground-based controls.

downregulated genes detected for proliferation in the mammary glands of rats exposed to microgravity were PIK3C2A (Fold 2.4, $P < 0.01$) and RRM2 (Fold -3.5, $P < 0.01$) (Fig. 1). Analysis of differentially expressed apoptotic genes in the mammary glands of rats exposed to microgravity showed that TRAIL (Fold 3.9, $P < 0.01$) was highly upregulated, while TRAF1 (Fold -2.0, $P < 0.01$) was significantly downregulated (Fig. 2).

Discussion

Microarray analysis revealed that microgravity exposure affected expression of genes associated with proliferation and apoptosis in the periparturient rat mammary gland. The canonical MAPK/ERK pathway is a central regulator of cell proliferation and modulates genes associated with cell cycle progression (Wen et al., 2022). Our results show that several genes critical for the transition of the distinctive phases of the cell cycle were downregulated in the mammary gland with exposure to microgravity (Fig. 1). It is plausible that

exposure to microgravity affects the mitotic cycle by altering the expression of regulatory genes, and merits further investigation.

Apoptosis is an evolutionary conserved program that is vital for cell turnover, organogenesis, morphogenesis and physiological output (Kaushal et al., 2023). The programmed cell death can be triggered by either extrinsic or intrinsic pathways (Kaushal et al., 2023). Our findings reveal that expression of a myriad of genes associated with both extrinsic and intrinsic apoptotic pathways were altered in the mammary gland of periparturient rats with exposure to microgravity (Fig. 2). The mRNA abundance of TNF, TNFR1, FASL, and FAS that play a pivotal role in the formation of the cytoplasmic death-inducing signaling complex connected to the extrinsic pathway were suppressed in the microgravity-exposed rats. Similarly, we found that exposure to microgravity downregulated the mRNA expression of BCL-related genes that govern the intrinsic apoptotic pathway. This suggests that microgravity exposure affects both extrinsic and intrinsic apoptotic pathways leading to a cellular homeostatic imbalance. However, further research is needed to confirm this.

Conclusion

These findings collectively show that exposure to microgravity impacts steady-state expression of genes responsible for cellular turnover in an organ that undergoes dynamic remodeling during pregnancy. Equally, it demonstrates that exposure to microgravity alters expression of genes associated with biological processes that are indispensable for organogenesis, morphogenesis and function.

Acknowledgements

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Effects of simulated microgravity on sperm function: An *in vitro* study evaluating sperm quality and function-specific genes

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Introduction

Microgravity, a state of near-zero gravity, has been shown to negatively affect multiple biological systems including the male reproductive system, with potential implications on male fertility. In humans, research findings remain inconclusive, with some studies reporting reduced sperm motility following exposure to parabolic flight (Kumar et al., 2013), while others find no significant effects (Boada et al., 2020). Additionally, studies on sperm motility, in mice exposed to microgravity, have shown conflicting results, with some showing a decrease in motile spermatozoa (Kamiya et al., 2003; Ogneva et al., 2020a), while others report no significant changes (Tash and Bracho, 1999; Usik and Ogneva, 2018). Similarly, fruit fly studies have indicated increased sperm motility under microgravity (Ogneva et al., 2020b; Ogneva,

2021), highlighting the variability in findings across species. Research on the broader impacts of spaceflight, microgravity and simulated microgravity (s-MG) on sperm quality and function remains limited, particularly in humans.

Current literature suggests that rats exposed to s-MG displayed degeneration and necrosis of spermatogenic cells, alongside severe structural damage of the testis (Ding et al., 2011), reduced testosterone levels and testicular weight (Kamiya et al., 2003), and damage to DNA mouse spermatogenic cells (Wakayama et al., 2017). In a recent study, it was also highlighted that spaceflight could lead to mutations in genes that affect male fertility (Omolaoye et al., 2023). The inconsistencies in prior research likely stem from differences in s-MG techniques, species, methodologies, and exposure durations. Given the variability in these findings and the limited number of human studies, this study aimed to address these gaps by investigating the effects of s-MG, using a Rotary Cell Culture System (RCCS), on sperm function (including sperm motility, viability, and acrosome reaction) and the genes that regulate these processes.

Material and Methods

Sample collection and study design

Participants for this research study consisted of five healthy males recruited through voluntary sampling methods. Inclusion criteria include individuals within the age range of 20 and 35 years. Normozoospermic semen samples used in this prospective *in vitro* experimental study were obtained and assessed in accordance with the World Health Organization (WHO) guidelines (WHO, 2021). Following the initial neat analysis, samples were double washed and thereafter divided into three different groups at 37°C. The groups consisted of a control group where semen samples were placed in a static incubator, a 2-dimensional (2-D) group where samples were placed on a shaker incubator moving in two dimensions at 15 rpm and a s-MG group where samples were placed in a RCCS at 15 rpm inside an incubator. Sperm parameters (motility, viability, and acrosome reaction (AR)) of the various groups were analyzed at 0 hour (Hr), 1Hr, 3Hr and 6Hr. Upon completion of

the experiments, the remainder of the samples were stored at -196 °C until further analysis.

Sperm parameters analysis

After liquefaction, 2 µL of sample was pipetted onto Leja® chamber slides kept at 37°C using a slide warmer. Evaluation of semen parameters including concentration, total motility (TM) and progressive motility (PM), was then performed using the computer-aided system analysis (CASA) through the SCA® automated software system (MicrOptic, Spain). For morphology analysis, smears were air-dried and stained with SpermBlue™ and analysed using the SCA® morphology module, as previously described. Viability was determined via eosin-nigrosine dye-exclusion staining to differentiate between viable and non-viable cells by counting 200 spermatozoa in duplicate under light microscopy (40x brightfield) using a hemocytometer desktop counter, with results expressed as a percentage.

Acrosome-reacted spermatozoa were analyzed using the Fluorescein-conjugated Pisum sativum agglutinin (FITC-PSA). A minimum of 100 spermatozoa was counted, classifying them as either acrosome intact (when the acrosome region fluoresced bright green) or reacted (when no acrosome was detected, and the spermatozoa appeared dull and empty). The result was expressed as a percentage of reacted cells.

Gene expression analysis

The assessment of genes specific to sperm motility (SPATA6, SPATA20) and AR (CABS1) was performed at 0Hr and after 6Hr. Briefly, total RNA was extracted from cryopreserved sperm samples using the RNeasy isolation Kit (Qiagen, USA) according to the manufacturer's instructions. cDNA was synthesized from 1 µg of total RNA (cDNA Synthesis Kit, SolisBiodyne, Estonia), and thereafter proceeded with PCR. qPCR was performed on 10ng/µl of cDNA using the custom Tagman gene expression probes of the genes of interest (*SPATA6*, *SPATA20*, and *CABS1*). Quantitative RT-PCR was

performed in duplicate in a QuantStudio™ 5 System (Applied Biosystems™ A28139, USA). Data were analyzed using the QuantStudioTM Design & Analysis Software v1.4.1, and the comparative Ct method 2^-(ΔΔCt) was used to quantify gene expression levels. Data of qPCR products were standardized to GAPDH, which was used as internal control.

Statistical analysis

Data were analyzed using GraphPad Prism 9.03 (GraphPad™ Software, Version 9.03, San Diego, CA, USA). When analyzing one independent variable, a one-way ANOVA with Tukey's post-hoc test was used, while a two-way ANOVA with Tukey's post-hoc comparison test was employed when two variables were measured. A probability level of $p < 0.05$ was considered statistically significant and results are expressed as mean ± SD.

Results

At 3Hr and 6Hr, there was a significant reduction in TM in the 2-D and s-MG groups compared to the control ($p<0.05$) (Figure 1A). Similarly, there was a significant decrease in TM in the s-MG group over time ($p<0.05$) (Figure 1A), while there was no significant difference in PM (Figure 1B). Although no statistically significant differences were observed in PM, the s-MG group showed nearly no PM at 6Hrs time point. Viability was significantly reduced after exposure to s-MG for 1Hr ($p=0.02$), 3Hr ($p=0.02$) and 6Hr ($p=0.01$) (Figure 2). Moreover, there was a significant increase in the percentage of acrosome reacted spermatozoa in the s-MG group at 3 and 6Hr, whereas at 6Hr, an increase was observed in the 2-D group compared to control ($p<0.05$) (Figure 3). While there was no statistically significant difference observed in PM and mRNA expression of genes of interest (Figure 4), the analysis reveals a discernible trend indicating the potential effects of s-MG.

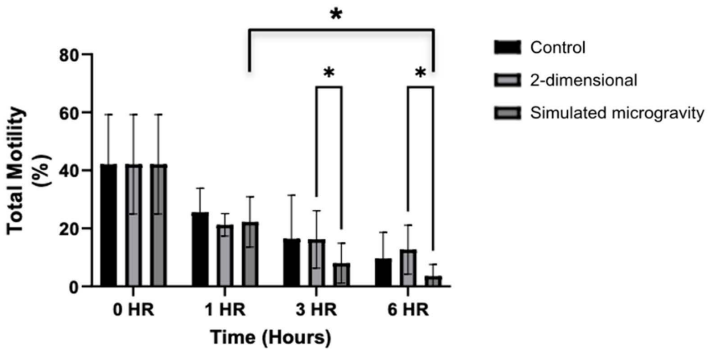

FIGURE 1A
Effect of simulated microgravity (s-MG) on sperm total motility (TM). *p<0.05, n=4.

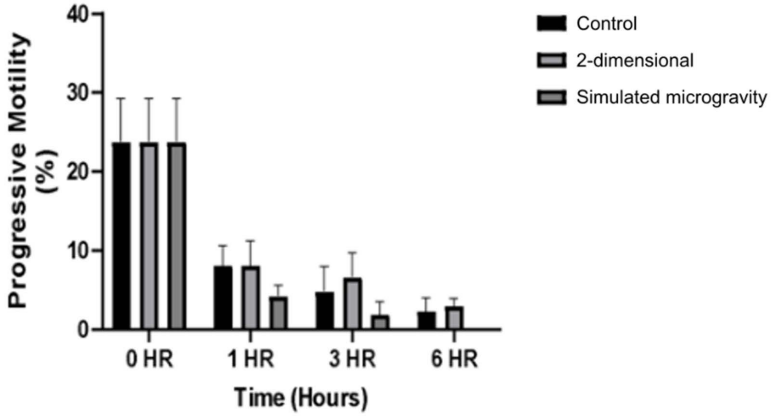

FIGURE 1B
Effect of s-MG on sperm progressive motility (PM). No significance. N=4.

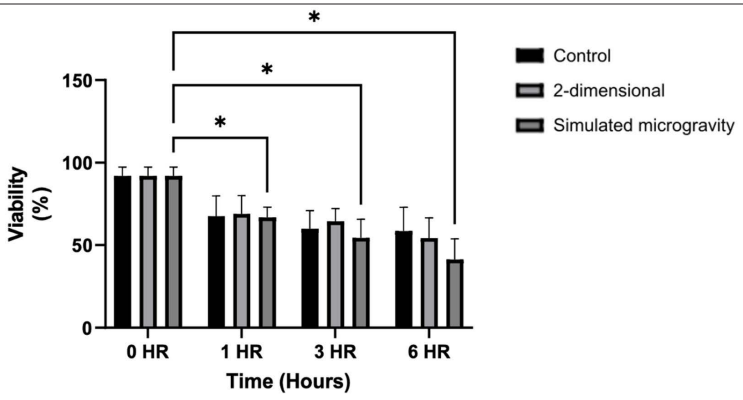

FIGURE 2
Effect of s-MG on viability. * p<0.05. N=4.

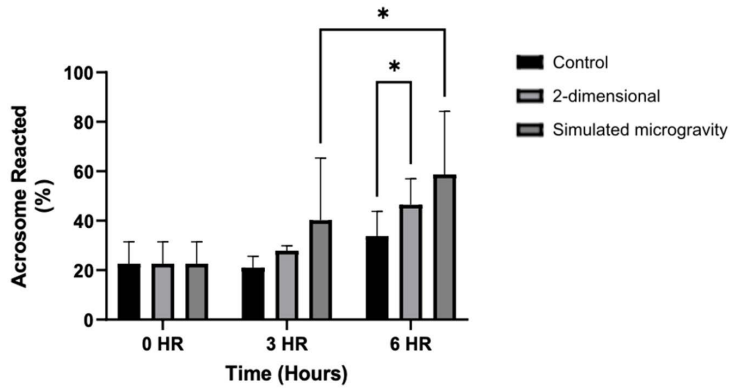

FIGURE 3
Effect of s-MG on acrosome reaction (AR). *p<0.05. N=2.

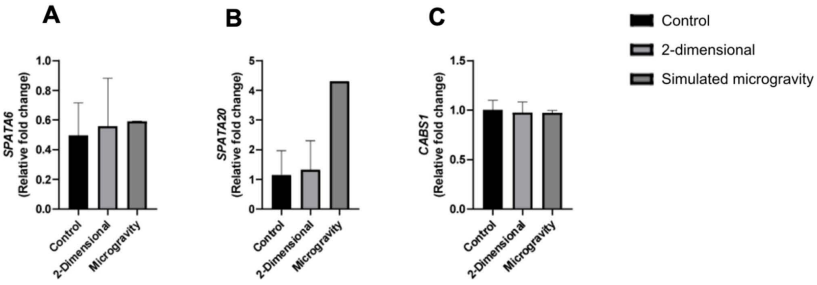

FIGURE 4
Effect of s-MG on the mRNA expression of SPATA6 and SPATA20 and CABS1. N =3.

Discussion

As space exploration and colonization gains momentum, understanding the effects of microgravity on male reproductive function is essential for future endeavors. Thus, this study was conceived as an initial step in experimenting on freshly collected human semen samples.

Sperm motility in the s-MG condition had shown significant decrease, which may primarily be a result of disruptions on various intracellular metabolic pathways, two of which are the calcium (Ca^{2+}) pathway and the influence of reactive oxygen species (ROS) (Pereira et al., 2017). Heightened levels of Ca^{2+} concurs in motility suppression due to disrupted protein phosphorylation and excessive ROS alters metabolic processes further impeding their motility. A scientific study on T-CAM2 cells, a model for mitotically active male germ cells, exposed to s-MG for 24-hours demonstrated an increase in levels of Ca^{2+} and ROS induced by oxidative stress (Morabito et al., 2017).

Of the s-MG group, the percentage of viable spermatozoa exhibited a statistically significant decrease. As previously hypothesized, the elevated levels of ROS may lead to lipid peroxidation, in result affecting the sperm's ability in regulating intracellular mechanisms responsible for motility and viability (Chakraborty and Saha, 2022). This further correlates with the

observed negative trend in terms of the TM and PM, as viability serves as an indicator of structurally intact and adequately functional sperm.

No statistical significance was found in the mRNA expression of motility-associated genes (*SPATA6* and *SPATA20*); however, a 73.2% increase in *SPATA20* expression was observed in the s-MG group, indicating a compensatory response to preserve their structural integrity in an altered environment. *SPATA6* is a gene localized to the connecting piece of spermatozoa. Its inactivation leads to acephalic spermatozoa syndrome (ASS) in mice models, attributing to *SPATA6*'s interaction with myosin subunits critical for the sperm's cytoskeleton assembly (Yuan et al., 2015). Additionally, studies on ASS in human revealed a loss of function in *SPATA20* causing the separation of the sperm head and flagellum, with a combined effect of decreased *SPATA6* (Wang et al., 2023).

The significant increase in acrosome reacted spermatozoa after exposure to s-MG was likely due to elevated levels of Ca^{2+} triggering premature AR (Aldana et al., 2021). Subsequently, no significance is observed with the *CABS1 gene*, which encodes the *CABS1* protein, localized in both the principal piece of the flagellum and acrosome of epididymal sperm of male mice (Shawki et al., 2016; Zhang et al., 2021). The study utilized anti-p*CABS1* antiserum to porcine sperm which significantly reduced the AR, highlighting p*CABS1*'s role in modulating Ca^{2+} signaling (Matsumura et al., 2019). The expected decrease in *CABS1* levels alongside the increase in the proportion of AR cells under MG, as suggested by Shawki's study, was not observed. This discrepancy raises the possibility that a prolonged duration may be required for *CABS1* action.

Conclusion

S-MG negatively affected TM, viability and AR, while PM and function-specific genes were not significantly impacted. These findings shed light on the potential detrimental effects of simulated microgravity on male reproductive function, providing valuable insights for experts in the field of space exploration. Thereby suggesting the importance of further investigation,

specifically in light of the impact that it could have in the advent of long duration space exploration.

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Crew-interactive AI-powered health applications via the ICE Cubes Media Set

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Introduction

The ICE Cubes Service Media Set is a pioneering platform that enhances real-time interaction and communication between Earth and the ISS. Central to its design is a GoPro camera connected to the ICE Cubes Facility within ESA's Columbus module. This setup allows for a broad range of activities, such as public engagement, education, media interactions, and private communications, including family conversations and medical consultations. These multifaceted capabilities support both health-related research and the development of practical applications. [1]

Achievements and utilisation

Since its inception, the Media Set has been employed in over 80 events on the ISS, particularly during commercial Axiom missions. Its versatility makes it an essential tool for various uses, from public outreach and educational sessions to media engagements and medical communications. [2]

Development of Media Set Mk2

Currently, the ICE Cubes Service is developing an upgraded version of the Media Set, known as Media Set Mk2. This next-generation system is designed to capture video and audio data across all modules of the USOS segment of the ISS in both full HD and 4K resolution. The Media Set Mk2 will enable real-time processing for full HD video and in-situ post-processing for 4K video. Additionally, it features a touch screen, allowing ISS crew members to receive real-time video feedback from ground control, thus enhancing their ability to visualize and interact with the data.

Advanced capabilities and applications

The Media Set Mk2 is engineered to integrate a variety of information sources and facilitate comprehensive data analysis using domain-specific algorithms. This is particularly beneficial in healthcare applications, where this system can be used to recognize, interpret and understand health-related challenges and diagnose medical conditions through multi-modal data processing. By harnessing the power of advanced computer vision and AI capabilities, the Media Set Mk2 can analyze facial cues to assess emotional states, stress levels, and potential neurological conditions. Additionally, it incorporates sophisticated audio processing techniques to detect anomalies in speech patterns, which may indicate respiratory or cardiovascular issues. The system's capability extends to analyzing body movement, gait, pace, and patterns, aiding in the identification of musculoskeletal disorders and the early detection of neurological conditions.

Comprehensive health assessment

The integration of these diverse modalities allows the Media Set Mk2 to offer a comprehensive and non-invasive health assessment. These capabilities extend beyond traditional diagnostic methods, aiming at providing a more nuanced and personalised health monitoring. The Media Set Mk2 supports both physiological and psychological research, enhancing decision-making

processes for healthcare professionals and providing actionable insights. Its advanced diagnostic capabilities can assist a significant advancement in health monitoring aboard the ISS, offering substantial value for human health assessment and research in space. [3] [4] [5]

Future implications

Looking ahead, the Media Set Mk2 holds substantial promise for future commercial space stations and planetary outposts, particularly as we anticipate long-term human exploration missions into deep space. Its innovative approach to health management and early disease detection stands to significantly improve patient care and enhance doctor-patient communication. The system's ability to provide detailed and accurate health assessments will be crucial in ensuring the well-being of astronauts during extended missions, contributing to the success of future space exploration endeavors. [6] [7] [8]

Conclusion

As technological advancements continue to progress, the ICE Cubes Media Set Mk2 is poised to facilitate proactive health management and early disease detection. This advanced system not only supports current missions aboard the ISS, but also sets the stage for future exploration efforts. By integrating state-of-the-art diagnostic tools and AI capabilities, the Media Set Mk2 can be of help in better health outcomes for astronauts, expanding our understanding of human health in space. Its comprehensive and innovative approach to health monitoring can make it an auxiliary asset for ongoing and future space missions, contributing significantly to the advancement of space healthcare technology.

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Self-organized criticality of heart rate variability during simulated weightlessness: Insights from lower body negative pressure

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Citation

Thomas, W., Navasiolava, N., Custaud, M.A., Fortrat, J.O. Self-organized criticality of heart rate variability during simulated weightlessness: Insights from lower body negative pressure.

Introduction

Self-organized criticality (SOC) is a theoretical framework that describes the behavior of complex systems near a critical point, where small events can trigger significant responses (Muñoz, 2018). This concept, observed in various natural and complex systems, is particularly relevant in the study of the cardiovascular system, especially regarding vasovagal syncope—a phenomenon often triggered by an upright posture, leading to a sudden loss of consciousness due to cerebral hypoperfusion (Fortrat & Gharib, 2016; Jardine et al., 2017; Fortrat & Ravé, 2023). Cardiovascular SOC

appears to play a crucial role in maintaining system stability in the context of environmental stressors such as standing (Fortrat & Ravé, 2023). The relevance of SOC in cardiovascular dynamics extends notably to spaceflight simulation, where it sheds light on cardiovascular deconditioning and the associated heightened risk of orthostatic syncope (Navasiolava et al., 2023). A comprehensive understanding of cardiovascular SOC is imperative for effectively characterizing cardiovascular deconditioning and exploring its potential utility in predicting orthostatic syncope. However, studying cardiovascular dynamics in the upright position poses a paradox in space physiology, given that weightlessness eliminates this orientation entirely. The lower body negative pressure test (LBNP) was developed to simulate the fluid shifts caused by gravity, reproducing the orthostatic stress encountered when standing on Earth (Patel et al., 2001; Esch et al., 2007). This test has since become a standard procedure for assessing orthostatic tolerance following spaceflight simulation conducted by the European Space Agency (ESA, Coupé et al. 2010; Robin et al., 2020). In this study, we aim to elucidate the dynamics of cardiovascular SOC during LBNP in a simulated weightlessness scenario.

Material and Methods

Simulated weightlessness protocol

Nine healthy men aged 25 to 43 years participated in a 5-day dry immersion (DI-5 CUFFS) simulation designed to mimic the effects of microgravity. Dry immersion involves placing the subject in a body-temperature water bath, separated by a waterproof fabric, creating a weightless-like environment by redistributing body fluids (Navasiolava et al., 2011).

Lower body negative pressure

The Lower Body Negative Pressure (LBNP) test followed the standard European Space Agency (ESA) protocol: 5 minutes of rest, followed by incremental -10 mmHg steps applied every 3 minutes until reaching -60 mmHg or until symptoms of orthostatic intolerance occurred. The LBNP

tests were conducted twice during an 11-day experimental plan: the first test was performed three days before the start of the dry immersion phase (B-3), and the second test immediately after the dry immersion phase (R0). Cardiovascular data, including electrocardiogram (ECG) and blood pressure, were continuously monitored for medical supervision.

Data analysis

RR intervals were measured beat by beat from the ECG recordings of the medical supervision. A recording of 180 heartbeats was selected for each LBNP step up to -40 mmHg.

RR-interval time series were manually filtered to eliminate artifacts. Bradycardia episodes define as consecutive RR-interval increase were then automatically identified, categorized, and counted based on their length. Zipf's plots were created using these data to analyze the distribution of episode lengths (Fortrat & Ravé, 2023). A criticality index for each LBNP session was calculated from these Zipf's plots by summing the maximal lengths of these episodes across all the assessed LBNP steps.

Statistics

Data are presented as mean $\pm$ SEM. Pre- and post-simulation data were compared using a paired t-test, with normality checked using the Kolmogorov-Smirnov test.

Results

The mean age of subjects was 33.9 $\pm$ 7.1 y, their height was 1.76 $\pm$ 0.06 m, and their weight 73.9 $\pm$ 7.5 kg. Details of the experiment and results have been previously reported (Robin et al., 2020). These previously reported results demonstrated the cardiovascular deconditioning through the resting tachycardia following the experiments and the drop of LBNP tolerance time

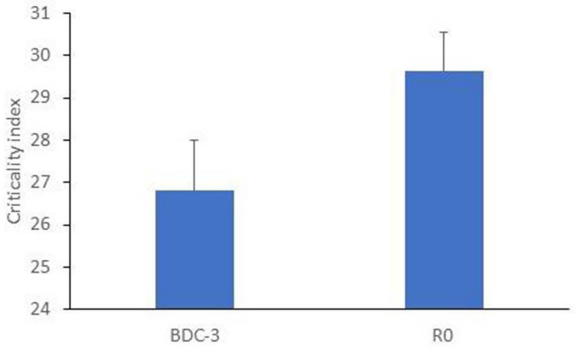

FIGURE 1
Cardiovascular criticality index during lower body negative pressure performed before and after a spaceflight simulation by dry immersion (BDC-3 and R0) respectively).

(Robin et al., 2020). The criticality index during LBNP was not significantly altered during the dry immersion simulation (Figure 1).

Discussion

The main finding of this study is that the ESA standard LBNP procedure failed to demonstrate an alteration of cardiovascular self-organized criticality during a spaceflight simulation by dry immersion.

Dry immersion serves as an effective simulation model for studying weightlessness, as it closely replicates the physiological effects of microgravity on the human body (Navasiolava et al., 2011). This method provides valuable insights into the body's adaptations to space-like conditions without requiring space travel. It is utilized mainly in research centers in Moscow and Toulouse.

Dry immersion is known to effectively induce cardiovascular deconditioning, similar to the effects observed in actual weightlessness. The lack of gravitational load during immersion causes shifts in fluid (Navasiolava

et al., 2011). The DI-5 CUFFS experiment reported here induced a cardiovascular deconditioning in the control group studied here as demonstrated by the subjects' resting tachycardia and by the decreased tolerance time during LBNP as previously reported (Robin et al., 2020).

We previously demonstrated parallel changes in cardiovascular self-organized criticality associated with cardiovascular deconditioning (Navasiolava et al., 2023). A deeper understanding of cardiovascular self-organized criticality is essential for advancing our knowledge of cardiovascular deconditioning and orthostatic intolerance after spaceflights. These adverse effects are currently assessed using the standard LBNP procedure defined by the European Space Agency. However, our study shows that this procedure does not allow for simultaneous assessment of cardiovascular self-organized criticality. To address this, specialized protocols incorporating longer RR-interval recordings are necessary, as the short duration of LBNP steps in the ESA procedure likely affects the quality of SOC evaluation (Fortrat, 2024).

To address these issues, future research should explore extending the duration of LBNP tests or adopting alternative protocols. Such modifications could provide more accurate insights into cardiovascular SOC and its implications for cardiovascular deconditioning under simulated conditions.

Conclusion

The European Space Agency's standard LBNP procedure is a valuable tool for demonstrating cardiovascular deconditioning following spaceflight simulations. However, the data collected during this protocol are insufficient for studying cardiovascular self-organized criticality, due to the short duration of the LBNP steps. Specialized procedures with longer recording times should be used for this purpose.

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Direct comparison of head-down bed rest and dry immersion effects on human cardiac baroreflex during orthostatic stress

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Zhedyaev, R., Tarasova, O., Borovik, A., Vinogradova, O. Direct comparison of head-down bed rest and dry immersion effects on human cardiac baroreflex during orthostatic stress.

Introduction

Head-down bed rest (HDBR) and dry immersion (DI) are the two most widely used on-ground models of space flight multisystem deconditioning. While both lead to centralization of blood flow, they differ in mechanisms inducing such a fluid shift (Navasiolava et al., 2011, Watenpaugh 2016). That distinction may lead to different cardiovascular outcomes, including different baroreflex response to orthostasis. However, no comparison was performed previously between two models on their long-term effects (>3 days) on cardiac baroreflex functioning.

The analysis of low frequency (LF) waves of systolic arterial pressure (SAP) and RR-interval (RRi) is widely used to assess cardiac baroreflex function. The amplitude relation and phase coupling of these waves may be assessed by α-coefficient ("spontaneous baroreflex sensitivity") (Pagani et al., 1988) and by phase synchronization index (PSI) (Negulyaev et al., 2019) accordingly. We applied such an analysis to data collected in supine and head-up positions to assess differences in HDBR and DI influence on baroreflex cardiac control.

The aim of the study was to compare the effects of 19-day HDBR and DI on amplitude and phase coupling of SAP and RRi LF waves during head-up tilt test.

Material and Methods

Two groups of healthy men were exposed to 21-day HDBR ("HDBR" group, n=9, age 31$\pm$5 yrs., BMI 24.2$\pm$2.8 kg/m2) or 21-day DI ("DI" group, n=8, age 29$\pm$4 yrs., BMI 22.3$\pm$2.9 kg/m2). Head-up tilt test (HUT, 65°, 15 min) was performed before, on 6–7 day, 14 day and 19 day of HDBR and DI. ECG (NVX52, MCS, Russia) and blood pressure (Finometer, Finapres Medical Systems, the Netherlands) were continuously recorded; RRi and SAP were calculated for every cardiac cycle. Mean power of RRi (S_{RRi-LF}) and SAP (S_{SAP-LF}) waves in LF (0.05-0.13 Hz) band were calculated using spectra obtained with fast Fourier transform. LF α-coefficient was calculated as square root of S_{RRi-LF}/S_{SAP-LF} ratio and reported as cardiac baroreflex sensitivity (cBRS) (Pagani et al., 1988); mean PSI for SAP and RRi in LF band (PSI_{LF}) was calculated as previously described (Negulyaev et al., 2019).

Percent differences between parameters in 15-min supine position and 15-min HUT position are reported as reaction to test. As two men in HDBR and one in DI skipped tests on day 19 due to medical considerations, instead of 2-way ANOVA we used Mixed-effects model with the Geisser-Greenhouse correction and Sidak's multiple comparisons to examine influence of time ("Time" factor) and different models ("Model" factor) during two gravitational unloading (GU) models on baroreflex reactions to HUT.

Results

Two groups didn't differ in any parameter collected during tests before HDBR and DI (Fig. 1). While S_{SAP-LF} didn't change in horizontal position during modelled GU (Fig. 1 A), S_{RRi-LF} tended to decrease ($p<0.05$ – "Time" factor) with no observable difference between models (Fig. 1 B); thus, cBRS in horizontal position also tended to decrease with GU exposure time ($p<0.05$ – "Time" factor, Fig. 1 C). PSI_{LF} in horizontal position didn't change with exposure time and didn't differ between models (Fig. 1 D).

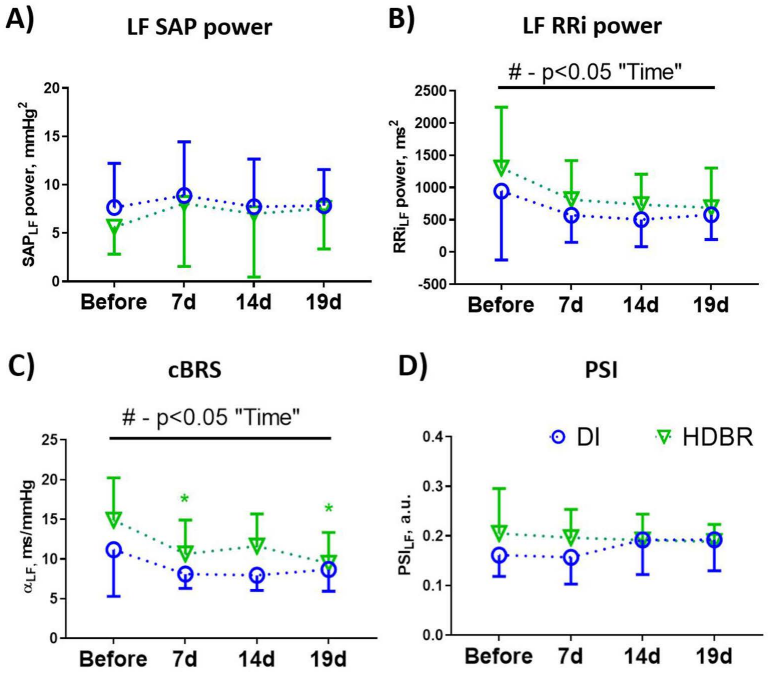

FIGURE 1

S_{SAP-LF} (A), S_{RRi-LF} (B), cBRS (C) and PSI_{LF} (D) in supine position obtained at different stages of DI (green) and HDBR (blue). * - $p<0.05$ – compared to "Before"; # - $p<0.05$ – for corresponding factor (mixed-effects analysis with Sidak's multiple comparisons test).

Responses to HUT are shown in figure 2. S_{SAP-LF} response increased with the exposure time without observable difference between models (Fig. 2 A), while S_{RRi-LF} response decreased (Fig. 2 B). Notably, in DI the decrease of RRi LF power in HUT was quite dramatic (the decrease close to -100%) and post-hoc tests revealed significantly higher decrease of S_{SAP-LF} in DI compared to HDBR on day 14. Thus, cBRS reaction to HUT aggravated with GU exposition

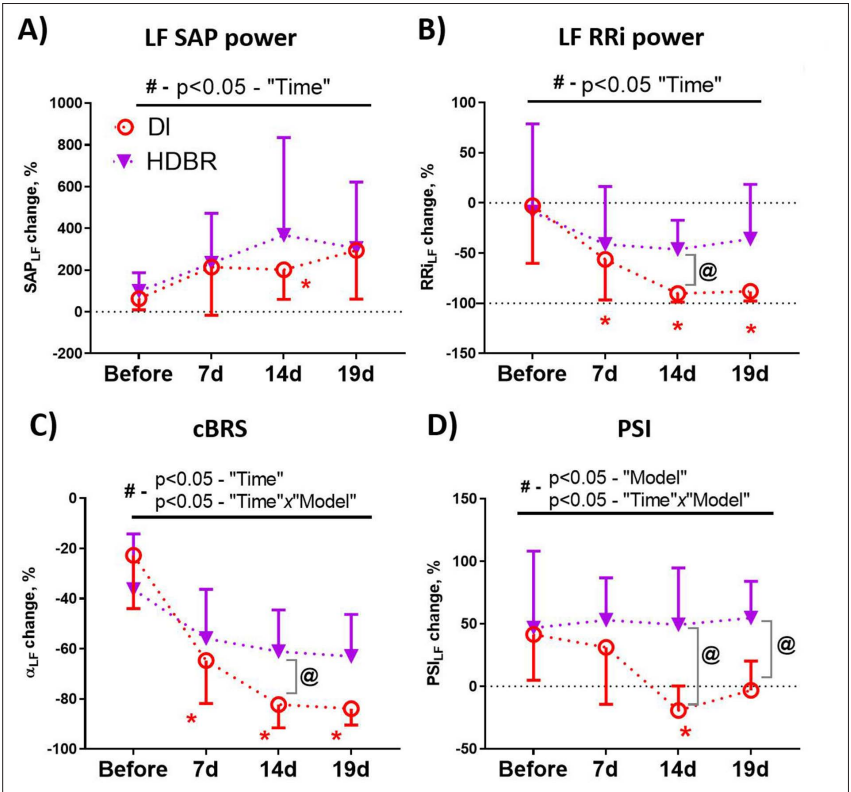

FIGURE 2

Response of S_{SAP-LF} (A), S_{RRi-LF} (B), cBRS (C) and PSI_{LF} (D) to HUT obtained at different stages of DI (red) and HDBR (purple). * - p<0.05 compared to "Before", @ - p<0.05 compared to other model, # - p<0.05 for corresponding factor (mixed-effects analysis with Sidak's multiple comparisons test).

time and showed greater decrease in DI compared to HDBR ($p<0.05$ – "Time"x"Model" interaction) (Fig. 2 C). PSI_{LF} reaction to HUT didn't change in HDBR while diminished completely in DI from day 14 (Fig. 2 D).

Discussion

Most spaceflight studies have shown a decrease in the sensitivity of the cardiac baroreflex, which correlates with the data obtained in model experiments in our work. According to several studies on astronauts (Gisolf et al., 2005; Beckers et al., 2009) cBRS in head-up position before spaceflight was 8-10 ms/mmHg, and after a 10-11-day flight it decreased to 5-6 ms/mmHg. In our study, cBRS before unloading in HUT was similar to those described in the literature. On the 7th and 14th days of HDBR cBRS in HUT decreased to an average of 4.4 ms/mmHg while in DI it changed more pronouncedly: on day 7, it decreased to 2.8 ms/mmHg, and on day 14 - to 1.4 ms/mmHg. Interestingly, in a study (Beckers et al., 2009) was shown that the coherence of LF oscillations of the AP and heart rhythm during orthostasis was maintained at the preflight level. At the same time, in our study, DI changed the response of PSILF (an analogue of coherence) to HUT. Thus, HDBR probably more accurately reflects changes in baroreflex regulation of heart rate, whereas DI leads to more profound changes over a similar period of time than space flight. However, that conclusion is preliminary, since, firstly, statistical verification is required to confirm it, and, secondly, tests in spaceflight studies were not carried out immediately after the flight, but only 1-3 days after landing, which can greatly affect the measured parameters.

One of the factors which may cause a deeper change in cardiac baroreflex function in DI compared to HDBR may be a difference in degree of plasma volume reduction/haemoconcentration, and, as a result, different effects on baroreception and volume reception. The two models differ in the mechanism of "centralization" of blood flow, which further leads to a decrease in plasma volume: DI causes compression of peripheral tissues, while HDBR creates a hydrostatic gradient from legs to head (see reviews by Navasiolava et al., 2011; Hargens, Vico, 2016; Tomilovskaya et al., 2019). A

comparison of 3-day DI and HDBR (Navasiolava et al., 2011), as well as 3-day DI and 21-day HDBR (Amirova et al., 2020) indicates that blood plasma loss in DI is more significant. These changes are likely to lead to greater activation of the sympathetic system and a decrease in vagal activity (through which cardiac baroreflex is predominantly carried out) in DI compared to HDBR.

Conclusion

According to our data, DI impairs cardiac baroreflex control more prominently than HDBR, leading to such dramatic changes in cardiac function during HUT as seen during parasympathetic blockade (Clemson, 2022). Notably, gravitational unloading exerts diverse effects on amplitude relation of LF RRi and SAP waves (cBRS) and their phase relation (PSI_{LF}), highlighting the fact that these characteristics reflect different aspects of cardiac baroreflex functioning.

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