Version of the program: May 24th 2024

General Overview of the Program

ISGP 2024 in Dubai

Venue: Mohammed Bin Rashid University of Medicine and Health Sciences

Time	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)	Plenary 2 Session dedicated to MBRSC (9:00 - 11:00)	Plenary 3 Rodents in Altered Gravity: Advances in Space Biology Research (9:00 - 11:00)	Coffee break available	Friday tour
09:30		Plenary 1 - CURRENT CONCEPTS Alamelu Sundaresan "The Lunar Challenge- Lunar dust and the human being in moon missions" Asa Berggeen "Insects as food for space travel and planetary colonisations"				
10:00					Plenary 4 LBNP as countermeasure (10:00 - 12:00)	
10:30						
11:00		Coffee break	Coffee break	Coffee break		
11:30		Young Investigators Session (1) 11:30 - 12:30	Young Investigators Session (2) 11:30 - 12:30	Young Investigators Session (3) 11:30 - 12:30		
12:00						
12:30		Lunch Break	Lunch Break	Lunch Break	Lunch Break	
13:00		Lunch & Poster (13:00 - 14:00)	Lunch & Poster (13:00 - 14:00)			
13:30				Institutional session (1) 14:00 - 15:00	Institutional session (2) 13:30 - 15:00	
14:00		Moon Exploration (14h:00-15h30) | Studies with gender differences (14h:00-15h30)	Muscle and movments (14h:00-15h30) | Medical issues for exploration (14h:00-15h30)			
14:30				VIVALDI dry immersion study (15h:00-16h30) | Animal Models (15h:00-16h30)		
15:00	Welcome of participants for registration				Closing Ceremony (15:00 - 15:30)	
15:30						
16:00		Coffee break	Coffee break		ISGP General Assembly (16:00 - 17:30)	
16:30		Space Exploration and extreme environements (16h:30-18h00) | Cells and plants (16h:30-18h00)	SIRIUS Isolation study (16h:30-18h00) | Cardiovascular system (16h:30-18h00)	Coffee break		
17:00				Immunology and inflammation (17h:00-18h00) | Hypergravity (17h:00-18h00)		
17:30						
18:00						
18:30		Welcome party (18:30 - 21:30)	Young Investigator event : Ghawa & Career (18:30 - 20:30)			
19:00				Gala Dinner & Young Investigators Award Ceremony (19:30 - 22:00)		
19:30						

مـــــركز محمد بن راشد للــــــــفضـــــــاء
MOHAMMED BIN RASHID SPACE CENTRE

Detailed Scientific Program

ISGP 2024 in Dubai

INTERNATIONAL SOCIETY FOR GRAVITATIONAL PHYSIOLOGY ISGP 1991

Monday, May 27th

Opening Ceremony

Plenary 1 - Current Concepts (9:30 – 11:00)

Chaired by Alexander Choukér & Marc-Antoine Custaud

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 (11:30 – 12:30)

Chaired by Anna-Maria Liphardt & Sami Al Ghayath

Ahmed Bakri
The impact of Microgravity on Experimental Periodontitis: An In Vivo Study (p.1)

Damien Lanéelle
Orthostatic tolerance according to cerebral arterial pattern variations during hemodynamic stress combining lower body negative pressure and head-up-Tilt (p.4)

Victorien Faivre-Rampant
Does gravity affect intrinsic cardiac function? Effects of different gravitational loads on the cardiac performance independent of the preload (p.6)

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 (p.8)

Parallel Session (a) "Moon Exploration" (14:00 – 15h30)

Chaired by Alamelu Sundaresan & Jay Bookbinder *(tbc)*

Salma Subhi
Chondrites: Understanding the Origins of the Solar System (p.10)

Chiara Pucciariello
The REGOLIFE project: Bio-Engineering Lunar Regolith for Moon Crop Cultivation (p.12)

Jay Bookbinder
SpinSat: a Novel Mission Architecture for Deep Space Radiation and Gravitational Studies (p.14)

Shannon Marchal
Research into "Lunar Hay Fever" on Earth – Finding Answers in an in Vitro Airway Model? (p.15)

Fawzan Mohamed Kareem Navaz
Utilizing bio-inspired hierarchical multi-shell structures (BHMSS) for radiation shielding in space exploration (p.17)

Parallel Session (a) "Studies with gender differences" (14:00 – 15h30)

Chaired by Vishwajeet Shankhwar & Mennatullah Khalil

Kunihiko Tanaka
Galvanic Vestibular Stimulation Decreases Parathyroid Hormone in Menopausal Women (p.18)
Ivan Vasilev
Parameters Of Venous Hemodynamics In Female Volunteers During Their Stay In A 5-day "Dry" Immersion (p.19)
Galina Vassilieva
Five-day "Dry" Immersion With Female Subjects ("Immersion-5F-LF"): Main Objectives And Results (p.21)

Parallel Session (b) "Space exploration and extreme environment" (16:30 – 18h00)

Chaired by Alain Maillet & Noora Al Mheiri

Yasmin Halawani
AstroBEAT: Cardiovascular Variability Analysis and Lunar Microgravity Twin (p.23)
Monica Monici
Mechanisms of Adaptation to Extreme Environments The Exposome Signature Project (p.25)
Elena Fomina
Methods for the prevention of monotony in interplanetary spaceflight (p.27)
Leonardo Surdo
Crew-interactive AI-powered Health Applications via the ICE Cubes Media Set (p.29)
Judith-Irina Buchheim
Support of a Crew Activity with the Crew Interactive Mobile Companion (CIMON) (p.31)
Sandeep Sureh Babu
Potential of Bioprinting in Space Missions: Challenges on the way forward (p.33)

Parallel Session (b) "Cells and plants" (16:30 – 18h00)

Chaired by Mohamed Jamal & Jack van Loon

Mahamed Ashiq
Hypergravity Confers Abiotic Stress Tolerance In Bread Wheat (Triticum aestivum L.) (p.36)
Irina Ogneva
The Drosophila Melanogaster Oocytes Demonstrate The Mechanoreception Under Short-Term Modelling Micro- and Hypergravity (p.38)
Devjoy Dev
The effect of short-term exposure to simulated microgravity on circadian clock gene expression in mouse embryonic fibroblasts (p.40)
Mohamed Jamal
Oral tissues and neural crest derived stem cells as a model to study oral health in microgravity environment (p.41)
Osman Patel
Impact of microgravity exposure on genes regulating cell turnover in rat mammary gland (p.42)
Mauro Maccarrone
Simulated Microgravity Affects Specialized Pro-Resolving Mediators and Human Inflammatory Homeostasis in a Cell-Specific Manner (p.43)

Tuesday, May 28th

Plenary 2 - Session dedicated to MBRSC (9:00 – 11:00)

Chaired by Angelique Van Ombergen & Noora Al Mheiri

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 (11:30 – 12:30)

Chaired by Angelique van Ohmbergen & Youssef Elsabban

Zeinab Ibrahim
Exploring Novel Therapeutics Targets Against Cardiovascular and Skeletal Muscle Deconditioning in Hindlimb Unloading Model (p.45)

E. Yu. Gorbacheva
The Ovarian-Pituitary Axis Of Mice After Antiorthostatic Suspension During The Full Estrous Cycle (p.47)

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 (p.49)

T.J. Pereira
Does an N95 mask improve Orthostatic Tolerance? (p.51)

Parallel Session (a) "Muscle and movements" (14:00 – 15h30)

Chaired by Anna-Maria Liphardt & Elena Tomilovskaya

Elena Tomilovskaya
Perspectives of electromyostimulation approaches for muscle strength and endurance maintenance under motor unloading conditions: from Space to Earth (p.53)

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 (p.54)

Karolina Almeida Borges
Space Tourism- MyotonPRO experiment on Muscle Tone (p.56)

Tatiana Shigueva
Effects of Electromyostimulation on Characteristics of Reflex Excitability of Calf Extensor Muscles Under Dry Immersion Conditions (p.59)

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") (p.61)

Anna Ganicheva
The Role Of Spaceflight Experience And Mission Duration In The Success Of Completing Model Tasks On The Planet Surface (p.63)

Parallel Session (b) "Medical issues for exploration" (14:00 – 15h30)

Chaired by Ilya Rukavishnikov & Monica Monici

Parallel Session (a) "SIRIUS & Isolation studies" (16:30 – 18h00)

Chaired by Elena Fomina & Shaikha Al Falasi

Parallel Session (b) "Cardiovascular system" (16:30 – 18h00)

Chaired by Victoria Ly & Jacques-Olivier Fortrat

Wednesday, May 29th

Plenary 3 - Rodents in altered gravity: Advances in Space Biology Research (9:00 – 11:00)

Chaired by Sara Tavella & Jack van Loon

Alexander Andreev-Andrievskiy
Sex differences in vasopressin regulation of water-salt metabolism in hindlimb unloaded mice (p.173)

Sara Tavella
Adaptation to 3g Hypergravity: A Multidisciplinary Tissue Sharing Program from a 27-Day Mouse Experiment (p.175)

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

Young Investigator session – 3 (11:30 – 12:30)

Chaired by Marc-Antoine Custaud & Alya Alowais

Victoria Ly
Self-Generated Lower Body Negative Pressure Exercise, a Low Power Countermeasure for Deep-Space Missions (p.90)

Zhiyao Ma
Exploring the Impact of Simulated Microgravity on Osteoarthritis Development: The Role of CD36 and Sex-Specific Responses in a Mouse Model (p.92)

Constance Badalì
SpaceBike – Preliminary Insights into Neuromuscular Adaptation through Bed Rest (p.94)

R. Yu. Zhedyaev
Direct Comparison of Head-Down Bed Rest and Dry Immersion Effects on Human Cardiac Baroreflex During Orthostatic Stress (p.96)

Plenary session "Institutional session - 1" (14:00 – 15h00)

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" (15:00 – 16h30)

Chaired by Rebecca Billette de Villemeur & Nastassia Navasiolava

Rebecca Billette de Villemeur
Of The Dry Immersion Model For ESA: Description Of The VIVALDI I And II Studies (p.98)

RK. Vergos
VIVALDI I And II: General Tolerance To 5 Days Of Dry Immersion In 38 Healthy Men And Women (p.100)

Nastassia Navasiolava
Dry immersion effects on circadian rhythms and day-night variability of core temperature, heart rate, and blood pressure (p.102)

Peter Fernandez
Exploring Bone Adaptation and Energy Metabolism Between Males and Females Under Dry Immersion Conditions (p.103)

Adrien Robin
Venous functions and leg volume changes during the two ESA Vivaldi dry-immersion studies in men and women (p.105)

Marc Kermorgant
Gender Related Differences On Dry Immersion-Induced Ophthalmological Changes (p.107)

Parallel Session (b) "Immunology and inflammation" (17:00 – 18h00)

Chaired by & Jean-Pol Fripiat & Alexander Choukér

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 (p.109)

Mei ElGindi

Effects of Simulated Microgravity on Immune System Potency in 3D Microenvironment (p.111)

Panel discussion on that topic

Parallel Session (a) "Animal models" (15:00 – 16h30)

Chaired by Alexander Andriev-Andrievsky & Angela Maria Rizzo

Theo Fovet

The NEBULA Project: Effect Of Pre-Flight Physical Training On Bone And Muscle In A Mouse Microgravity Analog Model (p.112)

Jack J.W.A. van Loon

Fetal mouse long bones under continuous microgravity or in-flight periods of 1×g centrifugation as countermeasure (p.114)

Timur Mirzoev

Spinal mechanisms triggering the spontaneous tonic activity of the postural soleus muscle under hindlimb unloading (p.116)

Ameneh Ghadiri

Femurs of Mice Exposed to Hypergravity Show Deregulation of Genes Mainly Associated with ECM-receptor Interactions and Protein Digestion and Absorption (p.117)

Angela Maria Rizzo

Hypergravity Exposure Induces Alterations Of Erythrocyte Membrane And Antioxidant Potential Of Mice Housed In The MDS Facility (p.119)

Parallel Session (b) "Hypergravity" (17:00 – 18h00)

Chaired by Ines Ebner & Maryam Almarzooqi

Rebecca Billette de Villemeur

A 60-Day Bed Rest With Artificial Gravity And Cycling Exercise: The BRACE Study – Description Of The Study Method (p.120)

Jan Millek

Does Artificial Gravity Tolerance Change Across seasons? (p.122)

Maryam Almarzooqi

Comprehensive exploration of artificial gravity solutions for optimizing long-term space exploration missions (p.123)

Alina Saveko

Effect of different short-radius centrifugation interval training modes on vertical stability (p.126)

Thursday, May 29th

Plenary 4- LBNP as countermeasure (10:00 – 12:00)

Chaired by Nandu Goswami & Andrew Blaber

Nandu Goswami
Physiological effects of LBNP *(p.177)*

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 *(p.178)*

Vishwajeet Shankhwar
Does Gender Influence Cardiovascular and Autonomic Responses to Central Hypovolemia? *(p.179)*

Plenary session "Institutional session - 2" (13:30 – 15h00)

Chaired by Ines Antunes & Cyndi Roman

Pierre Denise
SPACEMED Erasmus Mundus Joint MSc: The first European Master's program in Physiology and Medicine of Humans in Space and Extreme Environments *(p.128)*

Angelique Van Ombergen
ESA's Human Exploration Enabling Science Activities: recent highlights, where are we going and how can you get involved? *(p.130)*

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 *(p.131)*

Neil Melville
ESA's Parabolic Flight Activities: An overview of our campaigns, capabilities, and new application routes for Technological and Commercial proposals *(p.133)*

Marisa Covington
Navigating the NASA IRB and human research multilateral review board (HRMRB): an ethics perspective *(p.134)*

Cyndi Roman
ClinicalTrials.gov: Understanding the Clinical Trials Requirements at NASA *(p.135)*

Closing ceremony and announcement of our next meeting in 2025

Posters Session

Poster will be available during all the meeting.
Authors are kindly requested to be present during their session

Posters presented during the session on Monday

Chaired by Noora Al Mheiri & Nastassia Navasiolava

1 - Tatiana Kostrominova
Role of Inositol-trisphosphate Receptors in the Regulation of Signaling Pathways During Unloading-induced Rat Soleus Muscle Atrophy *(p.136)*

2 - Monica Christova
Activating Orthostatic Response with Motor Imagery: Potential Application in Returning Astronauts and Older Adults *(p.139)*

3 - Amira Sayed Khan
Novel GPR120 agonist modulates systemic and neuroinflammation *(p.141)*

4 - Aya Hesham
Space-Fit Far Infrared Suit for Back Pain Mitigation onboard the International Space Station (ISS) *(p.142)*

5 - Alexandru Nistorescu
Assessing Achilles Tendon Mechanics With MusTone Device: A Myotonometric Approach To Understanding Tissue Dynamics *(p.144)*

6 - Abdulrahman Alblooshi
Exploring the Therapeutic Potential of Gravitational Psychology in Disease Understanding *(p.146)*

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 *(p.148)*

8 - Adel Elmoselhi
Effects of Isolation and Confinement on Vascular Health during Space Travel: Insights from a SIRIUS-21 Analog Mission *(p.150)*

9 - Andreas Rössler
Effects of hemodynamic responses during stand test following 15 minutes of sinusoidal vibration of varying intensity *(p.152)*

10 - Masahiro Terada
Performing the bedrest study for the space medicine educational programs *(p.154)*

Posters presented during the session on Tuesday

Chaired by Noora Al Mheiri & Marc-Antoine Custaud

11 - Devjoy Dev
Exploration of the biomechanical stress on the body while performing functional and operationally relevant movement patterns under variable gravitational stress *(p.155)*

12 - Kristina Sharlo
Effects of Muscle Electrical Stimulation under 6-day Dry Immersion on Soleus Muscle Signaling *(p.156)*

13 - Natalia Vilchinskaya
Time-course of alterations in the expression of mechanosensitive ion channels in rat soleus muscle under simulated microgravity *(p.158)*

14 - Ameline Saouli
Effects of Simulated Microgravity on Sperm Function: An In Vitro Study Evaluating Sperm Quality and Function-Specific Genes *(p.159)*

15 - Tiffany Stead
Examining Hypercoagulability in Females Exposed to Dry Immersion: a mechanism for Development of Venous Thromboembolism in Microgravity? *(p.161)*

16 - Irina Bryndina
Sphingolipids as regulators of skeletal muscle phenotype at gravitational unloading *(p.163)*

17 - Victoria Gulimova
X-Ray Phase Contrast Microtomography Investigation Of Thick-Toed Geckos Caudal Vertebrae After A Long-Term Space Flight Using Machine Learning *(p.165)*

18 - Andrew Blaber
Exploring Cardio-postural Interactions in relation to Prolonged Space Missions *(p.167)*

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 *(p.169)*

20 - Karolina Almeida Borges
Repetitive Movements and Ultra Long Flights as Predictors Influencing Musculoskeletal Disorders Among Commercial Airline Pilots: A Cross-Sectional Study *(p.170)*

21- Sami Alghayath
Assessment of Hemodynamic and Autonomic Reponses to Changes in Posture in Diabetics in Dubai: A Prospective Cohort Study *(p.172)*

ABSTRACTS

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

Ahmed Bakri[1], Asmaa Ismail[1], Adel B. Elmoselhi[2], and Zahi Badran[1]

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[2] Basic Medical Sciences Department, College of Medicine, University of Sharjah, UAE

ABSTRACT

INRODUCTION: The United Arab Emirates (UAE) is rapidly gaining credit for its outstanding accomplishments in the field of space exploration. One of the aims of the UAE space program is to enhance astronaut's overall health. At present, there is a gap of knowledge on the effect of microgravity on human health and specifically on oral health and its implications. Periodontitis is the sixth most prevalent chronic disease worldwide and a major oral health disease affecting almost half of the adult population (Nazir MA. *et a.l*, 2017). It is well known as a multifactorial disease in which both environmental and genetic factors play a precise and controversial role in determining its onset (Isola G. *et al*, 2020). The prevalence of periodontitis is high, estimated to be around 50% of the adult population in the USA and approximately 10% of the global population affected by severe periodontitis. The prevalence of periodontal diseases is increasing in the United Arab Emirates (UAE). Despite the limited local data about periodontal diseases, statistics from Abu-Dhabi Emirate in 2015 showed that 24.6% of the adult population suffered from periodontal diseases, and that, in addition to dental caries, constitute 40% of the global expense of oral care (Abu-Gharbieh E. *et al.*, 2019). As alveolar bone loss is a distinguishing characteristic feature of periodontitis progression, the mechanisms by which the local and environmental factors disturb the homeostatic balance of bone remodeling to favor bone loss need to be studied further (Hienz SA. *et al.* 2015). Further investigations are needed to establish and measure the clinical manifestations and the rate of progression of this inflammatory response in microgravity environments.

OBJECTIVES:

The aim of this study is to understand and evaluate the clinical and radiographical changes that occur during space flights in microgravity environments on the progression and the clinical presentation of Periodontitis. It also aims to assess specific bone turnover/remodeling markers produced in mouse Gingival Crevicular Fluid (GCF) with induced periodontitis in response to microgravity using the Hindlimb Unloaded (HLU) model, consequently paving the way for the development of more effective preventative and therapeutic measures to protect against periodontal disease, in space and consequently on ground.

METHODS:

The ligature-induced model is the most used model for inducing periodontitis (Chipashvili O. *et.al*, 2022). The literature describes methods of inducing periodontal disease by placing a ligature around the mouse molar teeth (mostly 2nd molar) (Li D. *et.al*, 2020). In this study, Periodontitis was induced in a 3-5 months old male c57BL/6j mice using C+ nickel-titanium root canal files and stainless-steel ligature wires (fig. 1). Mice with induced Periodontitis (IP) were divided into two groups: ground control and hindlimb unloaded (fig. 2) and kept under controlled conditions for 7 days (Lin P. *et.al*, 2021) (Azeem M. *et.al*, 2021). Mice were then euthanized, and the alveolar bones were stored in 10% formalin for further investigation. Mice GCF was also collected from the test site and used to assess Alkaline phosphatase (ALP) enzyme activity, which is a biochemical parameter for the diagnosis of periodontal disease. Bone loss will then be assessed using micro-computed tomography analysis and bone turnover will be assessed using qPCR and immunohistochemical analysis. Mice were also clinically evaluated at the beginning and at the end of the experiment by recording tooth mobility score.

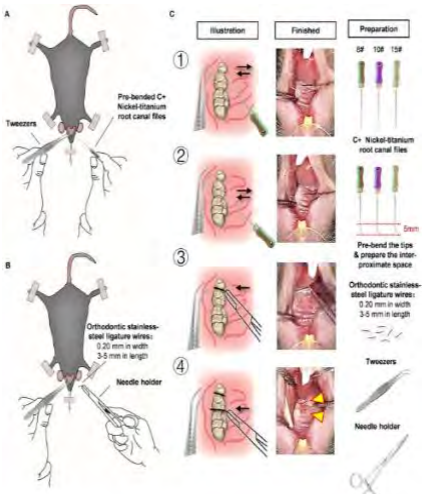

Figure 2: Ligature induced model of induced periodontitis.

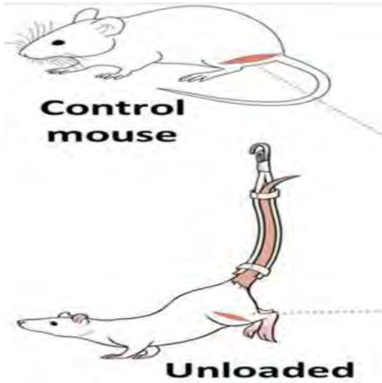

Figure 1: Control and Hindlimb unloaded mice models.

RESULTS:

Clinically, at the end of the experimental period, the access to the injury site was reported to be much easier, and that could be explained by the expected periodontal bone loss which made retrieving the ligature wire easier than inserting it. Intrestingly, ALP activity from the GCF retrieved from IP HLU mice was significantly higher (p<0.01) than the IP ground control mice indicating that microgravity plays a vital role in the exacerbation of Periodontitis (fig. 3).

CONCLUSION:

To the best of our knowledge, this the first attempt trying to elucidate the effect of microgravity on periodontal and oral health. Our preliminary findings reveal a statistically higher level of ALP in the HLU group compared to the control group, indicating an enhanced deterioration of periodontitis in the microgravity environment of space. This study would give us a first insight about how this common oral disease could evolve in space. The overall understanding will allow the scientific community to come up with preventive and therapeutic modalities to facilitate a safe route for humans to the moon and beyond.

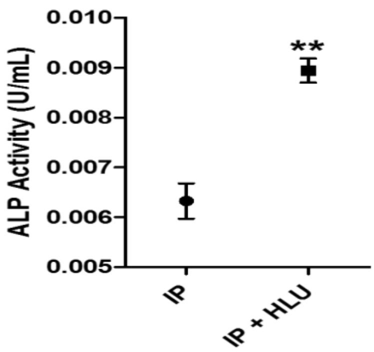

Figure 3: ALP activity in Gingival crevicular fluid collected from induced Periodontitis ground and hindlimb unloaded mice. IP: induced Periodontitis. HLU: Hindlimb unloaded.

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

Damien Lanéelle[1,2], Enora Fahss[1], Marion Hay[1], Olga Kuldavletova[1], Hervé Normand[1,3]
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BACKGROUND

On return to Earth, astronauts heterogeneously develop orthostatic intolerance, the etiology of which has yet to be elucidated (Nicogossian AE et al, 1992). An anatomical reason could explain this heterogeneity; the circle of Willis, a network of arterial anastomoses located at the base of the brain, shows significant inter-individual variability, in particular the presence of hypoplasia of the posterior communicating arteries (Jongen JC et al, 2002). It is possible that this anatomical variability (figure 1) is responsible for the heterogeneity of orthostatic intolerance.

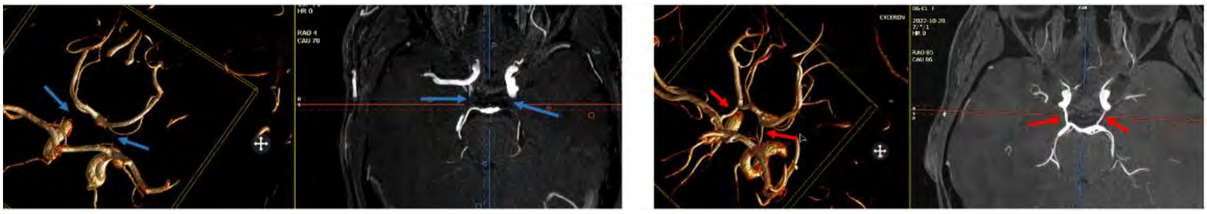


Figure 1. MRI of the Circle of Willis with incomplete Arterial Brain Pattern (ABP) to the left and full ABP to the right.

The aim of this study was to create 2 groups of subjects with different Arterial Brain Pattern (ABP), using a Doppler UltraSound (DUS) technique, confirmed with magnetic resonance imaging (MRI) technique and to assess the orthostatic tolerance according to this pattern during hemodynamic stress combining Lower-Body Negative Pressure (LBNP) and Head-Up tilt (HUT).

METHODS

Screening

From 76 healthy subjects, a GE Vivid-i DUS was used in combination with a short carotid compression (5 sec, Jatuzis D et al, 2000) to distinguish two groups: 12 with incomplete ABP and 12 with full ABP (52 exclusions due to intermediate ABP or temporal bone window not allowing DUS exploration). A secondary reclassification by 3 Tesla (Signa, GE) time-of-flight MRI was done for 24 subjects with 3 reclassifications (2 incompletes reclassified as completes ABP and 1 complete reclassified as an incomplete ABP) and 1 unclassified.

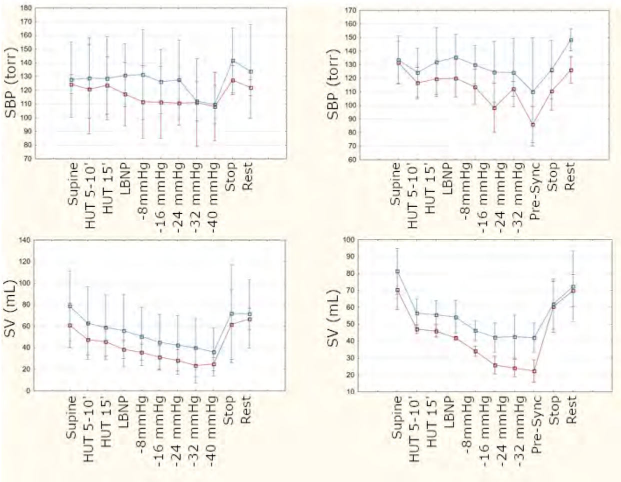
Hemodynamic stress

Measurements were obtained, for these 24 subjects, in the supine position and during an 80° HUT followed by a progressive decrease in LBNP of -8 mmHg every 3 minutes until pre-syncope, in accordance with the ESA protocol, slightly modified to limit the duration of stimulation to 30 minutes (minimum pressure: -40 mmHg). The definition of pre-syncope and the criteria for the end of test were those of the ESA. Subjects who did not complete the test were considered orthostatic intolerant (OI) and those who completed the test up to 30 minutes were defined as orthostatic tolerant (OT).

Measures

During the hemodynamic stress test, finger blood pressure (Finometer Pro, Finapress Medical Systems, The Netherlands) negative pressure in LBNP and standard electrocardiogram (ECG; Biopac, ECG 100 C, United States) were recorded continuously. Minimal systolic pressure (sAP) was recorded during the first minute of standing. Pre-syncope time was assessed according to ESA protocol.

RESULTS



OI subjects (stopping the test) and OT subjects (completing the test) have different physiological parameters during the test. Physiological data return to their initial values within one minute of the end of the LBNP (figure 2). No difference in was shown between the two ABPs or between the sexes. For OI subjects, sAP decreases during the first minute of standing (p=0,05; figure 3), whereas it does not in OT subjects (p=0,98). SV also decreases in intolerant subjects (p<0.001), whereas it does not in tolerant subjects (p=0,11).

8 OT subjects 13 OI subjects

Figure 2. Measurements of Systolic Blood Pressure (SBP) and Stroke Volume (SV) during the HUT and LBNP test in 8 subjects with Orthostatic Tolerance (left) and 13 subjects with OI (right). Data correspond to means with 95% confidence intervals with women in red and men in blue.

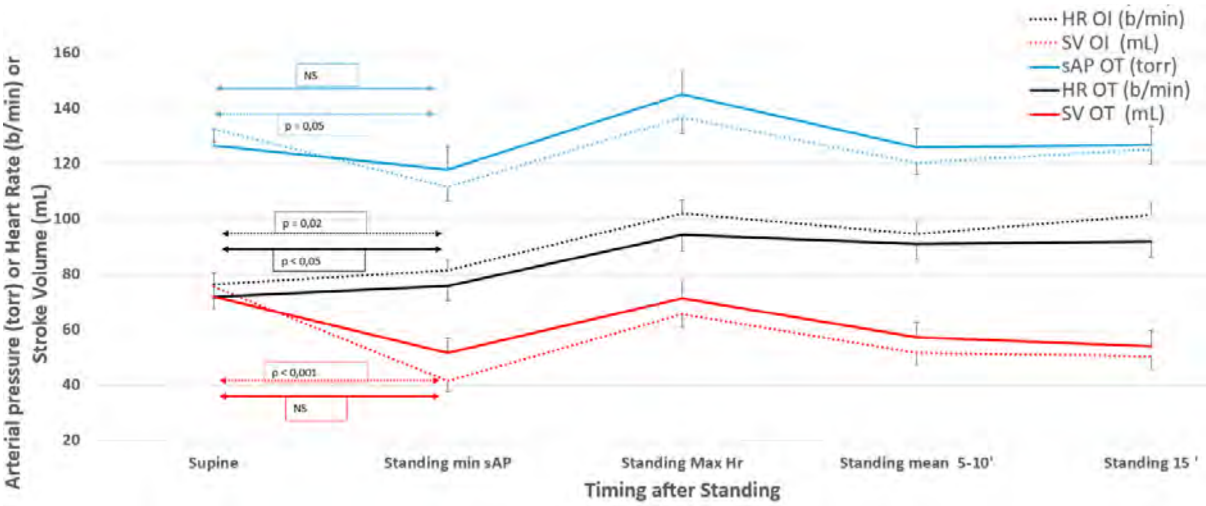


Figure 3. Percentage of evolution of minimal systolic pressure (sAP, blue), heart rate (HR, black) and stroke volume (SV, red) during the 80° HUT testing in 8 Orthostatic Tolerant subjects (OT, solid lines) and 13 Orthostatic Intolerant subjects with (OI, dotted lines). Data are expressed as means ± SE (Standard error of the mean).

CONCLUSIONS

The variability of the arterial brain pattern does not appear to result in changes in orthostatic tolerance during hemodynamic stress combining LBNP and HUT. In subjects with orthostatic intolerance, systolic arterial pressure appears to decrease during the first minute of standing.

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Does gravity affect intrinsic cardiac function?

Effects of different gravitational loads on the cardiac performance independent of the preload

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INTRODUCTION: Most cardiac performance indicators are dependent on the preload, which influences the left ventricular end-diastolic-pressure (LVEDP) and stroke volume (Spinale, 2015). *In vitro* experiments have demonstrated that LVEDP is also affected by intracardiac hydrostatic pressure gradients, which can be modified by the orientation of the heart in the thoracic cavity or the gravitational load in the head-to-foot direction (g-load) (Pantalos et al., 2005). As a consequence, cardiac function will be altered during exposures to reduced gravity such as would be experienced on Mars (3.71 $m.s^{-2}$) compared to that on Earth (9.8$m.s^{-2}$). We hypothesized that the heart position and the g-load could affect the cardiac function in the same manner as the preload, and should be taken into account to reflect the intrinsic properties of the heart. The aim of the present study was to isolate the effect of the intracardiac gradient of pressure independently of the preload by investigating cardiac function during reduction in the pressure surrounding the lower body (lower body negative pressure, LBNP) in a supine position (inducing fluid shift only) and compared it to several head-up tilt (HUT) angles without LBNP (simulating fluid shifts and altered intracardiac hydrostatic pressure gradients ranging from that anticipated on Mars to that on Earth).

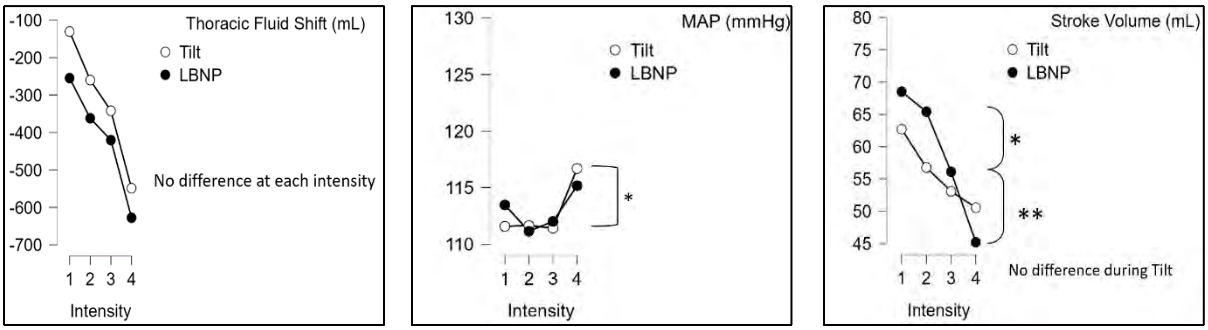
METHODS: Following a 10-min supine rest period on a tilt table with a LBNP compartment, 10 subjects (more data will be presented during the congress) were exposed to 4 levels of LBNP in supine position and 4 HUT angles simulating different levels of g-load on the cardiovascular system. The order between HUT and LBNP conditions and the intensities (angles and pressures) was randomized and interspersed with 6-min breaks in the supine position to restore a cardiovascular baseline. For each tilt angle or LBNP level, the condition was applied for 8min to reach a steady state (2min) before echocardiography (6min). The HUT angles were: 80° (simulating 1g on Earth), 58° (0.85g), 42° (0.66g) and 22° (simulating 0.37g on Mars). While the LBNP levels were: -10, -20, -35 and -50 Torr. Arterial blood pressure was monitored with a continuous non-invasive method. Fluid shifts in the different body segments were measured with bioelectrical impedance and then derived with Geddes's formula (Geddes & Sadler, 1973). The echocardiographic data were assessed with a Vivid IQ device.

RESULTS AND CONCLUSIONS: The effect of LBNP and tilt angles on fluid shifts in the thoracic region were equivalent, resulting in equivalent modifications in preload (Fig. 1, left panel). Fluid shifts in other regions (abdomen, pelvis and legs) exhibited differences most likely reflecting regional differences in blood flow distribution. In both conditions (HUT and LBNP), the regulation of blood pressure is appropriate, with a maintained systolic blood pressure, but the diastolic blood pressure is increased, especially during higher intensities. As a consequence, mean arterial blood pressure was elevated in both condition at high intensities

(Fig. 1, middle panel). The main difference between the conditions, and thus the main impact of the gravity on the cardiovascular responses, is on the hemodynamic parameters. In both conditions, the heart rate is similarly increased, certainly to compensate the decrease in stroke volume (Fig. 1, right panel). At low angles or LBNP levels, the stroke volume is equivalently reduced by the blood loss in the thoracic area only. At higher intensities the stroke volume continues to decrease during LBNP while it seems to stabilize during Tilt, which follow our hypothesis. An intrinsic heart property seems to take advantage of the heart position and gravity to compensate the reduction in preload. Indeed, at higher HUT, the gravity effect seems to assist cardiac filling and enhance the downward ventricle stretching due to the weight of the blood. A transthoracic echographic analysis will give us a non-invasive way to study this mechanism by measuring the cardiac filling and ejection at an equivalent preload. Equally, the color doppler M mode will provide a measure of the intracardiac gradient of pressure which will give us more information about the impact of gravity on mechanical heart function (Hodzic et al., 2020).

This is a part of a larger project aimed at developing a novel non-invasive method for assessing the heart function independent of the preload; which could potentially assist in monitoring astronaut's health in the future space missions.

Figure 1. Thoracic fluid shift (left panel), mean arterial pressure (middle panel) and stroke volume (right panel) during passive tilt and lower body negative pressure (LBNP).



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

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Under space flight conditions, a decrease in the impact of Earth's gravity on the musculoskeletal system is observed. That leads to mechanical unloading of skeletal muscles. At the same time, muscle atrophy occurs, slow muscle fibers are transformed into fast ones, muscle strength and fatigue resistance decreases. These alterations negatively affect human performance and the quality of life. The postural-tonic muscles in particular the soleus muscle are the most susceptible to negative changes caused by mechanical unloading.
In addition, as early as on the 2-3rd day of unloading, an accumulation of calcium ions is observed in the myoplasm of skeletal muscle fibers, which persists for at least 14 days (Ingalls et al., 1999, Ingalls et al., 2001). In contrast to the short-term accumulation of calcium during physical activity, under mechanical unloading, there is a relatively small amplitude but long-lasting increase in the content of calcium ions, similar to what is observed in various pathological conditions such as Duchenne muscular dystrophy or aging. It is known that excessive long-term accumulation of calcium in muscle fibers can lead to impaired mitochondrial function, including mitophagy activation, reactive oxygen species (ROS) generation, and ryanodine receptors oxidation (Andersson et al., 2011).

We assumed that ryanodine receptors oxidation by ROS can cause calcium store depletion in the sarcoplasmic reticulum, which leads to decreased maximum strength and increased muscle fatigue.

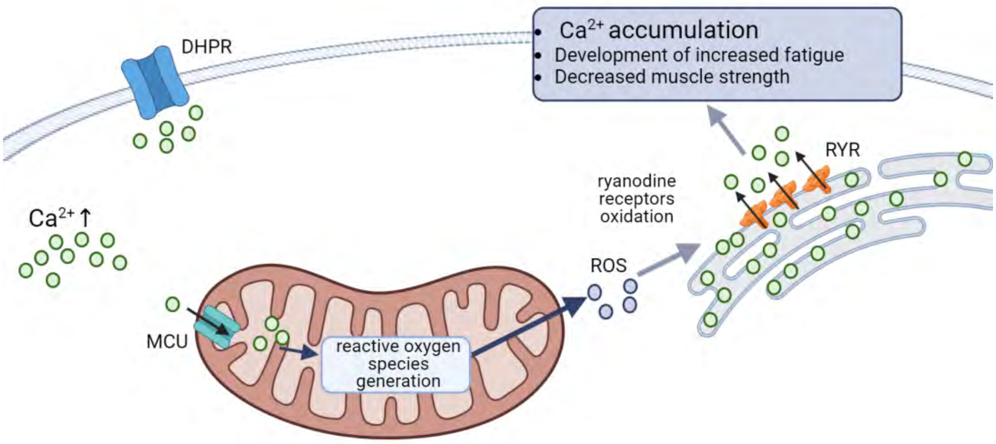

Fig.1.Functional unloading-induced calcium accumulation

Twenty-four male Wistar rats were randomly assigned to one of the three groups (eight animals per group): control rats with placebo (10% DMSO in food,“C”), 7 days of unloading/hindlimb suspension with placebo (“7H”), and 7 days of unloading with ryanodine receptor stabilizer S107 (90 mg/kg in 10% DMSO in food “7H+S”). Rodents were hindlimb suspended according to the Ilyin-Novikov method modified by Morey-Holton (Morey-Holton, Globus, 2002). After the experiment, the soleus muscles were dissected and placed in Ringer–Krebs solution.

Then we analyzed ex vivo soleus muscle fatigue. The muscle was attached via tendons to a force sensor at one side and to the fixed hook at the other side. The muscle was set to optimal muscle length, and it was electrically stimulated with a frequency of 40 Hz for 2 s. The maximum strength of the tetanic contraction was recorded. For the fatigue index, the ratio of the force of contraction after 20 tetanic contractions repetitions was normalized to the maximum contraction force measured during the entire test. Scores were normalized to the muscle physiological cross-sectional area.

The experiments were performed at the Institute of Biomedical Problems, RAS, Russia. The Committee on Bioethics of the Russian Academy of Sciences reviewed and approved all animal experiments for this study

After seven days of unloading, the soleus muscle mass was significantly decreased in both 7H and 7H+S groups (35.39 ± 3.41 mg and 41.10 ± 9.19 mg, respectively) when compared with the control rats (65.96 ± 12 mg). Despite atrophy, treatment with ryanodine receptor stabilizer prevented the unloading-induced decline of the muscle fatigue index (Fig. 2, A). Moreover, the 7H group had a significant decline in the maximum force, but S107 treatment prohibited these alterations (Fig.2, B).

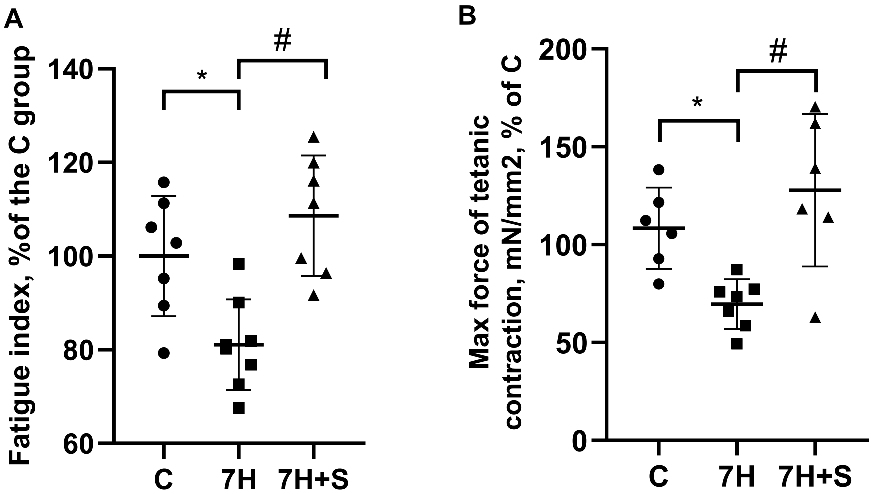

Fig.2.A – Fatigue index of soleus muscle. B – Special maximum force of tetanic contraction.
* – significant difference from the C group, # – significant difference from H, $p< 0.05$.

Thereby, changes in the muscle mechanical characteristics during functional unloading are most likely due to the calcium ions leakage from the sarcoplasmic reticulum ryanodine receptors.

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Chondrites: Understanding the Origins of the Solar System

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INTRODUCTION

Chondritic meteorites, comprising 80% to 85% of observed meteorite falls (Sanders and Scott, 2012), are among the oldest rocks to reach Earth's surface, offering invaluable insights into the early Solar System's formation. They may have originated from interstellar events preceding the formation of planetesimals in our solar system (Sears, 2004), where collisions and gravitational influences led them onto trajectories that intersect with Earth. The term "chondrites" refers to these formations, characterized by distinctive interior spherules called chondrules, which capture components from the protoplanetary disk, serving as cosmic samples (Trio-Rodriguez and Blum, 2009). The Meteorite Center at the Sharjah Academy for Astronomy, Space Sciences, and Technology is equipped with sophisticated facilities to conduct tests on samples brought by customers to confirm their authenticity by examining their elemental composition and mineralogy. This study focuses on both physical and chemical analyses, utilizing X-Ray Fluorescence (XRF) and X-Ray Diffraction (XRD) techniques. It contributes to the United Arab Emirates space sector knowledge base, revealing more about early solar system chondrites and enhancing the elemental examination methods.

METHODOLOGY

Some rocks resembling meteorites require physical tests to confirm their meteoritic properties. This includes documentation, a density test to understand solidification conditions (Ostrowski and Bryson, 2019), examination of magnetic susceptibility related to metal content, distinct fusion crust, surface texture, and inclusions. The samples showed a dense weight surpassing the terrestrial rocks. Stony meteorites are around 1.5 times heavier than Earth rocks, and their average densities are between 3 to 4 g/cm3 (Ostrowski and Bryson, 2019). The tested samples are magnetic as they contain iron-nickel metal. Some samples display a black fusion crust, while others exhibit a rusty, brownish-red crust due to iron-nickel metal oxidation from terrestrial weathering. The samples show a color difference between exterior and interior layers, with no bubbles or holes, and feature chondrules, from hundreds of micrometers to millimeters, in a fine-grained matrix.

We employed XRF and XRD techniques to analyze the studied samples, which enabled qualitative and quantitative analysis and crystallographic structure determination. Between 2017 and 2023, 123 samples underwent thorough examination, with 144 analyses conducted. Only eight samples were authenticated as genuine meteorites, four of which were classified as chondrites. Table 1 shows the physical features of these four stony meteorites.

Table 1. Physical characteristics of the four chondrites.

1	2	3	4	5	6	7	8	9
2018	S20	12.11g	3.0275 g/cm^3	-	attracted to magnet	XRF + XRD	ordinary chondrite	Lewa, Aldhafra Abu Dhabi
2020	S36-19/20	18.6 g		3.576	attracted to magnet	XRF + XRD	stony meteorite (chondrite)	KSA
2022	S21-22	64.46 g	3.22 g/cm^3		attracted to magnet	XRF + XRD	stony meteorite (chondrite)	Al-Rub' al-Khali – Oman
2023	S5-23	84.94 g	3.39 g/cm^3		attracted to magnet	XRF + XRD	stony meteorite (chondrite)	Al-Rub' al-Khali – Oman

Notes to Table 1. (1) Analysis Year; (2) Sample No.; (3) Sample Weight (g); (4) Density (g/cm3); (5) Specific gravity; (6) Magnetism; (7) Analysis Test; (8) Classification; and (9) Discovery Location

RESULTS AND DISCUSSION

Chondrites mainly consist of anhydrous silicates (olivine, plagioclase, clinopyroxene, orthopyroxene), nickel-iron minerals (kamacite, taenite), and iron sulfide (troilite) (Rubin, 1997). The dominant minerals found in the analyzed samples are Forsterite, Anorthite, Kamacite, Taenite (Burbine, 2014), Enstatite (MgSiO3) as well as Troilite. The mineralogy indicated their chondritic origins. Sample S5-23, weighed 84.94 g, attracted to magnet, and had a 3.39 g/cm^3 density. The mineralogy of the sample is composed of Forsterite (Mg2SiO4), Troilite (FeS), Kamacite, and Taenite, which are found only in meteorites (Fernini and Subhi, 2021). The analysis results are shown in Table 2 and Fig. 1. For sample S20, the dominant minerals are Forsterite, anorthite, Kamacite, and troilite. The mineral composition of sample S36-19/20 closely resembles the previous one, consisting of Forsterite, Anorthite, Kamacite, and Troilite. The common minerals in sample S21-22 are enstatite, Forsterite, Kamacite, Taenite, and Troilite.

Table 2. XRF analysis results of sample S5-23.

S5-23												
Fe2O3	CaO	SiO2	Al2O3	MgO	K2O	NiO	SO3	Na2O	TiO2	SrO	ZrO2	MnO
19.35	14.39	56.98	3.89	3.03	0.694	0.668	0.640	0.154	0.148	0.037	0.009	0.010

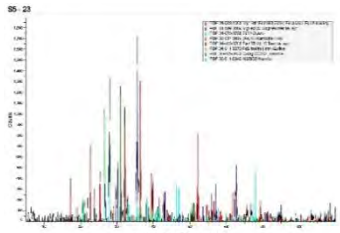

Fig. 1. XRD analysis pattern of sample S5-23

CONCLUSION

Studied samples consistently showcase chondrules and Fe-Ni metal, indicating their meteoritic nature. Sample S5-23, from Al-Rub' al-Khali, has minerals like Forsterite and exclusive meteorite minerals Kamacite and Taenite. Sample S36-19/20, from Saudi Arabia, mirrors S20's composition, suggesting similar origins or formation processes of chondrites. All samples consistently contain Forsterite, Troilite, and Kamacite, highlighting meteoritic characteristics. The analysis of chondritic meteorites enhances the UAE's knowledge of the solar system's origins and meteoritic compositions, positioning the UAE at the forefront of planetary research.

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

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Herein, we present the REGOLIFE project that aims at understanding how to engineering lunar regolith, the primary material covering the Moon's surface, into an environment capable of sustaining life and supporting crop cultivation. By leveraging the colonization of plants and earthworms, as well as shaping plant-microbiota interactions, REGOLIFE aims to mimic Earth-like soil properties in lunar regolith. REGOLIFE aligns with the current activities and plans of major space agencies of the world to return astronauts to the Moon (Smith et al, 2020). Central to REGOLIFE's objectives is the development of cutting-edge technology to support experimental activities and facilitate the manipulation of biological systems within lunar conditions. Through laboratory experiments conducted both in Earth gravity (g) and in simulated lunar gravity, the project will seek to understand the responses of different organisms, including *Arabidopsis thaliana* plants, earthworms, and microorganisms, to lunar regolith simulants and reduced gravity. Additionally, REGOLIFE will investigate the beneficial effects of these organisms on enhancing regolith fertility and promoting plant microbiota diversity. Key to the project's success is the exploration of lunar gravity's impact on plant-microbiota interactions and the design of innovative technological platforms enabling automated experimentation. By developing artificial cultivation systems, and microfluidic analytical platforms, tailored to lunar conditions, REGOLIFE seeks to study lunar soil fertilization and habitat development. Findings derived from REGOLIFE are expected to have broad implications for bio-regenerative life support systems and space farming, offering knowledge for future lunar exploration and colonization efforts.

Overall, REGOLIFE presents a multidisciplinary endeavor that epitomizes the intersection of biology, engineering, and space exploration, with far-reaching implications for the future of human presence beyond Earth.

PRELIMINARY EXPERIMENTS AND RESULTS

Assessing earthworms ability to colonize a lunar regolith simulant

We carried out a pilot study to test the impact of lunar regolith simulant (LHS-1) on the earthworm *Eisenia fetida* Savigny (Romano et al, 2023). This species tolerates several adverse environmental conditions and habitat perturbations, therefore it can also serve as a model bioindicator for the Moon soil environment. Assessing survival, reproduction, tunnel formation, and ingestion rates across various LHS-1 concentrations mixed with native substrates, the study aimed to gauge the earthworm's adaptability to lunar soil conditions (Fig. 1). While increased mortality occurred with higher LHS-1 concentrations, *E. fetida* demonstrated resilience, surviving through extended experiments. Tunnel formation increased with LHS-1 concentrations, indicating heightened mobility for food seeking. Notably, cocoon production and ingestion rates remained unaffected. These findings suggest *E. fetida*'s potential to adapt and colonize lunar regolith, offering insights into biological methods to promote Moon pedogenesis, and improve lunar soil habitability for crops.

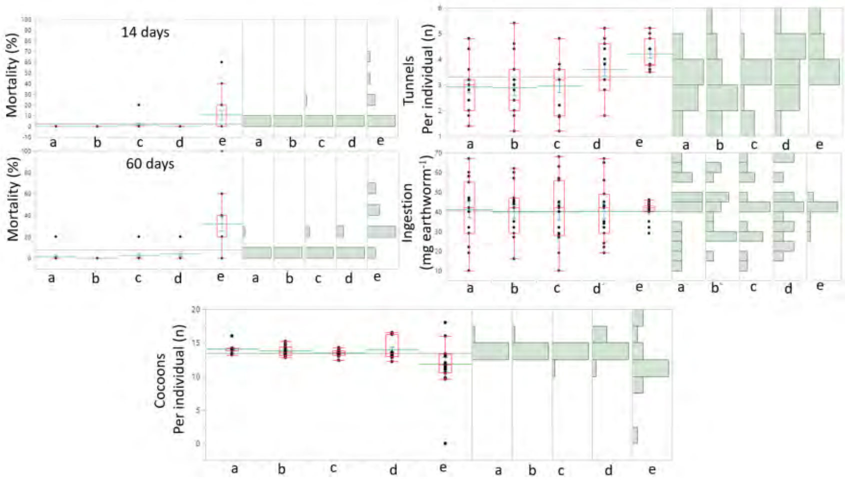

Figure 1 - Survival and fitness of *E. fetida* on lunar regolith. Lunar regolith simulant concentrations: a) 0%, b) 25%, c) 50%, d) 75%, and e) 100%. In each box plot the median (red line) and its range of dispersion (lower and upper quartiles, as well as outliers) are indicated. The mean (green line), and the standard error value (blue T-bars) are also included. Each box plot reports on its right histograms describing data distribution.

Effects of lunar regolith simulant colonized with earthworms on plants growth

Pilot experiments are in progress in order to preliminary identify the possible effects of lunar regolith, previously colonized with *E. fetida*, on *A. thaliana* plant growth. *A. thaliana* plants are seeded and cultivated in a plant growth chamber at optimal controlled conditions up to one month, checking the leaf area with LemnaTec LabScanalyzer (an example of the system is available in Fig. 2) and analyzing growth parameters at the end of the experiments.

Figure 2 - Representative result of experiments performed on *A. thaliana* plants using the LemnaTec LabScanalyzer for rapid phenotyping. Left: original image; right: image after plant tagging with different colors by the image analyser.

The REGOLIFE goals will be achieved through transdisciplinary efforts in plant science, molecular biology, genomics and ecology at the Biorobotics Institute and at the Center for Plant Sciences of Scuola Superiore Sant'Anna.

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

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The effects of deep space radiation and reduced gravity on biological systems remain largely unknown, thus posing a significant knowledge gap for both astronaut health and in-space agriculture.

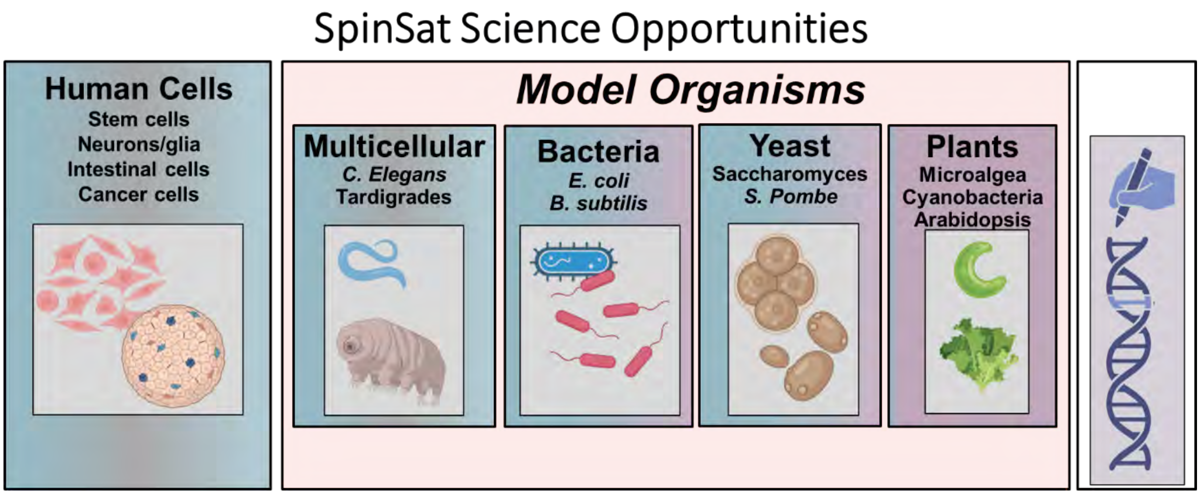
The SPINSAT spacecraft platform is designed to bridge a critical gap in our understanding of how deep space radiation and reduced gravity affect living systems. This research is crucial for astronaut health and space-based agriculture, but its implications extend far beyond, particularly in the field of cancer biology.

SPINSAT, a disk-shaped rotating bus, is tailored for biological experiments, ranging from human tissues to microorganisms and plants. It will explore the effects of long-term exposure to low-dose radiation in an environment similar to the moon and Mars' gravity. Key areas available to study are DNA damage, with direct implications for cancer research. Understanding how radiation impacts DNA in space can provide new insights into cancer development and progression, leading to potential breakthroughs in cancer treatment and prevention. Studies in SPINSAT could reveal novel ways to repair DNA damage, offering hope for more effective cancer therapies.

Additionally, the platform will enable investigations on cell membrane damage, immune defense, and the impact on bone and muscle function due to protein damage. These studies are directly relevant to cancer biology, as they could improve our understanding of tumor development and metastasis, and how the immune system can be harnessed to fight cancer.

The platform, accommodating various experimental setups, will facilitate significant advancements in understanding the space environment's effect on biological systems. This knowledge will not only be vital for long-duration human spaceflight but also holds the promise of groundbreaking applications in cancer biology, potentially leading to new treatments and better outcomes for cancer patients on Earth.

In summary, SPINSAT represents a significant step forward in space research with far-reaching implications for cancer biology. By studying the effects of space conditions on various organisms, it could pave the way for novel cancer treatments and a better understanding of the disease, greatly benefiting patients on Earth.



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

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ABSTRACT

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 (Xu F. *et al.*, 2023). 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 (Taylor L. *et al.*, 2005). This issue, and its potential impact on biological systems, is closely tied to the unique properties and behaviors (Miranda S. *et al.*, 2023).

Method

A tissue engineering approach (Maurer J. *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.

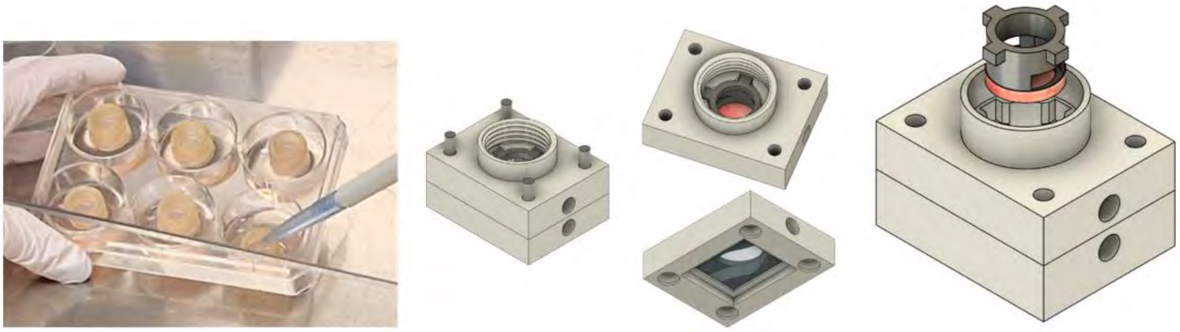

Figure 1. Artificial airway mucosa model. In this project we bring medicine, biology and technology together! Starting with the crowns for producing the airway model (left), the team is also working on the appropriate hardware for microgravity and hypergravity experiments.

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).

Conclusion & Outlook

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).

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UTILIZING BIO-INSPIRED HIERARCHICAL MULTI-SHELL STRUCTURES (BHMSS) FOR RADIATION SHIELDING IN SPACE EXPLORATION

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ABSTRACT

Space exploration presents numerous challenges, with radiation exposure being one of the most significant threats to astronaut health and safety. Traditional shielding methods have limitations in terms of weight, space, and effectiveness. This paper delves into the intricacies of space radiation and proposes a new way to tackle space radiation aided by BHMSS. This paper also proposes a novel approach leveraging Bio-inspired Hierarchical Multi-Shell Structures (BHMSS) as a promising solution for radiation shielding in space missions. Inspired by natural biological systems, BHMSS offers a hierarchical arrangement of materials with varying properties to effectively mitigate radiation. By mimicking the structure found in materials like bones or shells, BHMSS provides a lightweight yet robust defense against cosmic radiation. This abstract therefore discusses the theoretical framework behind BHMSS, its potential applications in space exploration, and the challenges and opportunities associated with its implementation. Through thorough research and analysis, the efficacy of BHMSS in shielding astronauts from radiation can be assessed, paving the way for safer and more sustainable space exploration endeavors.

Galvanic Vestibular Stimulation Decreases Parathyroid Hormone in Menopausal Women.

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Osteoporosis is a metabolic bone disorder that is characterized by low bone mass and micro-architectural deterioration of bone tissue. Postmenopausal osteoporosis, resulting from estrogen deficiency, is the most common type of osteoporosis. Recently, it has been known that the vestibular system is also involved in bone metabolism.

The vestibular system plays an important role in controlling motor and autonomic control such as posture and arterial pressure (AP) at the onset of postural change. Noisy galvanic vestibular stimulation (nGVS) improves these posture control and AP response. In the present study, to exam the effect of the nGVS for the bone metabolism, we measure markers of the bone metabolism in the menopausal women with and without nGVS.

This study was approved by the Institutional Review Board of the Gifu University of Medical Science, and written informed consent was obtained from all participants. 18 healthy women were recruited. The subjects were divided into 3 groups, i.e., menopausal with nGVS, menopausal without nGVS, and young with nGVS. The nGVS with a uniform intensity of 0.1 mA below the somatosensory threshold was then applied for 30 min. Before and after nGVS, blood was drawn, and parathyroid hormone (PTH), Calcitonin, osteocalcin, Bone Specific Alkaline Phosphatase (BAP), Calcium, inorganic phosphorus (IP), and 1,25-(OH)2 Vitamin D were measured.

In the menopausal with nGVS group, PTH, which stimulates the release of calcium from bones into the blood, was significantly decreased after nGVS, compared to that before GVS. The change was not observed in menopausal without nGVS group, and young with nGVS group. No significant difference was observed in the other measurements in all groups. Thus, nGVS or vestibular input might prevent the progress of osteoporosis in menopausal women due to suppression of PTH secretion. Further studies are needed in postmenopausal women or astronauts those who have issues of decalcification.

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

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The redistribution of fluid in the body of astronauts with its predominant accumulation in the upper part of the body, a decrease in plasma volume and changes in the ratio of fluid in the intra- and extracellular spaces are considered to be the most significant effect of weightlessness (Norsk P., 2020; Jirak P., 2022; Ercan E., 2021).

The absence of the usual hydrostatic fluid gradient directed towards the legs leads to an increase in central venous pressure (CVP) and dilation of large veins (Marshall-Goebel K., 2019). Activation of the sympathetic nervous system, systemic vasodilation, and a decrease in peripheral resistance occur indirectly. These changes are typical both for the acute period of adaptation and during the first week of flight. The additional effects of neurouretic and vasadilatory peptides, increased CVP and reflexes due to overstretching of the vessels of the upper half of the body lead to a decrease in systemic blood pressure, suppression of the sympathetic and the predominance of the parasympathetic nervous system (Miwa C., 1996; Gundel A., 2002; Mano T., 2005).

There is a limited number of works devoted to ultrasound assessment of the mechanisms of the body's adaptation to microgravity. A number of studies have described an increase in the diameter/sectional area of the internal jugular (IJV), common femoral and portal veins, and a decrease in the diameter of the deep veins of the leg. When measuring the blood flow velocity in the IJV, it was found to decrease, up to stasis or retrograde flow. Such non-physiological conditions and the observed hypercoagulability increase the risk of developing venous thromboembolic complications (TEC), cases of which have been described in astronauts (in particular, thrombosis of the internal jugular veins) (Arbeille P.,1985,2015; Kim DS.,2021; Pavela J.,2022). Currently, pulmonary artery thromboembolism (PATE) is the third most common cardiovascular disease (the estimated incidence in Europe and the USA is at least 250-300 thousand per year), as well as one of the main causes of mortality. At the same time, over the past two decades, a steady increase in the incidence of PATE has been recorded, with a relatively stable prevalence of deep vein thrombosis. The total prevalence of TEC syndrome varies from 100 to 200 cases per 100 thousand population.

Considering an increasing number of women participating in space flight (SF), the IBMP considered it necessary to continue research under “dry” immersion (DI) conditions and to increase the experiment duration to 5 days in order to more carefully study the physiological mechanisms of adaptation of the female body and possible gender peculiarities under the influence of SF factors. The study involved 23 female volunteers of reproductive age with a natural menstrual cycle (age 29.09±5.35). One of the objectives of this work was to study changes in venous hemodynamics under DI conditions and during the recovery period.

To assess hemodynamic changes 7 days before the start of DI (BDC-1), 24 hours after the beginning of the exposure, immediately after the end of the exposure (R+0), and on days 6/7 of the recovery period (R+6/7) in the morning, duplex ultrasound was performed on an empty stomach according to a standard clinical protocol (Logiq E scanner, GE, USA). Statistical analysis was performed using Statistica for Windows v. 12.0 (StatSoft, Inc.).

An analysis of primary data on changes in vessel diameters in the system of the inferior vena cava and internal jugular vein was carried out. A significant decrease in the diameters of the inferior vena cava, common iliac vein, and great saphenous vein was revealed at different periods of the examination ($p < 0.05$). Also, a significant decrease in the area of the internal jugular vein and the common femoral vein (CFV) with subsequent recovery (left IJV 0.71±0.05 cm2 (BDC-7); 0.33±0.03 cm2 (R1), 0.33±0.04 cm^2 (R+0) and 0.71±0.08 cm^2 (R+6/7); left OBV 0.77±0.06 cm^2 (BDC-7), 0 .77±0.07 cm^2 (R1), 0.64±0.05 cm^2 (R+0) and 0.83±0.07 cm^2 (R+6/7) was recorded during the time spent under DI conditions. A significant decrease in blood flow velocity in the inferior vena cava, common iliac vein, common femoral vein and internal jugular vein ($p<0.05$) was revealed.

The results obtained indicate reversible changes in the venous system of women occurring during the acute period of adaptation to experimental conditions. At the same time, the changes we recorded did not go beyond the physiological norm. But in contrast to the previous experiment with women exposed to DI conditions for three days (Vasilev I., 2021), we observe more pronounced changes in the system of the inferior vena cava and internal jugular vein by the fifth day of exposure. The next objective of the research is to conduct a comparative analysis of the state of the venous system of men and women in DI conditions, as well as to conduct longer studies with the participation of women.

The work was carried out within the framework of the Russian Science Foundation project No. 19-15-00435 (control group of female participants) and using the Transgenbank Unique Research Facility (groups with LF), Basic Topics of the RAS FMFR-2024-0039 for 2024–2026(comprehensive analysis).

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

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The tendency towards an increase in the number of women taking part in space flights (SF) of various durations poses a variety of special tasks for physiologists and physicians who ensure the maintaining of health of SF participants. Scientists are constantly turning to assessing the effect of cosmic field factors on women's body and searching for potential differences in the response of physiological systems to extreme effects. At this, the scope of biomedical experiments with the participation of women, both directly in space flight and in analogue studies, is not comparable with the scope of experiments conducted on men. A successful experiment with the participation of female volunteers in a 3-day "dry" immersion (DI) (NAIAD-2020, Tomilovskaya E., 2021) and the experience of European colleagues with 5-day exposure (VIVALDI, Robin A., 2023) allowed us continue research in DI.

Twenty three women of reproductive age (age 29,09±5,35, height 167,01±6,38 cm, weight 63,6±11,04 kg) took part in the "Immersion-5F-LF" experiment. The participants were staying in DI for 120 hours (5 days) and were divided into three groups. Two groups of participants took a human lactoferrin (hLF) biosimilar at a dose of 200 and 400 mg/day (9 and 7 women, correspondingly) from the first hours of their stay in the DI, and one group was a control (7 persons, placebo). All women had a negative pregnancy test taken 2-3 hours before the start of the DI. The time which female participants spent outside the immersion bath did not exceed 15 minutes per day. Taking of hLF or placebo continued until the 30th day of the aftereffect period. The choice of LF, an iron-binding glycoprotein of the innate immune system, as a means of preventing the negative effects of microgravity is due to its known pleiotropic effects (Cao X. et al, 2022). In addition to the well-studied antimicrobial, antiviral and anti-inflammatory properties, LF has an immunomodeling (Siqueiros-Cendón T., 2014) and osteogenic effect (Cornish J., 2004; Icriverzi M, 2020; Tian M., 2023), and also affects glucose metabolism (Maekawa Y., 2017) and is involved in the regulation of lipid metabolism (Laniro G., 2023). LF has high tolerability and has no side effects.

The main goal of the experiment was to obtain data on the influence of the early period of adaptation to support unloading on the physiological systems and microflora of women.

In the experiment the data was obtained that made it possible to analyze the state of the cardiovascular, respiratory and reproductive systems, mineral metabolism and bone tissue during the period of DI and after its end, to assess changes in body composition and the movement of body fluids. An extensive examination of the visual system (refractometry, biomicroscopy, fundusoscopy, electronic intraocular tonography, computer perimetry) and auditory system (otoscopy, tympanometry, otoacoustic emission) was carried out. Particular attention was paid to muscle physiology and activity and to sensorimotor reactions.

The composition of intestinal microflora, cervical canal and vagina was analyzed. A cytological assessment of the condition of the vagina was given.

The research program also included daily medical monitoring, which was carried out twice a day by the physician on duty: physical examination, measurement of body temperature, blood pressure, heart rate, filling out scales for assessing well-being, discomfort in the back and abdomen, appetite, urinary system function, night sleep.

Thus, a comprehensive assessment of the body condition of women who were exposed for 5 days to gravitational unloading simulating the initial period of adaptation of human body to microgravity was carried out.

The work was carried out within the framework of the Russian Science Foundation project No. 19-15-00435 (control group of female participants) and using the Transgenbank Unique Research Facility (groups with LF), Basic Topics of the RAS FMFR-2024-0039 for 2024–2026 (comprehensive analysis).

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AstroBEAT: Cardiovascular Variability Analysis and Lunar Microgravity Twin

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ABSTRACT

In preparation for human space exploration within the solar system, previous missions in Earth's orbit have shown that humans are capable of surviving and functioning in space over extended periods. Nonetheless, there remain technological, medical, and psychological challenges that need addressing before embarking on longer space missions. These challenges include protection against ionizing radiation, addressing psychological concerns, managing behavior and performance, and preventing bone loss. The advent of digital twins and Generative Artificial Intelligence (AI) heralds a new era in the study of human physiology under extraterrestrial conditions, particularly in understanding cardiovascular variability and the effects of microgravity on the human body on the Moon. This research aims to develop a comprehensive digital twin of the human cardiovascular system, leveraging Generative AI to simulate and analyze the intricate dynamics of cardiovascular variability in response to the Moon's microgravity. By integrating physiological data, environmental factors, and predictive modeling, the project seeks to uncover the underlying mechanisms of cardiovascular adaptation, potential health risks, and effective countermeasures to ensure astronaut health during lunar missions. The outcomes of this study are expected to provide vital insights for future manned missions, contributing to safer and longer space explorations.

OBJECTIVES

- To develop a digital twin of the human cardiovascular system that accurately simulates the effects of the Moon's microgravity on cardiovascular variability.
- To employ Generative AI techniques to predict cardiovascular responses under various lunar mission scenarios.
- To identify potential health risks and devise countermeasures to mitigate adverse effects on astronaut health.

Data Collection

Collecting diverse data types will provide a robust dataset for analyzing the effects of lunar microgravity on cardiovascular variability, facilitating the development of effective countermeasures to ensure astronaut health during prolonged lunar missions (Patel, S., 2020), (Baran, R.et al, 2021).

Physiological Data	
Cardiovascular Metrics	Continuous monitoring of heart rate, blood pressure (both systolic and diastolic), heart rate variability, and cardiac output to assess the cardiovascular system's response to lunar microgravity.
Radiation Exposure	Monitoring of cosmic radiation levels, which are significantly higher on the Moon compared to Earth, to assess their impact on cardiovascular health.
Atmospheric Conditions	Data on the habitat's atmospheric pressure, composition, and humidity, as can affect cardiovascular function.
Activity and Behavioral Data	
Physical Activity Logs	Detailed records of astronauts' physical activities, including exercise routines, to understand the impact of physical exertion on cardiovascular metrics in lunar gravity.
Diet and Nutrition Logs	Information on dietary intake to monitor the relationship between nutrition, fluid intake, and cardiovascular health.
Sleep Patterns	Monitoring sleep duration, quality, and circadian rhythm changes, as these can influence cardiovascular variability.
Technological and Instrumentation Data	
Wearable Sensor Data	Continuous data streams from wearable devices designed to monitor cardiovascular and other physiological parameters in real-time.
Environmental Control and Life Support Systems (ECLSS) Data	Information from systems regulating the habitat's atmosphere, including oxygen levels, pressure, and temperature, as these can indirectly affect cardiovascular health.

Role of Generative AI

Liverpool Hospital and Microsoft have partnered to develop a GenAI solution aimed at supporting cardiologists in navigating the extensive body of cardiology literature and guidelines, thereby enhancing decision-making in clinical settings (Hospital and Healthcare, 2023), (Health–Europe, T. L. R., 2023). Moreover, according to a joint report between Microsoft and the Tech Council of Australia, GenAI represents a substantial economic opportunity for Australia, with a potential contribution of $115 Billion annually to Australia's economy by 2030. And with the healthcare sector's total economic opportunity $5-13 Billion. GenAI can produce synthetic data, including medical images, which can enhance training datasets and foster the creation of varied datasets for both research and medical education purposes. Moreover, synthetic medical data offers a solution to challenges related to data scarcity and privacy.
GenAI facilitates the analysis of physiological data collected from astronauts during space missions, enabling researchers to better understand the impact of space travel and microgravity on cardiovascular variability. Additionally, GenAI can generate synthetic data to augment datasets, addressing issues of limited data availability from astronauts. This innovative approach not only enhances our comprehension of cardiovascular variability in space but also contributes to the development of tailored interventions and preventive measures to safeguard astronaut health during extended space missions.

Role of Digital Twins

Digital twins play a pivotal role in enhancing our understanding and management of the physiological effects of lunar microgravity on astronauts (Boyer, L. et al, 2023), (Piñal, O., & Arguelles, A., 2024). After the collection of data, digital twins serve several critical functions. Typically, Digital twins create highly accurate and dynamic models of the human cardiovascular system, allowing for the simulation of the body's response to lunar microgravity. These simulations can predict how various cardiovascular metrics might change over time in response to the moon's environment. They enable the testing of different scenarios, including varying levels of physical activity, radiation exposure, and dietary changes, to assess potential impacts on cardiovascular health. By incorporating specific astronaut data, digital twins can model individual responses to microgravity, allowing for personalized health monitoring and intervention strategies. Additionally, continuous comparison of real-time physiological data against digital twin predictions can help in the early detection of adverse changes in an astronaut's cardiovascular health, facilitating timely medical interventions.

Moving forward, digital twins allow for the virtual testing of countermeasures (such as exercise regimens, dietary adjustments, and medication) to mitigate the negative effects of microgravity on cardiovascular health without risking astronaut health. Moreover, through iterative simulations, the most effective and efficient countermeasures can be identified and optimized for individual astronauts or specific mission profiles. By simulating cardiovascular responses to various mission scenarios, digital twins assist in mission planning and the development of guidelines for maintaining astronaut cardiovascular health. They provide a tool for educating astronauts about potential cardiovascular risks and the effectiveness of countermeasures, using personalized data to enhance training programs.

Finally, digital twins contribute to a deeper understanding of the underlying mechanisms by which microgravity affects the cardiovascular system, guiding future research directions. They offer insights into the long-term effects of lunar missions on cardiovascular health, informing post-mission care and future mission planning.

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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 (Afshinnekoo E. et al., 2020). Chronic low-grade inflammation (LGI) is connected to these alterations and it is also a distinctive feature of major chronic diseases.

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 operative 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.

RESULTS

The results that will be presented have been obtained by assessing samples collected from submariners of the Italian Navy before, during and after some submarine missions of different length.

The outcomes of this projects are expected to have an impact on the ability to plan effective countermeasures, medical care procedures, diagnostic and therapeutic strategies for future interplanetary missions.

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Physiological methods for the prevention of monotony in interplanetary spaceflight

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INTRODDUCTION

The Russian system of prevention of negative effects of weightlessness on gravity-dependent body systems is based on physical training, where daily training on a treadmill is supplemented by a second training on an exercise bike or ARED strength-training machine, alternating every other day. When planning ultra-long duration missions and missions to Mars, it is necessary not only to ensure the prevention of negative effects of weightlessness, but also to avoid monotony in the implementation of preventive measures. To solve this problem, it is necessary to determine the effectiveness of each measure, i.e. the contribution of each measure to the preservation of functional reserves of the organism during weightlessness. It is important to assess the possibility of safe replacement of training using one countermeasure by another. The solution of these problems will allow to create a design of prophylaxis system for ultra-long flights avoiding the phenomenon of monotony. The first step in solving these problems was made in the implementation of a ground-based experiment with 520-day isolation and research is currently ongoing in an experiment with one-year isolation. The most important results were obtained in the conditions of the ISS in the framework of the space experiment "Prophylaktika-2", where the purpose of the study was to evaluate the effectiveness of experimental regimes of preventive measures in the conditions of long and ultra-long space flights (SF).

METHODS

Periods of physical training according to on-board documentation during the flight alternated with experimental periods. The first experimental period (2VELO) included days with two training sessions on the exercise bike and days with training on the exercise bike and training on the ARED strength training machine. The 2CSU experimental period alternated between days with 2 workouts with the support unloading compensator (SCU) and days with training with the SCU and training with the ARED. The effectiveness of the experimental periods was determined using a loading test on a treadmill in passive web running mode (MO-3). The test consisted of periods of walking (3 min), slow, medium, and fast running and walking. The total duration of the test was 11 min, the speed of locomotion at each stage of the test was chosen randomly according to the cosmonaut's well-being. Under SF conditions, an axial load of 60-70% of the cosmonaut's body weight was created using a special training-loading suit attached to the treadmill with carabiners. The duration of the experimental training periods was 3 and 6 days in semi-annual flights and 13 days in an extra-long flight.

Changes in heart rate (HR) and surface electromyogram (sEMG) recordings of the thigh and lower leg muscles were assessed. Based on HR, the physiological cost of loading was determined, which is calculated as the ratio of HR difference to the speed of locomotion multiplied by the value of axial load. sEMG was obtained using a Myograph device (Biophyspribor, Russia) from the soleus muscle (Sol). Linear (amplitude, frequency and stride length) and nonlinear (entropy, kurtosis, etc.) parameters were calculated from EMG recordings. Nonlinear parameters allow us to assess the predictability of the EMG signal, as well as to determine the degree of determinism of chaos.

RESULTS

After the experimental period of 2CSU, the physiological cost of the load at the steps of medium and fast running in the MO-3 test was increased (n=8, p<0.05). The speed of locomotion after the period of training with CSU was decreased in the steps of fast and slow running, and on the contrary, it was increased in the step of final walking.

When sEMG was analysed after the 2CSU period, mean amplitude during walking, slow and medium running increased after the training period, while mean velocity did not increase significantly in each of the test steps. Kurtosis decreased after a period of training with the 2CSU in all phases of the test. The kurtosis index approached the value of 3, indicating an increase in the synchronisation of the muscle units. Synchronisation was also indicated by a decrease in entropy during the walking and slow running phases.

Experimental training period 2VELO contributed to a decrease in the physiological cost of the load in the slow, medium and fast running stages (n=10, p<0.05) However, this training regimen did not result in a significant change in mean locomotion speed, distance, pulse sum of work and recovery.

CONCLUSIONS

Thus, we can conclude that in an ultra-long space flight it is possible to replace training on a treadmill with training on an exercise bike for up to 13 days without a significant decrease in functional reserves.

The replacement of training on a treadmill by training with a support unloading compensator leads to an increase in the physiological value of the load and an increase in the synchronisation of muscle units when running on a treadmill. CSU can be used as an additional countermeasure, but in the mode of twice-daily training for 30 minutes cannot serve as a full-fledged replacement of training on the treadmill and on the exercise bike.

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

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The ICE Cubes Service Media Set represents a state-of-the-art platform to communicate and interact, from ground, with the International Space Station (ISS) and/or its crew. Built around a GoPro camera, the ICE Cubes Media Set is connected to the ICE Cubes Facility on board the ISS, in the Columbus module of ESA.

A number of activities can be addressed through this setup, including public events, educational sessions, media interviews, inspiration and outreach, as well as private conversations with family or medical staff, serving also health-related R&D and applications. So far, the Media Set has been used for more than 80 events on-board the ISS during the commercial Axiom missions.

The ICE Cubes Service is currently designing and developing an upgraded version of the Media Set that will enable the acquisition of video and audio data from every module of the USOS segment of the ISS, in both full HD and 4K resolution. In-situ real-time processing will be possible for full HD, while 4K resolution will undergo post-processing, still in-situ. The Media Set Mk2 will also enable ISS crew members to engage in self-visualization through its touch screen, providing real-time video feeds from the ground as well.

The ultimate goal of the ICE Cubes Media Set Mk2 is to offer a comprehensive solution for information integration and data analysis. Coupled with domain-specific algorithms or a combination of these, researchers and practitioners in the field of affective computing in healthcare will be able to use the Media Set Mk2 to recognize, interpret, and understand aspects of health-related challenges, and ultimately diagnose medical conditions.

This innovative system will harness the power of multi-modal data processing to provide a holistic understanding of an individual's physiological and psychological well-being. It will enable the deployment of state-of-the-art computer vision algorithms and on-board AI capabilities to interpret facial cues, offering insights into emotional states, stress levels, and potential neurological conditions. Simultaneously, the system will allow for the utilization of advanced audio processing techniques to analyze vocal timbre, enabling the detection of anomalies related to speech patterns and potential indications of respiratory or cardiovascular issues. Furthermore, the system will also enable the analysis of body movement gaits, pace, and patterns, adding a dynamic dimension to health assessment. By leveraging sophisticated motion analysis algorithms, the ICE Cubes Media Set Mk2 could also be used for identifying irregularities in walking patterns, aiding in the detection of signs of musculoskeletal disorders, and contributing to the early detection of neurological conditions.

The integration of these diverse modalities will allow for a comprehensive and non-invasive health assessment, making the ICE Cubes Media Set Mk2, in combination with the on-board AI capabilities, a valuable tool for healthcare professionals. The system's diagnostic capabilities will extend beyond traditional methods, offering a more nuanced and personalized approach to health monitoring. This can support research in both physiology and psychology, and could also be used to enhance decision-making, and provide actionable insights. The ICE Cubes Media Set Mk2 represents an added value for supporting human health measurements on-board the ISS, and has the potential to serve as a valuable asset in advancing the development of applications and knowledge related to human health in space. This holds significance not only for forthcoming commercial space stations, but also for planetary surface outposts, particularly as we anticipate future long-term human exploration missions into deep space.

As technology continues to advance, the ICE Cubes Media Set Mk2 stands at the forefront of innovative solutions for proactive health management and early disease detection, improving patient care and enhancing doctor-patient communication.

Support of a Crew Activity with the Crew Interactive Mobile Companion (CIMON)

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BACKGROUND

The increase in workload and stress during long- duration space missions beyond low Earth orbit (LEO), along with limited ground support, highlights the need for autonomous technology advancements. Though robotic autonomy shows potential in mitigating stress and reducing workload, the demand for such support remains unmet. Previous efforts to deploy robots in space faced challenges, mostly due to technology issues. Ongoing advancements in machine learning promise intelligent, autonomous assistance systems as seen in healthcare applications.

METHODS

The Crew Interactive Mobile Companion (CIMON) is the first autonomous robot operating on the International Space Station (ISS) equipped with a ground-based artificial intelligence (AI) based on the IBM Watson system. Developed by Airbus Defense and Space, Friedrichshafen, Germany and owned by the German Aerospace Center (DLR), has been designed with the primary objective to assist astronauts in their daily tasks (DLR, 2018). Its spherical design features a front display, microphones, loudspeaker, ultrasonic sensors, cameras and a propulsion system for autonomous navigation and interaction with Crew. CIMON establishes WiFi connection to the ground station situated at the European Space Agency's User Support and Operations Centre (ESA USOC) in Lucerne, Switzerland as well as the IBM Bluemix cloud. The data link facilitates access to AI resources including neural networks for learning. CIMON is also trained in casual conversation and provides a wide range of social interactions. Commissioned on the ISS in 2018, the software and hardware have undergone further developments on board together with Crew input. We now report data from the first end-to end CIMON guided crew support procedure, involving the use of audio-visual instructions to execute a trained task, real-time video documentation and the evaluation of user experience in form of a spoken questionnaire. All studies were carried out according to the ethical code of the World Medical Association (Declaration of Helsinki). Ethical approval was obtained from the institutional review board of the LMU Munich, Germany and the medical boards of all participating Space agencies. Funding of the project was provided by DLR on behalf of the Federal Ministry of Economics and Technology/Energy (50WB2222, 50RP2260B).

RESULTS AND DISCUSSION

Following software updates and functionality checks, the subject was assigned to execute the "3D Kinetic Theory of Gases Model," a physics experiment from the Educational Payload

Operations (EPO) catalog, visually illustrating random particle motion and collision. The task involved shaking a box of metal balls in a specific manner to simulate particle motion. CIMON was trained to aid in this task with audio-visual tutorials on operational steps and scientific background. In the initial attempt, the subject followed written crew procedures without viewing the tutorials, resulting in incorrect execution, as evidenced by video documentation. In a subsequent attempt after viewing the audio-visual instructions, the experiment was nearly flawless. Notably, despite lacking prior training, the subject demonstrated improved performance with audio-visual instructions compared to written procedures. CIMON facilitated real-time video documentation and voice-controlled navigation at least once per scenario. Casual conversation was seamlessly conducted according to the subject's interests and preferences. User feedback collection via a spoken questionnaire was successful for yes/no questions as well as open-ended questions where the subject could provide input. The responses showed a generally positive experience and highlighted preferences for improvements in the voice-user interface. Multi-step procedures and science experiments may benefit from a free-flying crew support unit for training, documentation, and evaluation. Audio-visual instructions show promise in enhancing task training compared to written instructions, though further studies are warranted to validate this. Involving end users during the development phase of intelligent robotic assist devices ensures a user-centric design and fosters trust and acceptance.

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

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INTRODUCTION

The advancement of commercial space activities with players such as Space X, Virgin Galactic, and Blue Origin, and the announcement of deep space missions, including extraterrestrial human settlements on the Moon and Mars, demand several challenges to be addressed and technological advancements to be developed. As the distance between the target destination and Earth increases, beyond the Lower Earth Orbit (LEO), where the International Space Station (ISS) and other satellite space activities have been successfully explored and established, it becomes necessary that deep space missions become self-reliant and self-sustaining, especially with respect to medical treatment and medicine development technologies. In space, conditions like microgravity and radiation can have a direct influential effect on physiological state resulting in loss of muscle and bone mass. The immediate effect can be observed in the skin, muscles, bones, cartilage, and even organs such as the heart. Skin is the most exposed tissue comparatively and with the impairment of wound healing capacity in space conditions, it carries the most risk of injury and damage during spaceflight. The biological effects of the space environment include cell differentiation, regulation of genes, and tissue homeostasis due to hampering in mechanical stimulation and flow of blood.

A combination of regenerative medicine, advanced surgical instruments, and three-dimensional (3D) bioprinting are indispensable factors in developing autonomous deep space missions and extraterrestrial human settlements. The availability of 3D bioprinting techniques in space missions is expected to support the study of the space effect of cells by enabling the development of 3D tissue models, which are complex and multicellular in nature. Techniques such as extrusion-based and magnetic-based bioprinting are already tested on the ISS and improvements in such technologies are touted as a solution to cases of injury or diseases in astronauts, during long-duration space missions. The potential investment in 3D printing technologies on ISS experiment missions is visible, and some promising National Aeronautics and Space Administration (NASA) missions include the Redwire Regolith Print (RRP) mission, intending to explore the use of lunar material for sustainable manufacturing in space, related to the Artemis moon mission and the BioFabrication Facility (BFF) developed by Techshot company and shipped to ISS by NASA (Van Ombergen, A et al. 2023). It is to be noted that tissues that have been successfully bioprinted and studied in the microgravity conditions of the ISS include meniscus, skin tissue for wound healing purposes, cardiac tissues as well as some tumor models. The successful fabrication of complex bioprinted tissue models can aid in the successful treatment of microgravity-induced health deterioration and health risks in astronauts, with customized bioscaffolds and reduced risk of immunity rejection.

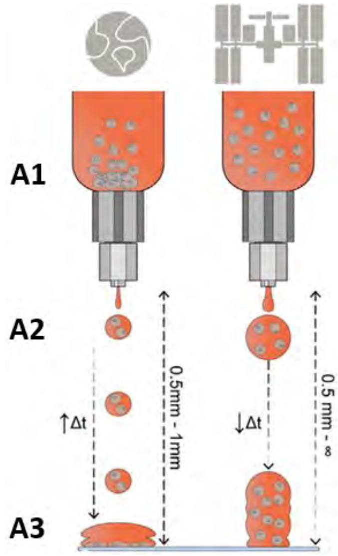

Figure 1. Illustration of differences in bioprinting characteristics in cell dispersion, printed droplet shape, and at the construct level for the cases on the Earth and the microgravity conditions at ISS.(Adapted with permission from Van Ombergen, A et al, 2023)

ADVANTAGES AND CHALLENGES

Bioprinting in microgravity can induce the development of complex tissue constructs with more well-defined fluid channels, which can aid in fabricating complex geometries and promoting vascularization. This enables the study of the lesser-explored pathophysiology effects at the cellular level. Bioprinted tissue models and organ-on-a-chip models are proven to be effective tools in studying tissue physiology, development of space medicine, and studying sites for automation in cell culture.

The positive influence of space conditions in improving the regenerative medicine application capacity of boundary cap neural crest stem cells (BCs) has been studied (Han, Y. et al 2021) by analyzing the gene expression of the BCs taken to the space region close to Earth and a control sample with a simulated microgravity environment in a random positioning machine (RPM) on Earth. The postflight effect on the BCs was evident in the activities of the genes related to survival and proliferation for the space-traveled BCs, whereas the ones in simulated microgravity related to inflammation and differentiation activities. Another area of investigation using bioprinting on the ISS is food production, where bovine muscle cells were printed and cultured with the support of a food-tech start-up, Aleph Farms (Van Ombergen, A. et al, 2023). Therefore, it can act as a food source for astronauts in long-duration space missions, which adds to its sustainability value. A handheld in-situ Bioprint FirstAid device (Van Ombergen, A et al, 2023), developed by the German Space Agency was tested on the ISS which enables the in-situ printing of the tissue as dressings and faster wound healing, by eliminating the need to cultivate and ensuring cell maturation.

Table 1. Role of 3D *Bioprinting Techniques in Microgravity*

Advantages	Challenges	Potential Solutions
• 3D constructs printed in space closest to native tissues, biocompatible, promote angiogenesis, and biodegradable. • Can be personalized based on the size and shape of any implant/injury site.	• Complex fluid dynamics phenomenon involved in the bioprinting process. • The nature of the shear stress on the cells, shape, and characteristics of the encapsulating droplet are affected by the microgravity conditions (as shown in Figure 1).	• Effect of microgravity on working mechanism of printers needs to be studied, variables quantified, and systematically optimized.
• Improved print fidelity of soft issues compared to Earth, as there is no requirement of high viscosity bioink to resist the gravitational force.	• Microgravity shown to adversely affect Extracellular Matrix (ECM) production and cell migration (such as fibroblast in wound healing).	• Need to establish protocols for cell maturation and material preparation of biomimetic bioink on-board to be developed.
• Low-viscosity hydrogel can be used (impossible on Earth) to form large-size droplets governed only by surface tension with uniform cell suspension possible.	• Unpredictable droplet behavior post printing printing. Liquids free to move in microgravity until surface impact. • Lack of buoyancy and cells may not reach the outer surface for adherence once ink reaches target.	• Proper study and preparation required before initiation of any process. • On-ground simulation using clinostats, rotary wall vessels, and random positioning machines are mandatory.
• No force of weight acting, thereby enabling bioink to have higher yield stress, easier to stack up layers for larger parts, and enforcing less shear stress on cells.	• Microgravity enforces slower motion of liquids, producing slower extrusion rates with higher chances of nozzle clogging. • Liquid drops in microgravity always tend to form a spherical shape, thereby introducing chances of wettability issues.	• Integration of printed constructs with systems such as microfluidic chips can aid in controlling the convective flow rate as well as maintaining suitable physiological environment for the cells.
• Easily scalable method, and ideal for setting up extraterrestrial human	• During space mission planning, the limited payload capacity, restrictions on storage	• Cryopreservation and leakproof designs improve cell longevity.

settlements or orbiting microgravity laboratories.	environment, closed system design, and on-site recycling are challenges in establishing a sustainable bioprinting solution. • Cargo transport expenses, material planning, autonomy for cell handling processes, and adherence to ethical regulations are also mandatory steps in full-scale application of bioprinted constructs.	• Biopolymers based on plants/ algae/ human blood constituents being utilized to produce bioinks. • Potential for automation has been proven in the online monitoring of astronauts' bodies and remote-control systems using Machine Learning (ML) techniques.

CONCLUSION AND FUTURE PRESPECTIVES

Bone degradation and radiation exposure from cosmic rays as well as solar flare particles are some of the common risks to astronauts in space missions. Bioprinting can act as a bridge to fill in the current knowledge gaps and enable first-hand experience in fabricating cell complex cell constructs while accounting for the respective influential factors. In one of the earlier studies (Domnin, P.A. et al. 2022), specialized bio-assembler equipment was utilized on the ISS to apply a magnetic field, based on the principle of magnetic levitation, and it was recorded how the magnetic force influenced the morphology and physiological behavior of an Escherichia coli probiotic strain M17 bacteria at a cellular level. The potential of the magnetic field in controlling cell response such as enzyme secretion and clustering was recorded. The integration of microfluidics with bioprinting has also been demonstrated (Silvani G. et al, 2021) to inhibit the blood-body barrier (BBB) functions and mechanics of a Glioblastoma multiforme (GBM)-based vascularized brain tumor model and this is a positive step in tumor therapies not only in space but on Earth itself. Another in-orbit bioprinting experiment (Mo, X. et al,2023) has successfully fabricated a viable and stable tumor model based on a microgel-based bi-phasic thermosensitive (MBT) bioink and intelligent machine learning-based control algorithms. Extensive research and analysis are required to countermeasure the adverse effects of space flight conditions and realize the full potential of bioprinted constructs, and the successful outcomes of the above-discussed advanced developments enforce the candidacy of bioprinting as a potential solution for medical treatments in long-term space expeditions, including extraterrestrial human settlements.

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

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This study explores the utility of hypergravity as a novel tool to induce reliable phenotypic changes in wheat (UAS-375 genotype), aiming at improving crop performance. In the first phase of experimentation, bread wheat subjected to hypergravity treatment (10g for 12 h) resulted in significant enhancement in root length and seedling vigor through the vegetative phase. Additionally, elevated total chlorophyll content suggested physiological benefits for wheat growth in response to hypergravity. To investigate the translational implication of these results, in the current study, wheat seeds were exposed to hypergravity and subsequently subjected to induced drought and salt stress at the seedling stage. The resultant data revealed significant improvement in shoot length, root length, total seedling length, and seedling vigor index compared to control. Further, a significant increase in the total chlorophyll content and proline levels in response to hypergravity suggests stress tolerance among hypergravity-treated seedlings. More importantly, hypergravity-induced robust changes in endogenous levels of 3-Indole acetic acid, Indole-3-butyric acid, and Abscisic acid (ABA) directly correlated with the root growth phenotype, and stress tolerance. These findings suggest that hypergravity seedlings are better equipped to thrive under drought and salt stress conditions, possibly opening avenues for developing better resilient crop varieties. Ongoing field studies should further validate these promising results.

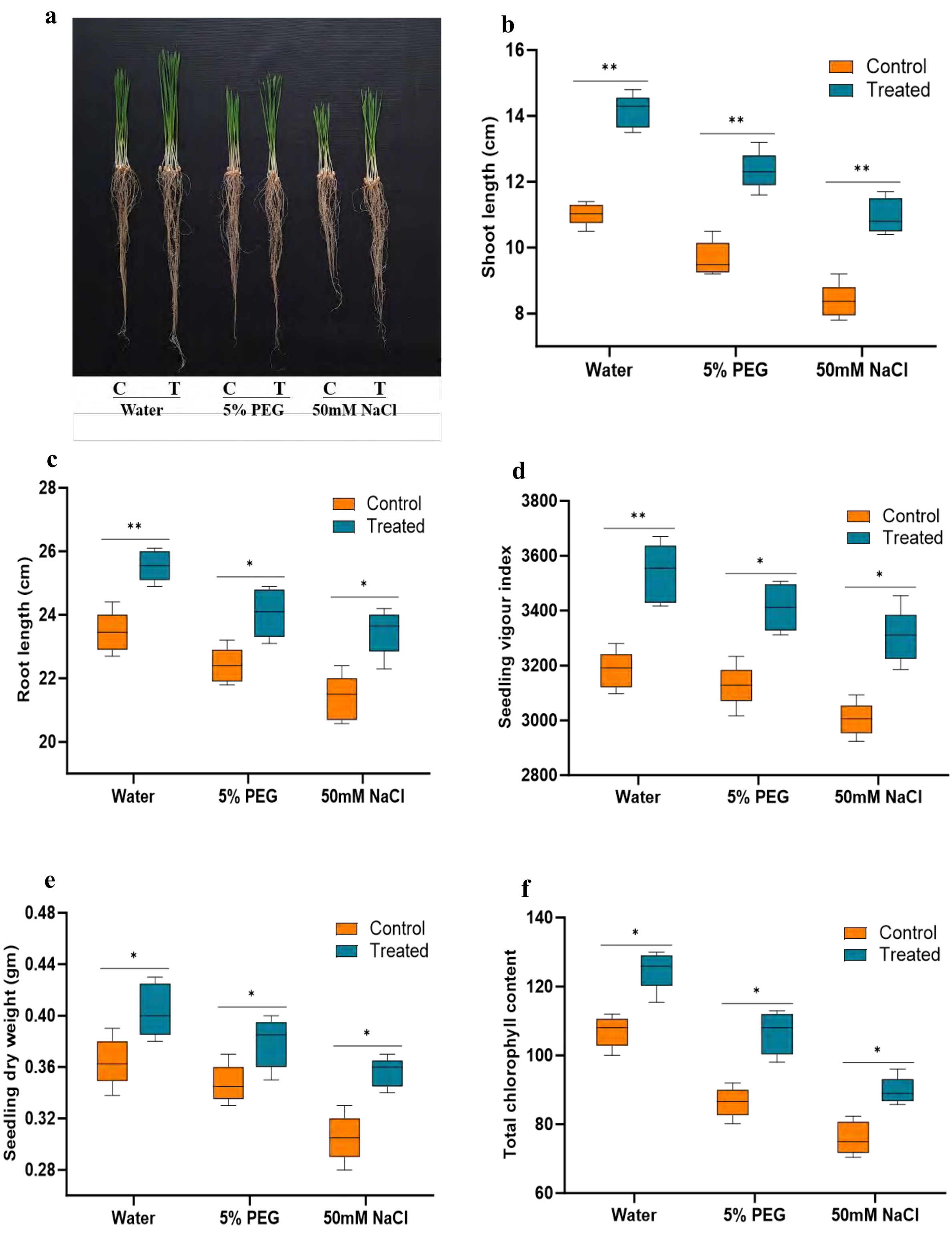

Figure: a Qualitative image of 10×g for 12 h treatment seeds exposed to induced drought (5% PEG) and salt (50mM NaCl) stress enhanced root and shoot length phenotype compared to control seed's seedlings recorded on the final count (7th-day) in laboratory conditions using between-paper-method (C: Control, T: Treatment). Hypergravity-induced changes in **b** seedling length (cm)**, c** root length (cm)**, d** seedling vigour index, **f** seedling dry weight and **g** Total chlorophyll content of wheat seedlings.

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

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AIM OF THE STUDY

The influence of physical fields on a cell, in contrast to various chemical stimuli, is one of the least studied issues in cell biology. Particularly difficult is the study of the interaction of a cell and a gravitational field. Despite a large amount of experimental data indicating various structural and functional changes in cells when gravity changes, there is still no unambiguous answer as to which structure acts as a receptor for changes in the gravitational field. Many works indicate changes in the structure of the cytoskeleton with changes in gravity and even propose to consider it as a whole as a sensor (Ingber D.E. et al., 2014). However, in the evolutionary series, not all cells have a developed network of filaments of different types that permeate the entire cell. But all cells have a cortical cytoskeleton, which supports the cell membrane – the boundary between the cell and the environment and, in fact, determines the shape of the cells. Therefore, it was previously proposed to consider the cortical cytoskeleton as a primary mechanosensor, various deformations of which (depending on an increase or decrease in external mechanical influence) lead to the launch of the corresponding signaling pathways and the formation of an adaptive structural and functional pattern (Ogneva I.V., 2013, 2022).

MATERIALS AND METHODS

The object of the study was dechorionized oocytes of *Drosophila melanogaster*. The choice of object is due to several reasons. The main reason is that dechorionized *Drosophila* oocytes can be exposed, for example, on agar plates in a humid atmosphere to avoid drying out, but not in a liquid, which eliminates artifacts associated with fluid shift. In addition, our previous results indicate that oocytes collected from flies immediately after space flight have structural changes, which are reflected in changes in the mechanical characteristics of the cells (Ogneva I.V. et al., 2022). *Drosophila melanogaster* oocytes were exposed to simulated 0g and 2g conditions for 30, 90 and 210 minutes without and with 2 μM cytochalasin B, 10 μM colchicine and 40 mM acrylamide. Cell stiffness and protein content were measured using atomic force microscopy in membrane and cytoplasmic fractions using Western blotting.

RESULTS AND DISCUSSION

The linear dimensions and area of the maximum frontal cross-section of oocytes were used as a marker for the occurrence of cell deformation. After 30 minutes, this area decreases in simulated microgravity and increases in hypergravity, which, assuming constant volume, indicates that the cells become more spherical or flattened, respectively. Therefore, one could expect a change in the structure of the cortical cytoskeleton of oocytes already at the early stages of exposure. For an integral assessment of the mechanical structure, cell stiffness, which is determined by the cytoskeleton, was used. Measurements carried out using atomic force microscopy showed that under simulated microgravity conditions, stiffness decreases after 90 minutes, under 2g conditions – after 30 minutes, which may indicate a change in the structure of the cytoskeleton. However, in the case of simulated microgravity, after 210 minutes the stiffness remains reduced, and in the case of hypergravity, it is restored to the control level.

To find out the reason for the decrease in stiffness, studies were carried out using disrupting agents, as well as the content of cytoskeletal proteins in the membrane and cytoplasmic fractions separately. Surprisingly, pre-incubation with disrupting microfilaments cytochalasin B did not lead to a change in stiffness relative to the group without incubation and did not affect the dynamics of changes in stiffness under conditions of simulated micro- and hypergravity. Also, there were no changes in the actin content in the membrane protein fraction, which determines the structure of the cortical cytoskeleton and cell stiffness, in the sμg and hg groups. At the same time, pre-incubation with disrupting microtubules colchicine did not change the onset time and level of hardness reduction, but prevented its recovery in the hg group after 210 minutes. This may indicate that microtubules are necessary to restore the structure of the cytoskeleton, but it is not this component that determines the mechanical characteristics of *Drosophila melanogaster* oocytes. This assumption is supported by data on the content of alpha-tubulin and its acetylated form as a marker of stable microtubules in the membrane fraction of proteins. Pre-incubation with acrylamide, which specifically disrupts intermediate filaments, leads to a decrease in stiffness in the control group to the same level as during exposure to simulated micro- and hypergravity. Moreover, in the dynamics of sμg and hg, the stiffness does not change and remains as reduced as at the beginning of the exposure. Recent evidence shows that Drosophila has cytoplasmic intermediate filaments formed by the Tm1-I/C protein (Cho A. et al., 2016; Sysoev V.O. et al., 2020). Indeed, the dynamics of changes in the Tm1 content in the membrane fraction of proteins in the sμg and hg groups correlates with changes in oocyte stiffness: it decreases after 90 minutes in the sμg group and remains reduced until 210 minutes, in the hg group it drops after 30 minutes and remains reduced after 90 minutes, but is restored to control levels after 210 minutes. Thus, the stiffness of *Drosophila melanogaster* oocytes is determined by intermediate filaments. The decrease in stiffness in the sμg and hg groups in both cases is associated with the destruction of intermediate filaments, the restoration of which requires microtubules. However, different dynamics of stiffness indicate the triggering of different signaling pathways in the case of simulated micro- and hypergravity. However, the question remains: how does a single cell perceive differences in changes in external mechanical stress?

Previously, we hypothesized that an increase or decrease in external mechanical stress leads to compressive or tensile deformation of the cortical cytoskeleton, leading to the dissociation of various proteins from it and the subsequent launch of various signaling pathways (Ogneva I.V., 2013, 2022), which in this study is confirmed by a decrease and an increase in the cross-sectional area of oocytes under simulated micro- and hypergravity, respectively. Given the stiffness data, proteins that interact with both intermediate filaments and microfilaments, such as fascin, alpha-actinin, and spectrin, may be involved in the mechanosensitivity of *Drosophila melanogaster* oocytes. The Singed protein, which is a homologue of fascin, a protein that binds microfilaments into longitudinal bundles, under simulated microgravity conditions decreases in the membrane fraction after 30 minutes and migrates to the cytoplasmic fraction, where its content increases after 90 minutes of exposure. Moreover, in hypergravity its content in the membrane fraction remains intact, and in the cytoplasmic fraction it increases only after 210 minutes. Alpha-actinin, which organizes microfilaments into another type of structure – loose networks, as well as spectrin, migrates from the membrane fraction to the cytoplasmic fraction after 30 minutes under hypergravity conditions, but remains intact under microgravity conditions. Thus, in response to simulated microgravity, a protein that organizes longitudinal filament bundles migrates from the cortical cytoskeleton of *Drosophila melanogaster* oocytes; in response to hypergravity, proteins that organize filament networks migrate. Such a difference may explain mechanosensitivity and the formation of different adaptive patterns of structure under conditions of simulated microgravity compared to hypergravity (Ogneva I.V., 2023).

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Title:

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

Abstract:

Space exploration poses a critical challenge to humanity. Human beings are not evolved to cope with the harsh conditions of microgravity. Numerous physiological, cellular and genetic pathologies are caused by microgravity. With the onset of commercial spaceflight driven by space agencies and global corporations, the need to understand and mitigate these risks for the amateur space traveler is more important than ever.

The human circadian rhythm is subject to change due to variable environmental cues such as gravity changes and high cortisol levels. The environmental cues during spaceflight differ dramatically from those on Earth, and thus they impose many critical adaptive challenges, including those for the human circadian clock. Altered circadian rhythms have been observed across many organisms in space conditions, and circadian misalignment leads to adverse impacts on adaptation, performance, and health. Much is still to be elucidated behind the mechanisms of how our sleep circuitry is affected by the effects of gravitational stresses.

Mouse embryonic fibroblasts (MEF) are an example of peripheral oscillators that undergo circadian rhythm under the influence of the principal oscillator, the suprachiasmatic nucleus (SCN). Here, we conducted an in vitro study on isolated cultured MEF cells to determine how expression of core clock genes, integral to driving biological rhythms, change under short-term simulated microgravity conditions. We targeted the clock genes; *Per1*, *Per2*, and *Bmal1*, and investigated their responses to these conditions across 3 days. Within 24 hours, the sinusoidal diurnal rhythmic expression of cells exposed to the same simulated microgravity conditions was compared to that of control samples in 1G. Hydrocortisone was also injected into the cultured mediums to better simulate the high cortisol level environment.

Significant changes were observed across all targeted clock genes across 72 hours, reinforcing the theory that microgravity plays a role in how mechanical forces affect the clock gene expression in vitro, independent of the external light-dark cycle. Our understanding of how the space environment affects our circadian clock and sleep performance is critical to space medical management, ensuring the safety of future astronauts and missions.

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

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ABSTRACT

It is very well established that microgravity environment at space flights or the International Space Station (ISS) affects human organs' normal functions and can cause degenerative diseases. Moreover, it has been reported that oral tissues normal functions are also affected by the microgravity and this could be the reason of reported cases of barodontalgia, periodontitis, alveolar bone loss and facial pain, suffered by astronauts or health individuals in a microgravity environment. Although these dental conditions can be prevented through pre-flight strict dental screening and oral hygiene routines for all astronauts, dental emergencies can occur. Furthermore, dental diseases can develop during mission, especially during extended travel time, mainly due to reduced salivary secretion and changes in bacterial virulence. With potential future extended stays in the space, limited dental expertise of crew medical officers and the absence of on-site dental professionals, it is very crucial to develop innovative preventive and treatment dental modalities that are tailored for astronauts in a microgravity environment. To develop these modalities, studies have to be conducted to understand and investigate the effect of microgravity environment on the normal biological and physiological functions of oral and dental tissues. However, the challenge of perfectly replicating microgravity environment on ground and the difficulty of conducting clinical studies on missions or at the ISS, represent an important hurdle limiting oral health scientists to conduct dental related projects.

This presentation will discuss these challenges, and the potential of dental/oral tissues derived stem cells (DSCs) and cranial neural crest derived mesenchymal stem cells (NC-MSCs) combined with tissue engineering stratigies as an alternative model to study oral and dental tissues development, normal functions and disease processes in microgravity environment. The presenter will also share recent efforts of investigators in the UAE to develop a research that focuses on oral health in the space.

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

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Introduction

The mammary gland is a dynamic organ that undergoes major cellular and molecular transformation during pregnancy. Our previous studies demonstrated that alteration in gravity-load disrupted metabolic homeostasis, endocrine axes, and circadian function in the mammary gland[1-5]. However, there is limited data on the effect of microgravity exposure on genes associated with cellular turnover and oxidative stress on a pregnant rat mammary gland.

Methods

Microarray (Rat 230 2.0 GeneChip® Array) analysis was used to compare the expression pattern of genes linked to proliferation, apoptosis, angiogenesis, and oxidative stress between pregnant Sprague-Dawley rats (n=4) exposed to microgravity (Space Shuttle, STS-70) and ground-based control group of pregnant (n=4) rats. TaqMan® Assays-on-Demand kits were utilized to validate microarray data.

Results

Functional analysis revealed that genes associated with proliferation (CCNB1, Ki67, NUF2, PTTG1, TYMS), apoptosis (BIRC5, FAS, TRAF1, TNF, NFKB1), angiogenesis (VEGFR1, CD93, NRP2, TIE2, Ephrin) and oxidative stress (CYGB, DHCR, NUDT1, PTGS, SCARA3) were downregulated ($P < 0.05$) in the treatment group. While CDH1, PIK3C2A (proliferation), TRAIL (apoptosis), EphA3, EphB4 (Angiogenesis), and BNIP3, LPO, SFTPD (Oxidative stress) genes were upregulated ($P < 0.05$) in rats exposed to microgravity.

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, our results demonstrate that exposure to microgravity alters expression of genes associated with redox homeostasis which is indispensable for cell proliferation and differentiation. (Supported by NASA Grant NCC2-2870 to Prof. Karen Plaut)

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

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Abstract

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. 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. 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. Here, we show that 24h exposure to rotary cell culture system (RCCS)-simulated microgravity rearranged SPM receptors both at the gene and protein level, in primary human monocytes. In particular, simulated weightlessness led to significant gene and protein upregulation of SPM receptors GPR32 and GPR18. We also found that microgravity significantly decreased the expression of 5-LOX – one of the three main enzymes responsible for the biosynthesis of SPMs – while significantly increasing its activity as previously observed aboard the International Space Station (Battista et al., 2012). Overall, RCCS treatment led to a significant reduction of the biosynthesis of resolvin (Rv) D1, a prominent SPM. Remarkably, both monocytes and lymphocytes displayed reduced levels of pro- and anti-inflammatory cytokines upon microgravity exposure, yet remodelling of SPM elements occurred exclusively in monocytes. These findings, summarized in Figure 1, strongly suggest that not only microgravity can impair the functioning of immune cells at the level of bioactive lipids directly involved in proper inflammation, but it does so in a cell-specific manner, possibly perturbing immune homeostasis with monocytes being primary targets (Leuti et al., 2024).

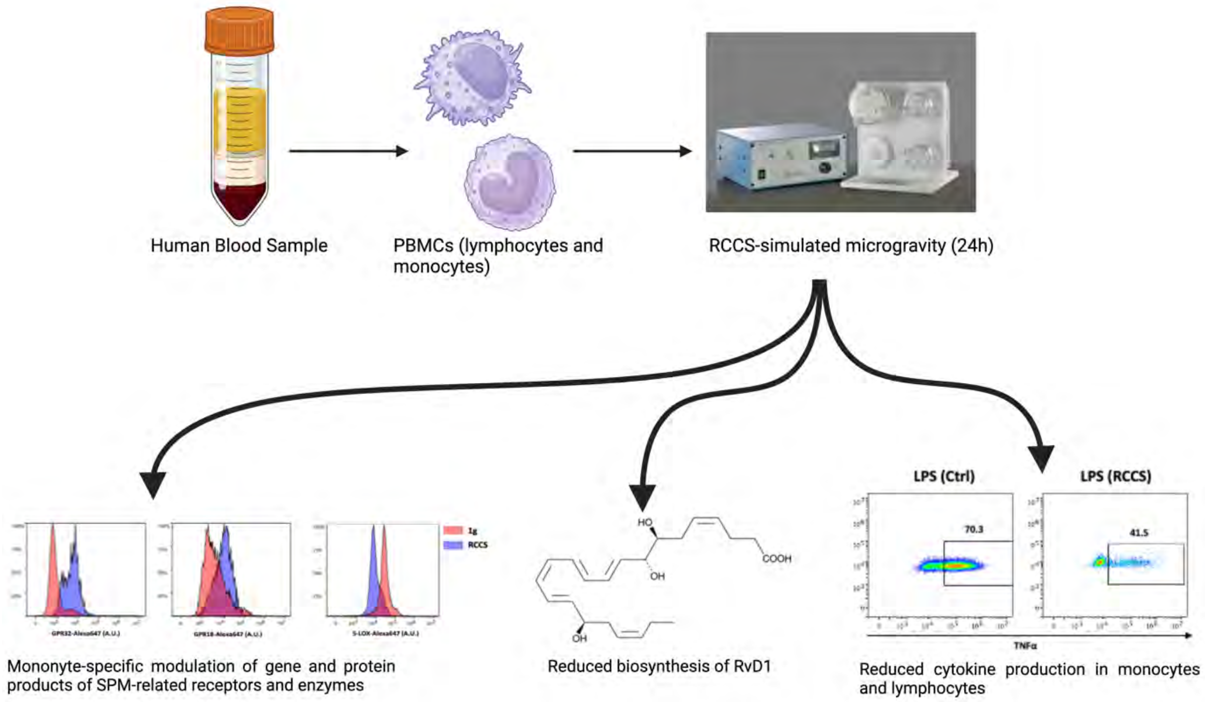

FIGURE 1

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

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INTRODUCTION: Microgravity and prolonged bed rest conditions both induce several abnormalities such as skeletal muscle mass loss and weakness, as well as cardiovascular deconditioning. In addition, dysbiosis of the gut microbiota has also been reported; however, the implications of the gut microbiota dysbiosis and alterations in its secreted metabolites in microgravity disorders such as cardiovascular and muscle deconditioning remain unknown. Our aim is to determine the mechanisms of cardiovascular and muscle deconditioning in microgravity and explore strategies to prevent and treat cardiovascular and muscle dysfunction in space travel as well as prolonged bed rest here on Earth.

METHODOLOGY: In this study, we used a hind limb unloading (HU) C57/Bl6 mice mouse model that mimics microgravity condition in space and we randomly divided them into ground-based controls, three weeks unloaded mice (HU), and a group that was unloaded for three weeks followed by two weeks recovery (HUR). Briefly, we measured the abnormalities in structure and functions of cardiovascular, skeletal muscle as well as gut microbiota in HU mice and their reverse in HUR mice using metagenomics and metabolomics for gut microbiota, organs histological staining, tail-cuff blood pressure, echocardiography, and muscle strength.

RESULTS: The effect of recovery on the gut microbiota of HU mice: Figure 1a displays the relative abundances of taxa within phyla. Interestingly, the most common phylum was Bacteroidata, however, its relative abundance was highest in the control group followed by HUR and not in HU. The Bacteroidota phylum is well-known for various beneficial species that supply nutrients and vitamins to the host and other gut microbes and their beneficial effect in the cardiovascular system (Yoshida et al., 2020) (Zafar and Saier, 2021).

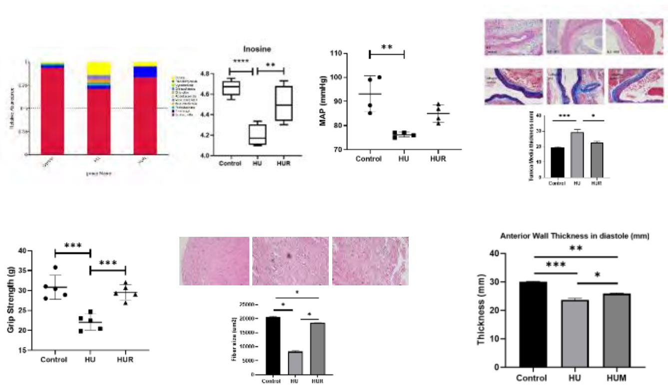
Metabolites alteration in microgravity environment: Metabolomics using Liquid Chromatography-Mass Spectrometry (LC-MS) was done to investigate the molecular mechanisms and the possible altered metabolites that can be used as biomarkers for microgravity as well as potential intervention targets to mitigate the cardiovascular and skeletal muscle deconditioning related to microgravity. We found that numerous metabolites may play a role in changes in cardiovascular and skeletal muscle function in microgravity and that they may also serve as biomarkers to monitor both functions (Ibrahim et al., 2024). Interestingly, among metabolomics results, we found inosine was significantly decreased compared to ground-based controls and HUR (figure 1b). The gastrointestinal tract of humans contains the bacterium Akkermansia muciniphila (A. muciniphila), which secretes inosine that is beneficial to the cardiovascular system. In HU mice, this bacterium is significantly reduced, hence the decrease in this metabolite (Ibrahim et al., 2024)."

Simulated microgravity induces alterations in cardiovascular function and structure: First, we assessed the cardiovascular function using the MC4000 Blood Pressure Analysis System, (Figure 1 c). Results showed a significant reduction in blood pressure of HU mice compared to ground-based controls and the HUR group indicating alterations in cardiovascular following microgravity conditions. Next, we performed hematoxylin and Eosin (H,E) stain and Masson trichrome stain for the aorta tissue of the three groups. Masson trichrome stain showed an increase in collagen (blue color) in HU mice compared to control and HUR mice. Moreover, there is an increase in tunica media thickness of HU mice compared to ground-based controls while HUR mice showed a decrease in tunica media thickness of HUR mice compared to HU mice. An increase in collagen and tunica media both are markers of arterial stiffness.

Simulated microgravity induces alterations in skeletal muscle function and structure: HU mice showed reduced muscle strength compared to both controls and HUR (figure). H,E stained gastrocnemius muscle showed a significant decrease in Fiber size of HU mice compared to ground-based controls, while HUR mice showed a significant increase in fiber size compared to HU mice (figure 1g,h).

Potential beneficial effect of inosine supplement on cardiovascular and skeletal muscle deconditioning: We currently investigating the effect of inosine metabolite known to reduce the oxidative stress in the cardiovascular system of HU mice. Figure 1i shows a very promising result as a supplement with the metabolite inosine (HUM) showed a significant increase in anterior wall thickness in diastole compared to HU mice. Moreover, HU mice showed a significant decrease in thickness compared to ground-based controls which indicate an atrophy in the cardiac muscle, overall heart muscle was also found to be significantly decreased (Ibrahim et al., 2024).

CONCLUSION: Our study reveals a connection between alterations in gut microbiota-secreted metabolites and the cardiovascular and skeletal muscle deconditioning observed in a microgravity-simulated environment in HU mice model. Additionally, our findings suggest potential positive effects of those metabolites, particularly inosine, in alleviating microgravity-induced deconditioning. Collectively, this implies that targeting gut bacteria and its secreted metabolites could offer promising therapeutic interventions to prevent and mitigate cardiovascular and skeletal muscle deconditioning not only for astronauts during space flight but also for individuals undergoing prolonged bed rest here on Earth.



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

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AIM OF THE STUDY

The availability of data on the state of the reproductive system of women before and after space flight is extremely limited, and the heterogeneity of the sample due to different anamnesis does not allow for comparison. The results obtained from ground-based experiments conducted with female subjects are also extremely limited. In our previous experiment lasting 5 days, it was shown that after being females in "dry" immersion, the content of anti-Müllerian hormone does not change, but inhibin B increases, which is produced by granulosa cells and can be a marker of an increase in their number, especially since the size of the antral follicles and dominant follicle increase (Gorbacheva E.Yu. et al., 2023). Also, a decrease in the level of progesterone and luteinizing hormone in the blood was noted, which, in our opinion, could be associated with an increase in the capture of the latter from the bloodstream by an increased number of its receptors due to an increase in the number of granulosa cells, which requires experimental confirmation, which is possible in animal experiments. In connection with the above, the main goal of the work was to test the assumption of an increase in the number of granulosa cells and, accordingly, the number of receptors for luteinizing hormone in the follicles of mice after antiorthostatic suspension during the estrous cycle. Moreover, in the pituitary gland of the same individuals, we determined the content of luteinizing hormone and a number of basic cytoskeletal proteins.

MATERIALS AND METHODS

The experiment was carried out on the ovaries and pituitary glands of BALB/c mice (n = 14). The effects of weightlessness were reproduced using the standard Ilyin-Novikov antiorthostatic suspension model modified by Morey-Holton (Morey-Holton E. et al., 2005). All animals were kept under standard conditions; animals received standard food and water *ad libitum*. Suspension was carried out for 96 hours, which corresponded to a complete estrous cycle. The mice were randomly divided into two groups: control group - C (n = 7, m = 27.9 ± 0.6 g) and hindlimb suspension group - HS (n = 7, m = 27.1 ± 0 .9 g). After euthanasia of the animals, pituitary and ovarian tissues were immediately frozen for subsequent immunohistochemical analysis, Western blotting, and qRT-PCR. All procedures with animals were approved by the Biomedical Ethics Commission of the State Scientific Center of the Russian Federation - Institute of Biomedical Problems of the Russian Academy of Sciences (Protocol No. 521 of September 25, 2019).

RESULTS AND DISCUSSION

After 96 hours of antiorthostatic suspension, the weight of the ovaries of the control and experimental groups did not differ (9.6 ± 0.8 mg vs. 9.0 ± 0.6 mg), but the thickness of the layer of granulosa cells in the antral follicles of the ovaries of mice increased by 54% ($p < 0.05$) in the HS group. A growing oocyte receives nutrients primarily from granulosa cells, an increase in the number of which is a good prognostic criterion regarding its quality (Fan W. et al., 2023). The interaction of the oocyte and the surrounding cells determines the growth of the follicle (Edson M.A. et al., 2009). It can be assumed that the previously put forward assumption about the growth of the dominant follicle after a 5-day "dry" immersion as a result of an increase in granulosa layers becomes more probable (Gorbacheva E.Yu. et al., 2023). In the antral follicles, as they grow, receptors for luteinizing hormone (LH) appear on granulosa cells, under the influence of which ovulation occurs (Yding Andersen C., 2017). At the same time, a moderate increase in the expression of the LH receptor on granulosa cells leads to an increase in the development potential of the future embryo, while an increase of more than 2.5 times reduces this potential (Maman E. et al., 2012).

The data obtained from immunohistochemical analysis indicate that the relative intensity of fluorescence of antibodies specific to the LH receptor increases after hindlimb suspension by 71% ($p < 0.1$). Since normalization was carried out to the thickness of the granulosa cell layer, it can be assumed that the content of LH receptors on these cells also increases. Indeed, protein content data show that in the HS group its content was 66% higher ($p < 0.05$) than in the control. It can be assumed that this increase is associated with an increase in the expression of the gene encoding it, since the relative content of the corresponding mRNA was also 2.1 times higher ($p < 0.05$), which, in turn, according to the principle of negative feedback, may be associated with a decrease production of LH by the pituitary gland.

However, literature data indicate that antiorthostatic suspension of rats led to a decrease in the concentration of estradiol in the blood, while the content of pituitary hormones did not change, although its weight decreased (Tou J. et al, 2004). We also noted a 32% reduction in pituitary mass ($p < 0.05$) after 96 hours of antiorthostatic suspension, and luteinizing hormone content did not change.

LH is synthesized by gonadotropic cells of the anterior pituitary gland, accumulates in secretory vesicles and is released into the blood by regulated calcium-dependent exocytosis (Constantin S. et al., 2022). At the same time, the content of cytoskeletal proteins that provide vesicular transport in the pituitary gland was changed after antiorthostatic suspension. Thus, the content of actin-binding proteins, alpha-actinin-1 and alpha-actinin-4 was higher by 64% ($p < 0.05$) and 49% ($p < 0.05$), respectively, compared to the control group. However, the relative content of beta actin did not change. The level of alpha-tubulin decreased by 40% ($p < 0.05$), as well as its acetylated form, the level of which was 44% ($p < 0.05$) lower than the control value under conditions simulating the effects of weightlessness.

Thus, summarizing the results obtained, it can be assumed that 96-hour antiorthostatic suspension leads to an increase in the number of granulosa cells in the follicles of mice. Moreover, the content of the luteinizing hormone receptor and its mRNA increases in granulosa cells. The latter may be associated with a change in epigenetic regulation, which we noted earlier (Usik M.A., Ogneva I.V., 2018). The assumption of an increase in the production of luteinizing hormone by the pituitary gland, as an inducer of the expression of its own receptor in granulosa cells, was not directly confirmed. That is why the change in the profile cytoskeletal proteins in the pituitary gland allows us to put forward hypotheses about changes in the intracellular transport of LH and the dynamics of its secretion into the blood, which requires further research.

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

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Introduction: Pre-flight exercise routines are associated with bone tissue adaptation in response to microgravity (Gabel et al, 2022). Physical activity may thus serve as a predictor for the rate of tissue deterioration. The aim of this study was to monitor individual physical activity levels during a 60-days of 6° head-down-tilt bed rest (HDT) study with three intervention groups (HDT only (CON), HDT+cycling (BIKE), HDT+cycling+artificial gravity (AG)).

Methods: Data were collected during the European Space Agency (ESA)-funded "Bed Rest with Artificial gravity and Cycling Exercise (BRACE)" study, a randomized controlled trial with three intervention groups (CON, BIKE and AG). Bike and AG performed a personalized loading protocol for 30 minutes (six days/week). HDT was preceded by 14 days of baseline data collection (BDC) and followed by 14 days of recovery (R). Physical activity parameters were collected by wrist-worn (non-dominant) accelerometry (GENEActiv, Activinsights Ltd, Kimbolton, UK, sampling rate: 50 Hz, dynamic range: ± 8 g) at the following time points: prestudy (during daily life) days -14 to -1, BDC-8 to BDC-4, HDT18 to HDT23, R+0 to R+13, and poststudy (during daily life) days R+14 to R+28. Physical activity levels were calculated (R-package GGIR (Migueles et al, 2019)) with the following validated cut-points: inactive (IN < 45.8 mg), light (LIG > 45.8 mg), moderate (MOD > 93.2 mg), vigorous (VIG > 418.3 mg) (Hildebrand et al, 2017). Sleep time (SPT) was detected by heuristic algorithm (van Hees et al. 2015). Data are presented as mean, minimum and maximum. A repeated measures ANOVA was computed to detect the effect of time and intervention and their interaction on physical activity parameters with Bonferroni corrected post-hoc tests (level for significance $p \leq 0.05$).

Results: In this preliminary analysis, we included the twelve healthy male participants of study campaign 1 (age 30.3 ± 5.9 years; body mass index 23.3 ± 2.1 kg/m²). Physical activity parameters are summarized in tables 1 and 2. At all time points, participants spent most of the daytime in IN with the highest percentage in HDT. Percentage of daytime spent in LIG and MOD was lowest in HDT (LIG = 3.9%; MOD = 1.8%) and highest in poststudy (LIG = 10.5%; MOD = 7.8%; table 1). Time significantly affected all physical activity parameters except VIG. There was no measurable difference in physical activity levels between interventions, and SPT was not affected by time or intervention (table 2). MOD had the greatest percent changes over time and a high inter-individual variation (prestudy vs. HDT: -68.4 % (-88.0; -47.3), prestudy vs. poststudy: 36.7% (27.7; 173.0)) (table 2).

Conclusions: Accelerometry allowed us to quantify the expected changes in physical activity levels resulting from participation in this study and the large inter-individual differences for these changes. Participation in the countermeasure groups does not lead to a quantifiable difference in physical activity levels. The greatest decrease was observed during HDT for light and moderate activity, which were restored in recovery and poststudy periods. The individual rate of change in physical activity is likely an important mediator of musculoskeletal changes in the bed rest and countermeasure response.

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Table 1. Average time spent in different activity levels for all participants across all measurement time points.

Physical activity parameter		prestudy	BDC	HDT	recovery	poststudy
		Mean (minimum; maximum); percentage/day (minimum; maximum)				
IN	minutes	726.0 (546.7; 840.7)	797.6 (692.1; 881.0)	897.8 (825.8; 1039.9)	812.2 (767.4; 862.0)	702.3 (635.4; 821.5)
	% of 24 h	50.4% (38.0; 58.4)	55.4% (48.1; 61.2)	62.3% (57.3; 72.2)	56.4% (53.3; 59.9)	48.8% (44.1; 57.1)
LIG	minutes	120.8 (81.5; 179.0)	93.9 (71.2; 113.0)	56.0 (40.2; 82.8)	116.7 (102.3; 136.4)	151.2 (88.4; 199.9)
	% of 24 h	8.4% (5,7; 12.4)	6.5%’ (4.9; 7.8)	3.9% (2.8; 5.8)	8.1% (7.1; 9.5)	10.5% (6.1; 13.9)
MOD	minutes	94.5 (57.6; 209.4)	55.1 (22.1; 97.7)	26.2 (15.4; 39.4)	70.3 (42.8; 117.6)	112.5 (75.9; 157.1)
	% of 24 h	6.6% (4.0; 14.5)	3.8% (1.5; 6.8)	1.8% (1.1; 2.7)	4.9% (3.0; 8.2)	7.8% (5.3; 10.9)
VIG	minutes	12.4 (1.6; 55.8)	1.2 (0.0; 8.6)	5.2 (0.0; 19.4)	2.6 (1.4; 3.8)	5.2 (1.3; 14.5)
	% of 24 h	0.9% (4.0; 14.5)	0.1% (1.5; 6.8)	0.4% (1.1; 2.7)	0.2% (3.0; 8.2)	0.4% (5.3; 10.9)
SPT	minutes	481.5 (420.2; 557.0)	492.2 (395.9; 601.5)	454.8 (319.9; 537.1)	438.2 (367.5; 473.5)	468.7 (362.1; 566.7)
	% of 24 h	33.4% (29.2; 38.7)	34.2% (27.5; 41.8)	31.6% (22.2; 37.3)	30.4% (25.5; 32.9)	32.6% (25.1; 39.4)
IN = inactive; LIG = light; MOD = moderate; VIG = vigorous; SPT = sleep in sleep period; prestudy = period before the study in daily life; BDC = baseline data collection; HDT = head-down-tilt bed rest; recovery = after HDT; poststudy = period after study in daily life						

Table 2. Results of repeated measure ANOVAs for all study time points by intervention. Percent change of average time spent per day in different activity levels of all participants across the study for prestudy vs. HDT and prestudy vs. poststudy.

Physical activity parameter	rm-ANOVA (p-value, based on absolute values in minutes)			Percent change (Mean (minimum; maximum))			
	Time	Intervention	Time * Intervention	prestudy vs. HDT	Bonferroni (p)*	prestudy vs. poststudy	Bonferroni (p)*
IN	< .001	.593	.357	25.2 (6.5; 63.3)	.008	-1.7 (-17.1; 21.3)	1.00
LIG	< .001	.533	.768	-49.9 (-68.1; -10.9)	.002	26.2 (-20.9; 85.8)	.377
MOD	< .001	.335	.770	-68.4 (-88.0; -47.3)	.005	36.7 (27.7;173.0)	.266
VIG	.072	.108	.221	-53.5 (-99.4; 203.2)	1.00	-22.5 (-74.1; 31.5)	1.00
SPT	.107	.351	.371	-5.1 (-40.9; 19.5)	1.00	-4.2 (-13.8; 15.0)	1.00
IN = inactive; LIG = light; MOD = moderate; VIG = vigorous; SPT = Sleep in sleep period; prestudy = period before study in daily life; HDT = head-down-tilt bed rest; poststudy = period after study in daily life; *Bonferroni adjustment for multiple comparison, tests applied to absolute values.							

Does an N95 mask improve Orthostatic Tolerance?

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INTRODUCTION

Upon return to Earth, astronauts experience greater orthostatic intolerance (OI) after both short and long-term spaceflight (Buckey J.C. et al, 1996; Meck J.V. et al, 2001). Recent investigations have demonstrated that wearing a surgical or N95 mask increases end-tidal carbon dioxide ($ETCO_2$) in healthy adults (Brooks J.P. et al, 2023; Özdemir L. et al, 2020; Karsli E. et al, 2023; Shechtman L. et al, 2022). Given that the cerebral vessels are highly sensitive to arterial CO_2 (Battisti-Charbonney A. et al, 2011), wearing a mask may protect brain blood flow during standing and reduce OI symptoms (i.e., lightheadedness, fainting, etc.). This study aimed to determine the influence of wearing an N95 mask on cerebral blood velocity during rest and upright tilt, compared to a mask free control. The results of this study could potentially aid in improving brain blood flow and OI during an astronaut's recovery upon return to Earth.

METHODS

Twenty-seven young healthy individuals (males, n=10; females, n=17) were recruited to complete two randomized trials (i.e., Control or Mask), which included 5mins rest and 10mins 70° head-up tilt. ECG was used to measure heart rate (HR). Mean arterial pressure (MAP) was continuously measured using beat-to-beat finger photoplethysmography (BMEye Nexfin, Amsterdam, NL). Transcranial doppler (Multigon Industries Inc., Yonkers, USA) was used to measure right middle cerebral artery blood velocity (MCA_V) via a 2-MHz probe positioned on the temporal window and supported by an adjustable headband. Due to technical issues, gas analysis was not measured; however, multiple studies introduced above demonstrate that mask-wearing increases $ETCO_2$. To quantify OI symptoms, a Vanderbilt Orthostatic Symptom Score was summed based on scoring (0-10 scale) the severity of multiple symptoms of OI during the final minute of tilt (Raj S.R. et al, 2005). 2-way repeated measure ANOVAs were used to compare cardio- and cerebrovascular responses to tilt and mask-wearing. Total and OI symptom scores were compared with one-way repeated measure ANOVAs.

RESULTS

Blood pressure decreased and HR increased in response to tilt similarly between trials (all $p<0.05$; *Table 1*). Cardiac output index (Qi) was unaffected by tilt or mask-wearing (all $p>0.2$; *Table 1*).

Table 1. The cardio- and cerebrovascular response to head-up tilt while wearing or not wearing an N95 mask.

	Mask		Control		P-value		
	Rest	Tilt	Rest	Tilt	Mask	Tilt	Mask x Tilt
HR (bpm)	70±10	88±10	67±8	88±12	0.176	**<0.001**	0.304
MAP (mmHg)	84±11	80±12	86±11	81±12	0.254	**0.001**	0.219
SBP (mmHg)	114±16	105±18	118±16	107±15	0.192	**<0.001**	0.303
DBP (mmHg)	71±10	69±11	73±9	69±11	0.536	**0.018**	0.258
Qi (L/min)	6.7±1.4	6.5±1.6	6.7±1.4	6.8±1.5	0.378	0.999	0.245

All values are mean±SD. HR, heart rate; DBP, diastolic blood pressure; MAP, mean arterial pressure; Qi, cardiac output index; SBP, systolic blood pressure. Significance is represented by bold text (p<0. 05).

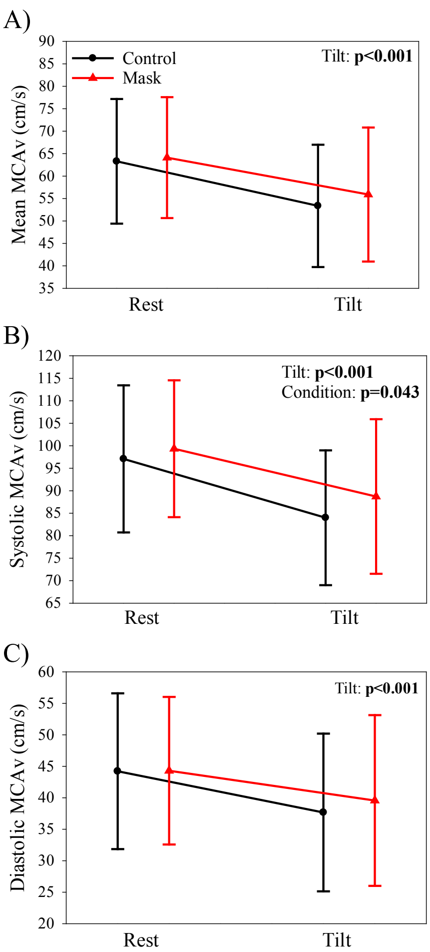

Figure 1. The mean (A), systolic (B) and diastolic (C) cerebrovascular blood velocity response to head-up tilt while wearing or not wearing a mask.

Mean (*Figure 1A*), systolic (*Figure 1B*) and diastolic MCA$_V$ (*Figure 1C*) decreased during tilt similarly in both trials (all $p<0.001$). However, during rest and tilt, systolic MCA$_V$ was higher throughout the mask trial compared to the non-mask trial ($p<0.05$; *Figure 1C*). Interestingly, experience of nausea, rapid heartbeat, and shortness of breath were higher during the mask trial than control (all $p<0.05$; *Table 2*). Total Vanderbilt Orthostatic Symptom Score was also higher during the mask trial ($p=0.002$; *Table 2*).

Table 2. Total OI score and individual symptom scores during mask and control trials.

	Mask	Control	P-value
Blurred Vision	2±2	1±1	0.285
Chest Discomfort	1±2	1±2	0.414
Headache	2±3	1±3	0.058
Lightheadedness	3±3	2±3	0.090
Mental Cloudiness	3±3	2±2	0.099
Nausea	1±2	0±1	**0.034**
Rapid Heartbeat	3±3	2±2	**0.011**
Shortness of Breath	4±3	1±2	**0.001**
Tremulousness	2±2	1±2	0.052
Total Score	20±17	12±12	**0.002**

All values are mean±SD. Significance is represented by bold text (p<0. 05).

DISCUSSION

The major findings of the current study are that 1) there were no differences in the hemodynamic response to tilt while masked or not masked, 2) mask-wearing increased systolic MCA$_V$ at rest and during tilt, and 3) OI symptoms were worse while wearing a mask. This disconnect between symptom experience and physiological responses may suggest that the symptoms were psychologically induced. Indeed, some individuals may find wearing a mask uncomfortable or too hot and may worry about being short of breath or receiving sufficient oxygen (Martin G.M et al, 2020). The psychological effects of mask-wearing may outweigh any potential benefit to brain blood flow. Future studies should 1) exclude participants who are uncomfortable with mask-wearing and 2) be conducted in participants prone to OI.

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

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INTRODUCTION

The method of electromyostimulation (EMS) is rather widespread in medicine and sports. It is also has been used for many years by Russian crew members in orbital flights. Two types of electromyostimulators are used onboard of ISS: low-frequency, LF (25Hz) and high-frequency, HF (2500 Hz modulated to 50Hz) ones. Electromyostimulation is not the obligatory countermeasure mean for space flight, but it can be used as addition mean or can help to maintain muscle strength and endurance in the short periods when cosmonauts cannot perform active physical training by some reasons. In 2020 the new temporal consortium – the World-class Research Center, the "Pavlov Center for Integrative Physiology to Medicine, High-tech Healthcare and Stress Tolerance Technologies" was created on the base of 4 organization. One of the branches of this center is the "Center of study and countermeasure for the effects of long-term isolation" at the Institute of Biomedical Problems of the Russian Academy of Sciences. One of the goals of this center is to develop on the base of space countermeasure experience the new electromyostimulation protocols for prevention of muscle properties loss under conditions of motor inactivity. The main idea of this project is to develop the electrostimulator which allow to combine the two mentioned types of stimulation. For this purpose, some of the experimental studies under conditions of Dry Immersion and in clinics with the patients with motor deficit were performed.

METHODS

20 healthy subjects took part in the experimental sessions in Dry Immersion (DI) conditions. 10 of them was in the control group (were exposed to 7 days DI without countermeasures) and 10 other – in the experimental one (7 days DI coupled with 2 sessions of EMS daily – LF and HF).

The study with patients was carried out in three groups of participants (average age 74.5±7.5 years). In the stimulation group (n=27), an HF EMS course consisted of an average of 7.9±2.4 sessions over 14 days. In the sham group (n=7), sham stimulation was performed at similar times. In the control group (n=10), no EMS course was performed.

RESULTS

Preliminary results of the study showed good patient acceptance of EMS. After the course, the EMS group showed a significant improvement from baseline in the standard neurological test (Tinetti Test, Rivermead Mobility Index and Timed Up and Go Test). There was also a significant improvement in balance quality and maximal voluntary strength of the shin muscles). No significant changes were observed in the control and the sham groups in the same tests. It can be concluded that stimulation of the hip and shin muscles with HF regimen has a positive effect on the motor system of elderly neurological patients. However, the results of combination of 2 sessions of EMS – low and high frequency under motor unloading conditions (DI) showed the positive results only for the hip muscles; for shin muscles we observed even opposite effect by some of the parameters. These results showed that the protocol which was offered is not optimal and some further studies of the other protocols efficacy are needed.

FUNDING

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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

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Introduction

In the conditions of space flight, maintaining physical performance is one of the important tasks. In space flight, such negative effects as skeletal muscle atrophy [1], decreased endurance and contractility of muscles [2,3], and decreased muscle tone [4-6] develop. The observed effects occur due to a decrease in the flow of proprioceptive information from muscles and tendons in response to the absence of mechanical loads. To ensure adequate mechanical load, a number of countermeasures means are needed, such as a treadmill, resistive training equipment, etc. The main disadvantage of countermeasures is that the training equipment need much space, while the station volume is limited. One of the promising directions in solving this problem is the use of passive training methods, such as electromyostimulation (EMS). EMS as a method of increasing muscle functionality is used in clinics for the rehabilitation of patients [6] suffering from diseases of the peripheral and/or central nervous system [7,8]. In the light of these observations, EMS may be an alternative method for maintaining the contractile properties of the human muscular system in conditions of prolonged support unloading.

The main purpose of this study was to evaluate the effect of combined EMS on contractile and viscoelastic properties of muscles under conditions of ground-based modeling the physiological effects of weightlessness.

Method

20 healthy males participated in the study, 10 of them made up the control group (30.5 ± 4.3 years) and 10 (33.10 ± 4.3 years) – the experimental one (using combined EMS). A Dry Immersion (DI) model was used to simulate the effects of microgravity [9]. The DI procedure was standard [10], the duration of exposure was 7 days.

EMS training was performed in the form of 2 sessions daily for 7 days of DI. The morning EMS session was conducted for 35-45 minutes on all stimulated muscle groups simultaneously, the evening EMS session was conducted for 10 minutes on each muscle group. The optimal frequency of EMS depends on the type of stimulated muscle group: for postural muscles with a predominance of slow fibers, low-frequency EMS (LF; 25 Hz) has the greatest effect, for muscles with a high content of fast fibers, the most effective is EMS with a high frequency (5 kHz) modulated to 50 Hz (HF) [11].

Table 1. Characteristics of the stimulation sessions

	Signal type	Stimulator
Morning	Bipolar symmetrical rectangular electric pulse with a duration of 1 ms with a frequency of 25Hz in cyclic mode (1s - stimulation, 25 - break), duration 45 minutes	«low-frequency» "Stimulus-NCh"
Daytime	Alternating current oscillations with amplitude modulation, frequency of carrier oscillations - 2500Hz; frequency of sinusoidal modulating voltage - 50Hz (duration of series - 10s, duration of pauses - 50s), duration 20 minutes	«high-frequency» "Amplidin-EST"

To assess the viscoelastic properties of soft tissues, the MyotonPro surface palpation method (Myoton AS, Estonia) was used. During the measurement, the sensor of the device is pressed into the skin with a slight force (0.18 N) perpendicular to the direction of the muscle fibers of the muscle under study. The sensor of the device outputs three short (15 ms) mechanical pulses of stable strength (0.4 N) with an interval of 1 s. The parameters describing the viscoelastic properties of the muscle are calculated based on their response vibrations. In this study, we analyzed only the transverse stiffness parameter.

The tensiomyography method (TMG-BMC Ltd.; Ljubljana, Slovenia) was used to assess the contractile properties of muscles. The amplitude of the muscle response was recorded during an electrically induced isometric contraction using a highly sensitive sensor with a constant elasticity of 0.17 N/mm, which was placed perpendicular to the muscle belly. The studied parameters were the maximum amplitude of the sensor displacement (Dm) and the time to achieve from 10% to 90% displacement of the muscular belly (Tc) [12]. The data were analyzed using the ANOVA repeated measurement method (GraphPad Prism, version 9).

Results

The most noticeable and stable changes were observed in the medial head of Gastrocnemius muscle. Thus, in the control group, the Dm value increased by 47% ($p<0.05$) on the first day after DI, while the reverse pattern was observed in the group with EMS. The Dm value decreased by 23% ($p<0.05$) on the first day after DI (Fig. 1A). At the same time, muscle stiffness in the control group decreased by 6% ($p<0.05$) on the first day after DI, whereas in the group with EMS, stiffness did not significantly change (Fig. 1C). The Tc value in the control group increased by 50% ($p<0.05$) on the first day after DI, while the Tc in the group with EMS at the same time decreased by 16% ($p<0.05$).

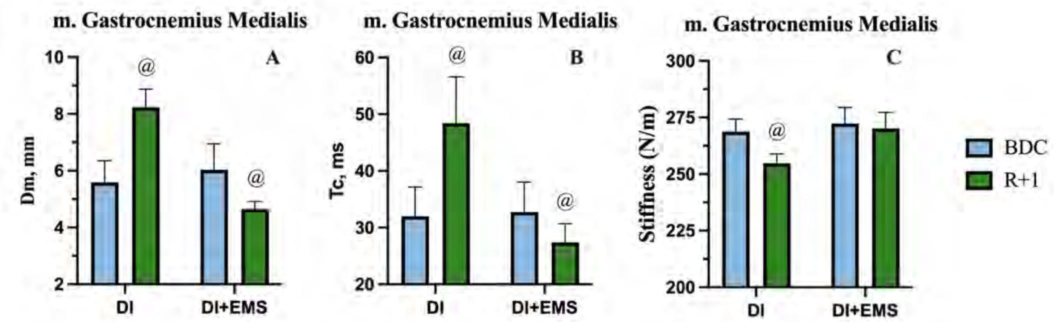

Figure 1 - Changes in responses of the medial head of Gastrocnemiusmuscle after 7-day Dry Immersion in the control (DI) and experimental (DI+EMS) groups. A - Maximum displacement amplitude - (Dm); B - Construction time (Tc); C - Stiffness. BDC – data collected before DI exposure. R+1 – post-DI results on the 1st day of recovery. M±SEM, @ – significant changes compared to the initial values, $p<0.05$

Conclusion

The use of a combined EMS regimen can help to reduce the effect of 7-day support unloading on the contractile and viscoelastic properties of the lower extremities' muscles.

Funding

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Space Tourism- MyotonPRO experiment on Muscle Tone

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BACKGROUND

As NASA experiment with MyotonPRO device is still in progress and it is being performed on the professional astronauts (https://nlsp.nasa.gov/view/lsdapub/lsda_experiment/de607d34-c881-54ba-8f89-953f0de1ed00), my area of interest is to investigate opportunity of civilian space traveling in terms of health and preparation for it. As prices of space tourism are coming down, “Space Tourism Market in the United Arab Emirates goes under Potential Development” (M. Amer Khan et al, 2022), “The UAE has put space exploration as one of its top priorities” said Andrew Cole, CFO of satellite firm Yahsat- Arabian Business, prognosis is that space tourism will be soon available for us. Going through the recent researches, main points of my abstract are:

- Experiments about microgravity effects on the human body were done on Astronauts who are in excellent physical condition but they do not represent general population health status. What results would we get if the experiment was performed on a 'regular population' (sedentary job, mild health conditions, low physical activity level, overweight, muscle atrophy). I would like to expose regular commercial passengers to the effects of microgravity and measure muscle tone changes using MyotonPRO device.
- Commercial space travels are launching now and so far were accessible to wealthy people, however prognosis is that in 15-20 years many of us will have this opportunity as price is coming down, making it available to the general population. Investigation if muscle tone goes under the same changes in a person with a sedentary lifestyle would give an insight into effects of microgravity on them and if they are tremendous.
- As a physiotherapist, I would be able to create a protocol to cope with a vast number of physical conditions, besides top shape.
- How does "regular" commercial passenger recover is another objective of my experiment. Nowadays civilians are able to purchase the flight from 3-6 days and more. Book your mission (https://www.spacex.com/human-spaceflight/). Measuring ratio of exposure time to microgravity and changes in muscle properties followed by extrapolation of results to estimate changes over a longer period will lead to design of Physiotherapy Protocol for Civilians as a preparation for space travels.

EXPERIMENT DESIGN

$$\text{Ratio of} = \frac{\text{time exposure to microgravity}}{\text{changes in muscle properties}}$$

↓

Extrapolation of results to estimate changes over longer period

↓

OUTCOME: Create Physiotherapy Protocol for Civilians as a preparation for space travels

1st Step:

5 single measurements of the muscle properties would be obtained using MyotonPRO which works in extreme environments like space. Measurement time is 350 ms and build in software registers the measurements.

2nd Step:

Proper positioning of the participant during parabolic flight will allow the measurement with the MyotonPRO device. As the calf muscles are mostly affected, I will be focusing on Gastrocnemius and Soleus Muscle.

3rd Step:

Obtaining 5 measurements from the muscle:

Tone / State Of Tension

1. F – Natural Natural Oscillation Frequency [Hz], characterizing Tone or Tensicillation Frequency [Hz], characterizing Tone or Tension

Biomechanical Properties

2. S – Dynamic Stiffness [N/m]

3. D – Logarithmic Decrement [relative unit], characterizing Elasticity

Viscoelastic Properties

4. R – Mechanical Stress Relaxation Time [ms]

5. C – Ratio of Relaxation and Deformation time [relative unit], characterizing Creep

CONCLUSION

Space tourism will also allow people, who are not professional astronauts, to venture into space. The space tourists will most likely be not so physically fit and in good physical condition as the carefully selected and trained astronauts. In addition, the very few chosen space tourists will experience physiological changes that are much similar to certain aspects of accelerated aging in space. Among others, these include cardiovascular changes, muscle wasting, osteopenia, etc. Therefore, understanding the mechanisms of physiological deconditioning induced by microgravity are crucial for success of manned space missions, preventing unnecessary mishaps in space travelers as well as in helping the aging population on Earth (Anna Hawliczek et al, 2022).

Finally, my study would explore medical restrictions for commercial space travelers, make it possible to launch people with disabilities to space with precautions for the necessary adaptations and explore what are the “acceptable” disabilities for a safe mission (Tania Gres et al, 2022). Also, contribute to development from aviation tourism to space tourism, add the value to the medical space platform, expanding capacity to conquer new space frontiers and bring more possibilities for space travelers. Moreover, using physiotherapy framework to enhance human performance in space and build up adaptation program of human body to microgravity environment.

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

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Results of previous studies have shown that hypogravitational motor syndrome is characterized by changes in all components of the motor system (Kozlovskaya I.B. et al, 1988; Reschke M. F. et al, 1998). The alterations in afferent systems' activity, in particular a decrease of volume of proprioceptive afferentation activating motor neurons of skeletal muscles, are considered to be one of the main reasons for changes in motor system following exposure to weightlessness. Currently, the search for effective approaches to preventing the development of muscle weakness in conditions of motor unloading is a crucial task. Electromyostimulation (EMS) can be used as a countermeasure mean to maintain contractile activity of skeletal muscles and additional proprioceptive afferentation. At the same time, the question of the most effective protocols of electrostimulation training to prevent the identified problems remains open.

Therefore, the aim of this work was to study the effect of different modes of EMS on spinal reflexes of human shin muscles during 7-day Dry Immersion (DI).

According to the tasks of the study, subjects were divided in four groups of ten people each. In the control group, the subjects were exposed to DI without any additional influences. In the second group, a low-frequency (LF) EMS of both legs' thigh and calf muscles (anterior tibial muscle, triceps surae muscle, quadriceps and back thigh muscle) was applied daily for 3 hours in the course of DI with the use of "Stimul-01 NCH" (Russia) device. LF stimulation session lasted for 1 sec and was followed by rest period of 2 sec. Stimulator generated bipolar 1 msec impulses with frequency of 25 Hz. In the third group, a high-frequency (HF) EMS of the same thigh and calf muscles was applied in the course of DI with the use of "Amplidin-EST" (Russia) electromyostimulator. Subjects were stimulated by electric pulses with a carrier current frequency of 2500 Hz, modulated into a sinusoidal signal with a frequency of up to 50 Hz and a duration of 10 msec. The training HF EMS session consisted of 10 repetitive stimulation periods, with 10 seconds of stimulation and 50 seconds of rest, a total of 10 minutes for each muscle group. In the fourth group, a combined EMS method was applied: in the morning, LF EMS was used for 45 minutes, in the evening – HF according to the protocol of the third group. During all of the EMS training sessions, the intensity of electrostimulation was selected individually – up to the tolerance threshold.

Effects of EMS training were evaluated by amplitude characteristics of H-reflex – the essential spinal mechanism of locomotion and posture control systems – in m. soleus and m. gastrocnemius lat. H-reflex was elicited by single 1 msec electrical pulses applied to the area of tibial nerve projection under the knee. Reflex responses testing was performed before, during and after DI completion. Reflex threshold and maximal peak-to-peak amplitudes of responses were evaluated.

Study of the recruitment curve of H-reflex in the control group showed that during 7-day immersion, an increase in the overall level of excitability of the motor neuron pool was present, manifested in a decrease in reflex thresholds and an increase in its absolute amplitude. In the group with LF EMS, the thresholds of the H-reflex on the 3rd day of DI did not differ from the baseline values, but on the 5th day they significantly decreased. The amplitude of the H-reflex did not differ significantly during immersion exposure. In the group with HF EMS, there were no significant changes in the parameters of the H-reflex during DI. However, on the second day of recovery, enhanced inhibitory effects were noted due to an increase in the thresholds of the H-reflex. In the group with the combined EMS method, no significant changes in the characteristics of the H-reflex of the shin muscles were found compared with the baseline values.

The data obtained confirms that under the conditions of support unloading excitability of motor neurons of calf extensor muscles increases due to reflex changes. Additional proprioceptive stimulation with the use of properly selected EMS training leads to lessen of this effect, i.e. helps to maintain the level of motor neurons excitability. The developed methods of electromyostimulation and a device for their implementation can be used to maintain muscular qualities in individuals with limited motor activity, including elderly people and patients who are unable to engage in active physical training for medical reasons.

ACKNOWLEDGMENTS

The study was supported by the Ministry of Science and Higher Education of the Russian Federation under agreement № 075-15-2022-298 from 18 April 2022 about the grant in the form of subsidy from the federal budget to provide government support for the creation and development of a world-class research center, the «Pavlov Center for Integrative Physiology to Medicine, High-tech Healthcare and Stress Tolerance Technologies».

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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")

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INTRODUCTION

The purpose of the work was to study the strength properties of the muscles of the lower extremities and muscle tone in patients with stroke and lack of motor activity before and after a course of modulated electrical myostimulation (EMS) in a hospital setting.

METHODS

The study was carried out in three groups of patients (average age 74.5±7.5 years). In the stimulation group (n=27), an EMS course was carried out with an average amplitude of 21.4±5.1 mA, consisting of an average of 7.9±2.4 sessions over 14 days. In the sham group (n=7), sham stimulation was performed at similar times with an average amplitude of 4.4±2.4 mA. In the control group (n=10), no EMS course was performed. Dynamometry was carried out in the supine position using a load cell mounted in a frame under the plate that took the loading from ankle extension. The measurement was taken from the leading leg with the angles of 90º at the hip, knee and ankle joints. The maximal voluntary force (MVF) in kg was determined - the patient performed three extensions in the ankle joint with the maximum possible force; during the analysis, the attempt with the maximal force was taken into account. Muscle tone of the lower extremities was assessed at rest using a MyotonPRO myotonometer (MyotonLTD, Estonia). The device applies five short mechanical impulses (15 ms) of a stable force (0.4 N) and records the response from the tissue under study, calculating the resistance of biological soft tissues to deformation force (Stiffness) (Schneider et al., 2015). The viscoelastic characteristics of the soleus, medial and lateral gastrocnemius, tibialis anterior, semitendinosus, biceps, rectus and vastus lateralis muscles were recorded as well as of the Achilles tendon. During this examination, the patient was in the prone or supine position. To standardize the position of the lower extremities, special support was placed under the knee and ankle joints in the prone or supine position, respectively.

RESULTS

After the EMS course, in the stimulation group there was a significant increase in MVF during ankle extension by 19.3% ($p<0.05$). In other groups, no significant changes were observed: in the control group, a slight increase in MVF was recorded by 7.1%, and in the sham group - a decrease by 8.24%.

A study of muscle tone in the stimulation group before and after EMS showed significant changes in the Stiffness parameter in the soleus, tibialis anterior and rectus femoris muscles. In the soleus muscle, transverse stiffness significantly increased from 354.6±6.2 to 370.8±7.6 N/m ($p<0.05$). In the tibialis anterior muscle, stiffness significantly increased from 363.0±11.4 to 400.9±13.8 N/m ($p<0.05$). In the rectus femoris muscle, stiffness increased from 276.1±9.4 to 295.6±13.1 N/m ($p<0.05$). In the control group and the sham group, no significant changes in muscle tone were observed.

The results of studies of maximum voluntary force and muscle tone of the lower extremities indicate that modulated electrical myostimulation can be considered as a promising method in the rehabilitation of patients with chronic cerebrovascular insufficiency and a lack of motor activity, leading to deterioration of postural stability and accompanied by the risk of falls.

FUNDING

The study was supported by the Ministry of Science and Higher Education of the Russian Federation under agreement № 075-15-2022-298 from 18 April 2022 about the grant in the form of subsidy from the federal budget to provide government support for the creation and development of a world-class research center, the “Pavlov Center for Integrative Physiology to Medicine, High-tech Healthcare and Stress Tolerance Technologies”.

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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-distance and long-term spaceflights pose an issue of describing the influence of prolonged staying in weightlessness on the physical performance and completing tasks after returning in the gravity conditions. Currently, flights to the ISS are the best model for analysis of changes in gravity-dependent physiological systems after long-term missions (Fomina et al., 2022; Rusanov V.B. et al., 2022).

The assessment of the cosmonauts' physical performance in the early period of adaptation after spaceflight was carried out with "Field Test" (Tomilovskaya E. et al, 2014). Based on this test, we have created an "express test", which includes tasks for subjective assessment of the weight of loads, physical activity (push-ups and squats) and a specific task for coordinating hand movements.

The purpose of the work was to determine the role of spaceflight experience and the duration of stay in weightlessness in the success of completing model tasks.

METHODS

Four cosmonauts participated in the study. Two of them made a six-month flight, two others – a year-long flight. In each of the two named crews one of the cosmonauts made his first flight, and the second already had flight experience behind him. In the early period of adaptation to Earth conditions, on the 1st, 2nd, 3rd and 4th days after a six-month spaceflight and on the 3rd and 4th days after a year-long flight, the cosmonauts performed the "express test". On the 1st and 2nd days after the six-month flight, the test was not performed due to medical restrictions. The test included hand movement control task, Romberg test, voluntary standing up from supine and prone positions, collecting loads around the perimeter of a designated area, estimating the weight of loads, throwing them at specified points, a dual task – walking in tandem while simultaneously counting down in threes from a given number and performing physical exercises - push-ups and squats - in 10 seconds. Throughout the test, heart rate (HR) was recorded.

RESULTS

The hand movement control test showed significantly higher accuracy of touching the target on the second day, compared to the first, after a six-month spaceflight. This confirms the hypothesis of rapid readaptation when returning to gravity conditions.

The cosmonauts who completed a six-month flight showed an improvement in the performance of the Romberg test from the first to the third days – an increase in the duration of maintaining balance, a decrease in swaying. In cosmonauts who completed a one-year flight and had different flight experience, no differences in maintaining posture were found between the 3rd and 4th days.

The strategies for standing up from the prone and supine positions after a six-month flight had some specialties at the 1st and 2nd days after the flight – cosmonauts turned on the side and support on both hands. On the 3rd and 4th days, the cosmonauts used less support points. 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 decrease. Thus, after a long flight, the time for readaptation and recovering of habitual getting up strategies increases.

During performing a double task, the ratio of the amount of named numbers to the time of tandem passage turned out to be less for cosmonauts who completed a year-long flight. Consequently, the duration of weightlessness influenced the success of the dual task. Moreover, according to our results we can suggest the positive impact of flight experience.

Subjective assessment of 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.

Among the cosmonauts who completed a six-month flight, there was a tendency for the performance of 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). At the same time, the best result was shown by an astronaut with flight experience. After a one-year flight, the crew completed this task only on the 4th day due to medical restrictions, and no differences were found between cosmonauts of this crew.

CONCLUSION

The results of the study suggest that the mission duration factor has a greater influence on the performance of model tasks in the early period of adaptation after the spaceflight than the cosmonaut's flight experience. However, experience was important to the success of the dual task. The assessment of the recovery dynamics after the flight shows an improvement in the "express test" results by the third day and subsequently its stabilization.

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

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BACKGROUND

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. 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. 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 EXPERIMENT

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) was launched on 26th November 2022 with SpX-26(Cargo Dragon 2), Expedition 68, and 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 SiS experiment aimed to study the behavior and healing of *ex vivo* sutured wound models (SWMs) in unloading conditions. The SWMs were prepared from human skin and blood vessel samples derived from plastic, vascular and cardiovascular surgery on subjects not affected by oncological, metabolic or infectious diseases, with Ethic Committee approval. 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. The experiment HW was developed by OHB and Kayser Italia.
For the in-flight experiment, the tissue samples were collected at Careggi Hospital in Florence, Italy, stitched to specific frames, 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. 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. 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, temperature profile, and tensile strength in the tissues 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.

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 the morphology of the tissues. Significant differences were found between ISS- and control samples. The most important of them concerned the ECM turnover, composition and structure, suggesting that microgravity could strongly affect the remodeling phase of WH. Also the expression of genes involved in the regulation of fibroblast activation and fibroblast-myofibroblast transdifferentiation changed greatly, suggesting an impairment in fibroblast behavior throughout the WH phases.
These results may give interesting insights to understand 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. Last but not least, 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.

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Risk of Thromboembolism in Space: Current Evidence and Perspectives

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Abstract:

Spaceflight has effects on many important physiological systems. These include the musculoskeletal system, the cardiovascular system, cerebral autoregulatory control and, recently, the coagulation system. There are recent reports of the development of thrombo-embolism in astronauts as well as venous stasis in the neck veins.

There is a general consensus that vascular endothelial function can be regarded as a marker of the net harmful effects of cardiovascular risk factors on the vascular wall. As COVID-19 syndrome is associated with multisystem inflammation, the pattern of organ damage caused by COVID-19 occurring in patients with COVID-19 is still incompletely understood, current treatment options are limited and improved understanding of the risk for severe and fatal COVID-19 outcomes is urgently needed. Patients with severe COVID-19 can develop COVID-19-associated coagulopathy, with features of both disseminated intravascular coagulation and thrombotic microangiopathy, resulting in widespread microvascular thrombosis that may involve consumption of coagulation factors and the liver. This appears to have a causal relationship with the inflammatory and reparative processes involving diffuse alveolar damage (DAD), because thrombi are frequently detected in small pulmonary arteries, most likely secondary to endothelial damage. The endothelial damage could occur due to the direct viral infection of the endothelial cells, which express ACE-2 receptors, or to a host response. Furthermore, the alveolar fibrin deposition in DAD may affect the delicate local balance of fibrinolysis and coagulation. A combination of alveolar and endothelial damage of smaller vessels may be followed by microvascular pulmonary thrombosis, which could then extend to larger vessels. Additionally, elevated D-dimer has been seen in patients with COVID-19, especially those at a severe stage. It is well known that elevated D-dimer concentrations are associated with acute pulmonary emboli (APE), deep venous thrombosis (DVT), cancer, peripheral vascular disease, inflammatory diseases and pregnancy. Patients with COVID-19, including those not on respirators but confined to bed, develop DVT and APE much earlier than expected. Despite the usage of prophylactic anticoagulation, autopsy reports have shown that deaths in COVID-19 may be caused by the thrombosis in segmental and subsegmental pulmonary arterial vessels.

This talk summarizes how thromboembolism research in spaceflight can help towards global health, including in the fight against COVID-19.

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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

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The hemostatic system is one of the important components involved in the formation of homeostatic balance in the human body. The response of this system to extreme environmental influences and its local reactions largely determines the body's resistance to stress and its ability to adapt (Subbotina L.A., 2008). Decrease in the compensatory capabilities of the regulatory components of blood coagulation system can lead to both the appearance of thrombophilic conditions and the development of hypercoagulation, and contribute to the development of hypocoagulation phenomena. It is known that the coagulation potential and activity of hemostasis components depend on the parameters of hemodynamics and hemorheology, which are largely determined by the level of motor activity, body position, and the effect of inertial and gravitational forces on it (Bentur O.S. et al., 2018).

Despite great achievements in the field of preventing cardiovascular risks in astronauts, many aspects of changes in venous hemodynamics and hemostasis require closer attention from researchers and an integrated approach to the development of this problem, especially in connection with planned human flights to the Moon (Ball JR, Evans CH Jr, 2001). An analysis of the literature on risk stratification of venous thromboembolic complications during space flight shows that in the last 25 years, a few scattered studies have been carried out in this direction. In this regard, it is especially important to conduct a targeted analysis of the state of the venous system under the influence of space flight factors using modern clinically significant examination methods to identify possible risks, including when exposed to similar factors in ground-based conditions (Dry Immersion, long-term isolation model) to identify the triggers of venous changes and hemostatic reactions and propose countermeasure means.

The study was supported by the Ministry of Science and Higher Education of the Russian Federation under agreement № 075-15-2022-298 from 18 April 2022 about the grant in the form of subsidy from the federal budget to provide government support for the creation and development of a world-class research center, the "Pavlov Center for Integrative Physiology to Medicine, High-tech Healthcare and Stress Tolerance Technologies"

LIVER TISSUE CHANGES DURING 6-MONTH SPACE FLIGHT MEASURED BY ULTRASOUND RF SIGNAL PROCESSING.

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The ultrasound RF signal (Radio Frequency) processing showed that the carotid wall structure was significantly different at the end of the spaceflight compared to pre-flight. This new ultrasound parameter derived from the RF signal seems to bring additional information on the Vessel wall. Our hypothesis is that this parameter when measured into the Liver tissue should also be disturbed by the spaceflight.
The objective of the present research was to check using the ultrasound RF signal processing if the Liver tissue response to ultrasound changed duringand 4d ays after 6months spaceflight.
Method: A 2D echography of the liver with the Portal vein at the bottom of the image was displayed and the RF signal of the entire image recorded. The RF signal was displayed along 6 adjacent vertical lines inside a square of 2cm² of liver tissue. (sample area). The coefficient of reflectivity was calculated from the Excel table showing the values of energy back-scattered by the liver tissue. Coefficient of reflectivity = Energy backscattered by the whole Liver sample selected (2cm²), divided by the energy received by this part of the liver tissue.
Results: Seven astronauts were investigated preflight at inflight day 150 and at day 4 postflight. The coefficient of reflectivity of the Liver tissue sample was found significantly lower higher at the end of the flight as well as at day R+4 postflight
Conclusion: The coefficient of reflectivity measured inside the Liver tissue evidenced physical property changes at the end and immediately after 6-month spaceflight which suggest that particle of low reflectivity to ultrasound have entered this organ (more liquid) or that structural/cellular change occurred in the organ. The coefficient of reflectivity provides an easily acquired, noninvasive index characterizing changes in tissue properties as a consequence of spaceflight.

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

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INTRODUCTION

Ground-based model experiments are an analog of a space expedition, in which such effects of space flight as autonomy, isolation, artificial habitat, reduced level of motor activity, restriction of living and working space can be simulated (Ushakov I. B. et al., 2014; Gushchin V. I. et al., 2019). To study the effects of these factors on the human body, the Institute of Biomedical Problems conducted the 240-day isolation experiment SIRIUS-21 in 2021-2022, and the one-year isolation experiment SIRIUS-23 was launched in 2023. Both projects investigated the dynamics of physical performance of volunteers against the background of application of the physical training system (the SIRIUS-23 experiment is ongoing at the moment). In the SIRIUS-21 experiment, performance was assessed using such an indicator as the lability of the cardiovascular system response to changes in the load. In the SIRIUS-23 experiment, the effectiveness of new 15-minute individualized locomotor training protocols is being evaluated. The creation of shortened effective training protocols is driven by the request to the scientific community to shorten the duration of preventive measures in spaceflight. The purpose of physical performance dynamics studies in the Sirius-21 and Sirius-23 experiments is to ground-test experimental individualized exercise protocols and new test procedures for space missions.

MATERIALS AND METHODS

There were 5 participants in the Sirius-21 study, three males and two females. The mean age of the volunteers was 36±7 years, height 171±7 cm, body mass index 26±7. Physical performance was evaluated using the locomotor integral test on a treadmill before, during (every month) and after isolation. The locomotor integrative test was performed on two different treadmills. The first part of the test was performed on the h/p/cosmos treadmill in active web motion mode and included one-minute running intervals from 3 km/h to 12 km/h, performed to assess the lability of the cardiovascular response to changes in load. The crewmember then proceeded immediately to a BD-1 treadmill, where he or she performed the second part of the test in passive web motion mode, starting with walking from 3 km/h with an increase in speed of 1 km/h every minute until the load was abandoned based on subjective sensations of fatigue. After that the subject performed walking in passive mode at a speed of 3 km/h for 1 minute. The tests included measurement of gas exchange in each respiratory cycle (Metalyzer 3B) and heart rate (CustoGuard chest pulse sensor).

In the first month of isolation, participants did not perform any physical training to assess the impact of reduced motor activity level without the influence of preventive measures. Starting in the second month, the SIRIUS-21 physical training system consisted of strength and locomotor training, which was structured as a 4-day microcycle. The microcycles were combined into six 20-day mesocycles. Intervals of high-intensity running with a heart rate of 80-90% of the individual maximum, intervals of low-intensity running, and intervals of walking were alternated in the training. Locomotor training was performed on treadmills with active (motor-assisted) and passive (leg power) modes of web movement. Strength training was performed on an Atlas strength training machine (USA).

There are 6 participants in the Sirius-23 experiment, four women and two men. The average age of the volunteers was 31.5±5.5 years, height 173.5±13.5 cm, body mass index 23.6±3.4. Physical performance was assessed by means of three different maximal exercise tests: on a h/p/cosmos motorized treadmill, on a Koenigsmann mechanical treadmill and on a Via Sprint cycle ergometer. The treadmill test protocols are identical and consist of 5 minutes of rest, three minutes of walking at a speed of 3 km/h, followed by a step-increasing load, increasing the running speed by 1 km/h every minute, and the test is performed to the subjective maximum (to failure). The bicycle ergometer test has a similar structure: 5 minutes of rest, 3 minutes of pedaling the cycle ergometer at a power of 50 W, then every minute the power is increased by 25 W, the test is performed until failure. The pedaling frequency is 70-75 rpm. The tests included measurement of gas exchange in each respiratory cycle (Cosmed gas analyser) and heart rate (chest pulse sensor).

In the first month of isolation, subjects did not perform physical training, similar to the SIRIUS-21 experiment. In the second month, subjects began to perform physical training on a h/p/cosmos and Koenigsmann treadmills, and a Via Sprint cycle ergometer. Training was organized into a 4-day microcycle with three training days and one rest day, 5 microcycles constituted a training cycle, and a battery of tests was performed after each training cycle. In each microcycle, workouts were performed on two treadmills and a cycle ergometer, the order was determined randomly. In the second month, the training protocols were similar to those used in the SIRIUS-21 experiment. Next, subjects performed experimental exercise 15-minutes protocols. The creation of shortened effective training protocols is due to the request of Russian cosmonauts and planning groups to shorten the duration of preventive measures in space flight. The new protocols were created based on the Tabata methodology (Tabata I, 2022; Tabata I, 2019). The training protocol contained a warm-up (4-5 minutes of walking and quiet jogging), the main part (high-intensity intervals of 20 seconds with a rest between intervals of 10 seconds) and a warm-down (4-5 minutes of walking and quiet jogging). Exercise intensity was calculated based on step-increasing test results.

RESULTS

in the Sirius-21 experiment, the maximum power achieved in the bicycle ergometer test did not change significantly on average for the group during the isolation with a tendency to increase power by month 3-5. Maximum oxygen consumption increased significantly by the fourth month compared to the first test in both absolute (l/min) ($p=0.02$) and relative values (ml/kg/min) ($p=0.003$). After the sixth month of isolation, maximum HR was significantly decreased compared to the first month ($p=0.04$). During the period of absence of preventive measures in isolation, a significant decrease in the functional capacity of the cardiovascular system was observed when performing a battery of performance tests. The proposed scheme of twice-daily trainings allowed to compensate the influence of the reduced level of physical activity on the crew members' health. By the 4th month of isolation in the bicycle ergometer test, there was a tendency to increase the indices of maximum achieved power, a significant increase in maximum oxygen consumption, a decrease in the physiological cost of work, and by the 6th month there was a decrease in HR in response to the load. The lability of the cardiovascular system response to load changes in the locomotor test increased in the periods with the use of prophylactics.

By the third month of Sirius-23 experiment no reliable change in physical performance was found in the group of volunteers, the changes were individual. However, there is a tendency to decrease HR and oxygen consumption level when performing standard load, which indicates a positive effect of training measures. The application of new 15-minute training did not lead to a decrease in the level of physical performance, on the contrary, there is a tendency to decrease HR and VO2 peak when performing the standard load, which can be interpreted as a decrease in its physiological cost. Interestingly, the absence of training activities in the first month of isolation did not significantly affect the level of physical performance, which is not consistent with the results obtained in the SIRIUS-21 experiment. Apparently, a greater loss of physical performance level under hypodynamic conditions in the absence of physical training should be expected in more trained testers. On the contrary, the short-term (up to 1 month) effect of hypodynamia and isolation has no appreciable negative effect on the level of physical performance of crewmembers who did not exercise regularly before the beginning of the experiment.

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MBRSC SPONSORED ISOLATION AND ISS STUDIES: DATA FROM THE UAE

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

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Abstract

Isolation, confinement and Extreme (ICE) analog environment are used to simulate the effects of prolonged isolation on human health. By creating controlled environments that mimic the challenges of space travel, such as isolation, confinement, limited resources, and communication delays, ICE analogs are routinely used by researchers to study the effects of these conditions on human physiology, psychology, and (team) performance as well as team cohesion. These factors, which have been shown to affect the success of long-duration human spaceflights, are used by researchers to evaluate the effects of long-duration space travel on humans and, more importantly, to develop strategies to overcome challenges.

Reasons for conducting Analog missions include: Cost effectiveness of simulating isolation seen in spaceflights; Human factor research (stress physiology and psychology); Effects of complex stressors (isolation, confinement, sleep deprivation) on human health; Ensuring success of future space-flight missions; Technology and systems testing; Mission preparation, especially as we prepare for deep space missions; training and skills development for future astronauts; and scientific research and knowledge generation. By performing analog studies, scientists from MBRU and space agencies like the MBRSC can gain insights into the challenges of space travel, enhance understanding of human adaptability in extreme environments and assist in developing countermeasures. These studies play a crucial role in advancing space exploration and ensuring the wellbeing and success of astronauts on future missions. While analog studies do not fully replicate the unique challenges of spaceflight and/or the microgravity environment encountered in space, which could affect the accuracy and applicability of the findings, they are the closest to what can be utilized to understand the effects of long-time isolation and confinement in extreme environments.

MBRSC, together with NASA, ESA, and the Russian space Agency, recently concluded an 8-month isolation study at the SIRIUS facility at IMBP in Russia. MBRU researchers and international collaborators from Austria and Canada examined the effects of isolation on cardio-postural interactions.

That is, how does the isolation affect hemodynamic and autonomic function, which play important roles in cardiovascular regulation, especially when standing up from a sitting or a lying down position. MBRU team collected data from Emirati, American and Russian analog astronauts. Due to the limited number of participants that can be included in the SIRIUS isolation study, MBRSC has now sponsored several short-term isolation studies but with greater sample size at HERA facility in Texas. In collaboration with NASA, and supported by MBRSC, the MBRU team is conducting the same study in HERA participants. These studies are foreseen to run over four isolation periods at HERA, with several participants in each mission. Finally, MBRSC has supported exactly the same project to be carried out on the ISS in the mission of the Emirati astronaut, Sultan Al Neyadi. This project, implemented by the Canadian Space Agency, is currently ongoing and provides a validation of the data that were generated on Earth from the SERIUS isolation campaign as well as those that will be generated during the upcoming-HERA missions.

This talk provides an overview of analog studies, which are a cost-effective way to simulate space-related isolation conditions on Earth, whereas space stations offer a real environment for conducting experiments. Indeed, data generated from the ground based analog studies such as those in SERIUS or in HERA provide insights into the both the physiological and psychological effects of complex stressor such as those encountered in space. Once these data are generated, the proof of concept is then tested in space environment such as the ISS. The data generated from the recently completed SIRIUS project CARDIOPOST are ongoing project CARDIOBREATH that is being carried out in space is being compared. Both approaches have their unique advantages and limitations, and they complement each other in advancing our understanding of space exploration. This talk concludes by acknowledging the financial and logistical support provided by the MBRSC for these ground-based analog studies as well as those that are currently being carried out in space.

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

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BACKGROUND

In the extreme environment of space, the human body undergoes profound physiological changes that challenge its adaptability. Microgravity, for instance, disrupts the equilibrium between bodily fluids, leading to fluid shifts which could result in health consequences such as cardiovascular diseases. While analog studies do not fully replicate the space environment as they lack the effects of microgravity and cosmic radiation, they can simulate long-term space missions by subjecting participants to prolonged confinement in isolated settings. Therefore, they represent invaluable tools to develop countermeasures and strategies to mitigate the health risks associated with space flights and enhance the well-being of astronauts during extended missions.

Objectives

To study the impact of an 8 month-isolation period, mimicking the condition of life in the International Space Station, with no microgravity and radiation, on body composition and cardiorespiratory function.

METHODOLOGY

An 8-month isolation study was conducted at the Institute for Biomedical Problems in Moscow, Russia, from November 4, 2021, to July 3, 2022, as part of the SIRIUS-21 program. Six healthy international adults crew members (50% female) were included. The study simulated a restriction of social contacts, restriction of social contacts in a limited space. Extravehicular activities (EVA) were performed using Virtual Reality technologies and physical EVA models. Crew members were engaged in endurance and resistance exercise.

Physical activity was monitored using Garmin device over periods of 10 consecutive days. The average duration in hours per day was calculated. A Cardiopulmonary Exercise Test (CPET) was performed at baseline and at the end of the study. Markers of the cardiorespiratory function were obtained from the CPET: Heart Rate (HR, bpm), VO_{2max} (mL/min/kg body weight), Respiratory quotient (RQ), %Fat and %Carbohydrate (CHO), Energy expenditure per minute (Eem, kcal/min) and METs. Body Composition, including Total weight (kg), %fat mass, %lean mass, Body Mineral Density (BMD, g/cm2) and Body Mineral Content (kg), was evaluated before and after the mission using DEXA.

Data were analyzed by using SPSS v28. Normality was tested by using the Shapiro-Wilk test. Data were presented as mean±SD and median (Q1-Q3) for normally distributed and skewed variables, respectively. Baseline and end-of-study values were compared by using paired t-test for normally distributed variables and Wilcoxon signed rank test for skewed variables. The statistical significance level was set at p=0.05.

RESULTS

One crew member did not complete the final assessment and was excluded from the comparisons. The mean age was 34.20 ± 2.40 years. The mean weight decreased from 79.53 ± 16.76 to 73.80 ± 13.72 kg over the whole study, but it was not statistically significant. In contrast, the percentage of fat mass significantly decreased from 34.40 ± 3.91 to 30.86 ± 6.12% ($p = 0.04$), and bone mineral density (BMD) significantly increased from 1.19 (1.16-1.18) to 1.21 (1.18-1.20) g/cm2 ($p = 0.04$). In contrast there was an increase in mean METs from 6.21 (5.46-6.64) to 6.35 (5.9-6.5). The results indicated a substantial increase in physical activity (PA) duration over the 8 months study duration (p-value=0.025).

According to the METs value, the crew members reached a high moderate to low vigorous level of intensity during the CPET. The median value for RQ indicated that mainly carbohydrates were oxidized to cover the energy needs related to the exercise (Table 1). All variables did not change after 8 months isolation except %Fat and %CHO which reduced and increased, respectively (Table 1). This may be related to the increased practice of physical activity over time inducing a metabolic adaptation towards a greater use of glucose and potentially a delayed anaerobic threshold.

HR and Eem did not significantly change over the 8 months period of isolation although there were a trend indicating a reduction of both HR and Eem during the exercise phase of the CPET. %Fat significantly reduced while %CHO significantly increased during the exercise phase of CPET. All this may be related to the practice of physical activity inducing cardiorespiratory and metabolic adaptations towards a more efficient heart muscle contraction and a greater use of glucose.

Table 1: Markers of the cardiorespiratory and metabolic functions during the exercise phase of the CPET at baseline and after 8-months isolation. Data are presented as mean±SD, median (Q1-Q3) as appropriate.

Marker	**Baseline**	**End-of-study**
HR (bpm)	140.73 (137.16-159.31)	136.91 (127.13 – 146.75)
VO_{2max} (ml/min/kg)	22.92±4.01	22.14±1.10
RQ	0.98 (0.92 -1)	0.99 (0.95 – 0.99)
%Fat	17.06±5.17	14.17±3.43*
%CHO	82.94±5.17	85.83±3.43*
Eem (kcal/min)	9.13±2.80	8.30±1.76
METs	6.21 (5.46-6.64)	6.35 (5.9 – 6.5)

Paired t-test or Wilcoxon signed rank-test were performed for comparisons. *Statistical significance set at $p<0.05$

CONCLUSION

After 8-months isolation, the body composition improved with greater BMD and reduced fat mass. In addition, cardiorespiratory fitness improved as shown by the decreased HR during the CPET, and first signs of metabolic adaptations were detected as indicated by the greater contribution of glucose to the energy production during exercise. This may be partially related to the increased time spent on physical activity. The results highlight the importance of the practice of physical activity to potentially counteract the negative adaptations to a long isolation as it happens during long-term space missions.

Acknowledgement:

We extend our sincere appreciation to the Mohammed Bin Rashid Space Centre for their invaluable support and collaboration.

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

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BACKGROUND

During space missions, the human body undergoes profound biological, physiological, and metabolic changes due to the unique conditions of the environment. These adaptations present significant challenges to the health and performance of astronauts. One notable effect observed in astronauts is a diabetes-like profile, resembling the conditions seen in isolated individuals on Earth. This phenomenon has been consistently documented in ground-based analog studies, which simulate aspects of spaceflight conditions by subjecting participants to extended periods of isolation. Therefore, identifying effective preventive measures to mitigate the risks associated with these changes is essential for the well-being of astronauts during long-term space missions. Although analog studies cannot fully replicate the space environment, as they lack factors such as microgravity and cosmic radiation, they provide valuable insights into the physiological responses to prolonged confinement and can play a crucial role in this endeavor. Physical activity has emerged as a potential countermeasure to mitigate the adverse effects of spaceflight on health. However, its specific impact on glucose homeostasis in isolated and confined conditions remains to be fully elucidated.

OBJECTIVE

To determine the impact of 8-months isolation ground-based study, simulating the conditions of long-term space mission, combined with regular exercise, on the body composition and blood glucose homeostasis in healthy adults.

METHODOLOGY

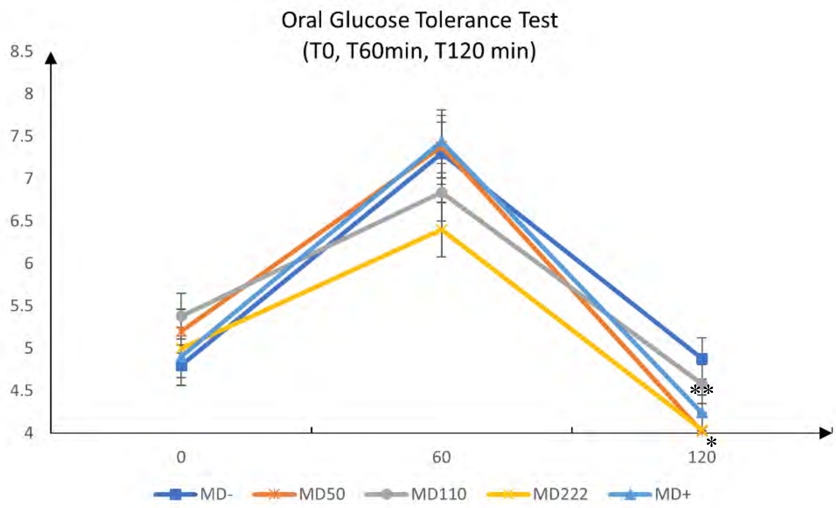
Six healthy adults from diverse international backgrounds (50% female) were recruited to participate in the SIRIUS-21 program. The study was conducted at the Institute of Biomedical Problems (IBMP) in Moscow, Russia, from November 4, 2021, to July 3, 2022. SIRIUS-21 simulated a mission to the Moon, lunar orbit operations, and extravehicular activities, with participants residing in the IBMP's ground-based facility for 240 days (8 months). Throughout the experiment, crew members engaged in tasks relevant to space flight operations, including daily medical monitoring, comprehensive health assessments, activities aligned with the flight scenario and regular physical training.

Body composition parameters (weight (kg), %fat mass, %lean mass, Bone Mineral Density (BMD, g/cm2), and Bone Mineral Content (BMC, kg) were assessed using DEXA scans before and after the isolation period. An oral glucose tolerance test (OGTT) was conducted five times: at baseline, on days 50, 110, 222, and at the end of the study. The area under the curve (AUC) was calculated using the trapezoidal method. Blood levels of HbA1c were measured before and after the isolation period. Physical activity was monitored using the GARMIN device over periods of 10 consecutive days, before, during, and after the isolation period, with daily duration measured in hours. Appetite was evaluated using a 10-point visual analog scale consisting of eight items.

Data analysis was performed using SPSS v.28. Normality was assessed using the Shapiro-Wilk test. Normally distributed variables are presented as mean±standard deviation (SD), while skewed variables are presented as median (Q1-Q3). Comparisons between time points were conducted using paired t-test, repeated measures ANOVA, and post-hoc Tukey tests for normally distributed data. For skewed data, the Friedman test and Dunn's multiple comparisons tests were employed as non-parametric alternatives. Statistical significance was set at 5%.

RESULTS

One crew member did not complete the final assessment and was excluded from the comparisons. The mean age was 34.20±2.40 years. While the total weight did not significantly change (from 79.53±16.76 to 73.80±13.72 kg) over the isolation period, the percentage of fat mass significantly reduced from 34.40±3.91 to 30.86±6.12% ($p=0.04$). BMD significantly increased from 1.19 (1.16-1.18) to 1.21 (1.18-1.20) g/cm2 ($p=0.04$). The crew members became more active, with an average time spent in physical activity of 1.16 hours/day at baseline and 3.20 hours/day at the end of the isolation period ($p<0.01$). The appetite level tended to decrease at the three main meals, but significantly at breakfast only. The blood level of HbA1c decreased from 5.10±0.29 to 4.88±0.08% ($p=0.62$). During the oral glucose tolerance test (OGTT), the blood glucose level at 2 hours after glucose ingestion decreased over time (from 4.88±0.22 to 4.04±0.29 mmol/L, $p=0.02$). This decrease began within the first 50 days of isolation and continued until day 110, after which it remained stable but still lower than the baseline value (Figure 1). Similar changes were observed for the AUC.



MD-: baseline; MD50: Day 50; MD110: Day 110; MD+: End of isolation. **Different from MD-, *Different from MD50

Figure 1: Blood glucose levels at T0, T60min and T120min during oral glucose tolerance tests at different timepoints

CONCLUSION

Overall, the SIRIUS-21 8-months isolation was associated with improved blood glucose control and body composition. This might be explained by the regular practice of physical activity which would support a counteracting effect of regular physical activity against the side effects related to long-term isolation and the importance of incorporating physical activity to the daily program of astronauts in the future space missions.

This study was conducted with the support from the Mohammed Bin Rashid Space Center, Dubai, UAE.

Effects of Isolation on Cardiovascular and Autonomic systems

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Abstract

The isolation and confinement of a spacecraft or habitat can lead to psychological and physiological changes in the human body, including changes in sleep patterns, mood, and cognitive function. Analog astronaut studies aim to identify these changes and develop strategies to mitigate them. In the 9-month long SIRIUS study, examined each month were the effects of the alterations in hemodynamic and autonomic responses during a 5-min sit-to-stand test. The sit to stand test is a physical performance test that measures a person's ability to stand up from a seated position and return to a seated position. It is used to assess cardiovascular reactivity and autonomic function and is a particularly important test for assessment of orthostatic hypotension. It was hypothesized that due to the reduced physical activity in isolation and the complex stressors induced by isolation, the hemodynamic and autonomic parameters will be affected and reflected during the sit-to-stand test.

This 9 month long analog study was conducted in the SIRIUS facility in Moscow, Russia. Participants were selected across the globe based on inclusion and exclusion criteria specified by space agencies and candidates with any cardiovascular pathologies, musculoskeletal and psychological / psychomotor perturbations were excluded. All participants performed a sit-to-stand test before entering the SIRIUS isolation facility, and this recording was used as baseline. During the mission, they performed sit-to-stand tests every month at an interval of 30 days. The same test was consecutively performed once a week for two weeks after completion of the isolation mission. The sit-to-stand test consists of 5 min sitting, 5 min standing and 5 min of re-sitting. For the sitting phase of the test the participants were required to sit quietly, and the feet placed in a parallel formation with both heels 10 cm apart. The Task Force Monitor (TFM, CNSystems, Graz) was used to collect all the physiological signals. Isolation and confinement show negative effect on the cardiovascular and autonomic systems, including: 1) Increases in heart rate during standing up; 2) Response in each participant was always similar across the isolation period; 3) Increase in sympathetic activity across the isolation period. Few analog astronauts showed high stress level at baseline and during the first three months of isolation. This increase in stress levels, especially during the first three months of isolation, agrees with what has been previously reported in the literature.

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Altered Cardiorespiratory Interactions with Spaceflight: Preliminary Results from CARDIOBREATH

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INTRODUCTION

Astronauts undergo significant changes in their cardiovascular and respiratory systems during spaceflight, which can severely impact their exercise capacity and ability to maintain blood pressure when standing upon return to gravity. Cardiovascular deconditioning, including post-flight orthostatic intolerance, remains a persistent problem associated with time spent in microgravity during spaceflight (Antonutto G. et al., 2003). Post-flight cardiorespiratory deconditioning has also been documented after short- (Verheyden B. et al., 2007) and long- (Cooke W.H. et al., 2000) duration spaceflights where the respiratory sinus arrhythmia (RSA), a well-recognized modulation of respiration on heart rate, was diminished post-flight. However, the cause of the reduced cardiorespiratory coupling post-flight is still controversial, perhaps due to the differences in the spaceflight duration and the limited number of participating astronauts. Here, we analyze data from the Causality Analysis of Respiratory Dynamic Interactions in Orbit: Breathing, Rest, Exercise And The Heart (CARDIOBREATH) study and present the cardiovascular and cardiorespiratory responses of four astronauts before, during, and after 6-month spaceflight on the ISS.

METHODS

Data Collection

Data were collected before (2 sessions 2 days apart: L–100 to L–60 (BDC1, BDC2)), during (3 sessions: L+20 to L+40 (IN1), L+80 to L+100 (IN2), and R-30 to R-10 (IN3)), and after (2 sessions: R+6 to R+8 (POST1) and R+13 to R+15 (POST2)) spaceflight. During each session, the test protocol (Fig.1) includes a 6-min quiet stand (on ground) or 7-min quiet float (inflight), followed by a 25-min exercise on cycle ergometer (on ground) or CEVIS (inflight) and a 2nd quiet stand (on ground) or float (inflight). Cardiovascular and respiratory measurements were collected using the Bio-M garment (Carré Technologies Inc., Montreal, QC, Canada), including ECG, heart rate (HR), RR interval (RR), systolic blood pressure (SBP), breathing rate (BR), minute ventilation ($\dot{V}E$) and continuous respiration signal (RESP).

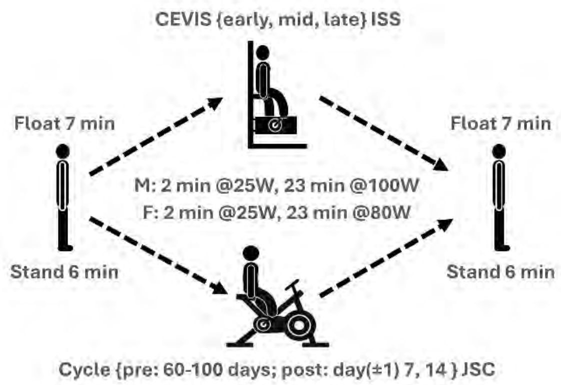

Figure 1: CARDIOBREATH exercise protocol. Ground sessions include a quiet stand before and after cycle ergometer exercise. Inflight sessions included a quiet float before and after CEVIS exercise.

Data Analysis

Data collected during cycling and standing (or floating) were analyzed to study the cardiovascular and respiratory responses. BDC1 was excluded from the analysis because of missing data points. Fraction time active (FTA), the active interaction time between two measurements (Xu D. et al., 2020), was calculated to characterize the coupling of three regulatory mechanisms: SBP→RR (cardiac baroreflex), SBP→RESP (cardioventilatory coupling), and RESP→RR (respiratory sinus arrhythmia). Venn diagrams were constructed to represent overlapped regions where two or more mechanisms were active at the same time (Garg A. et al., 2014). A repeated measures two-way ANOVA (Exercise (cycling/standing or floating) X Test Day) was used. Significance was assessed post hoc with Tukey's HSD. Data are reported as significant ($p<0.05$).

RESULTS

FTA of cardioventilatory coupling (SBP→RESP) decreased inflight (IN1 and IN2) compared to those on the ground (BDC2 and POST1) while RSA (RESP→RR) exhibited an opposite trend with FTA of IN2 significantly higher than that of POST1 (Fig. 2b). Cardioventilatory coupling was also more active during standing/floating after cycling. While cardiac baroreflex exhibited no changes, its co-activation with RSA showed more prominent coupling activities during inflight (IN2) than on ground (BDC2 and POST1,2), as well as during stand/float before exercise (S/F1) than cycling (Cyc) and stand/float after exercise (S/F2) (Fig. 2b). Cardioventilatory coupling and RSA worked less closely during exercise than stand/float, which was also true when all three mechanisms were active at the same time (Fig. 2b).

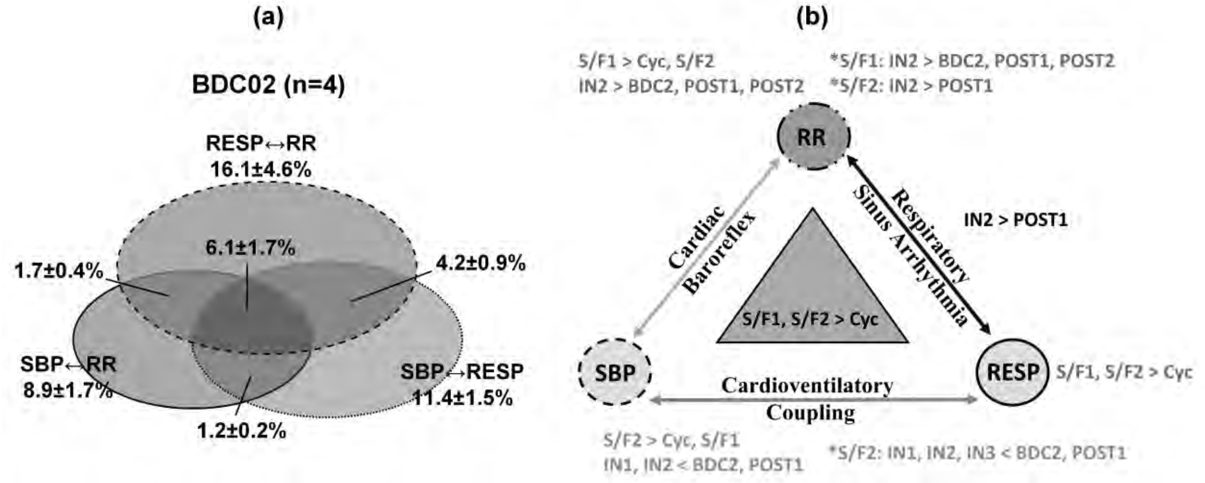

Figure 2. (a) Example of Venn diagram of session BDC2 from the four astronauts. (b) Major changes observed from the two-way ANOVA. Arrows: regulatory mechanisms between two measurements. Circles: two mechanisms were active at the same time. Triangle: all three mechanisms were active at the same time. *: interaction effect existed between factors Exercise and Test Day. Cyc: cycling. S/F: stand/float.

DISCUSSION

Cardiorespiratory interactions, including cardioventilatory coupling (SBP→RESP) and RSA (RESP→RR) were found to be significantly affected by spaceflight. Cardioventilatory coupling was diminished during spaceflight, indicating impaired responsiveness of respiration to blood pressure changes. The enhanced RSA activities under microgravity compared with post-flight could be attributed to altered ventilation and pulmonary perfusion inflight, which changed the cardiac vagal tone. Although no changes were observed with cardiac baroreflex alone, the synergy between cardiac baroreflex and RSA was higher under microgravity, suggesting increased modulation of heart rate from both central feed-forward respiratory driven (Skytioti M. et al., 2022) and peripheral reflex mechanisms. While no significant pre- and post-flight differences were found, a trend of post-flight decrease in cardioventilatory coupling as well as its co-activation with RSA was observed. Therefore, with more data collected and analyzed in the CARDIOBREATH study, it is reasonable to hypothesize impaired cardiorespiratory responses after long-term spaceflight.

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Understanding mechanisms and unveiling countermeasures for the bedrest-induced decrease in cerebral blood flow: Preliminary data

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Studying the cerebral circulation in actual or simulated microgravity remains difficult, yet a common finding of bedrest studies is a decrease in cerebral blood flow (Kramer et al., 2017; Marshall-Goebel et al., 2016; Ogoh et al., 2020). Because the brain has an extraordinarily high metabolic demand, but very limited energy storage, changes in cerebral perfusion may impact the cognitive function of astronauts, or bedridden patients on Earth. Indeed, evidence for cognitive impairment has been seen during experimental bedrest (Lipnicki et al., 2009; Liu et al., 2012) and hospitalisation (Calero-García et al., 2017). Therefore, identifying the underlying mechanisms causing a reduction in cerebral blood flow after bedrest is important and may include: 1) an increased concentration of red cells due to plasma volume contraction (Greenleaf et al., 1989; Ogoh et al., 2020) and the subsequent increase in oxygen carrying capacity, 2) cerebral autoregulation causing excessive vasoconstriction due to the persistently higher arterial pressure at brain level relative to brain level in the upright posture.
We are currently performing a series of 2-day bedrest studies whereby we hypothesize that normalizing haemoconcentration via plasma volume expansion will restore cerebral blood flow to baseline values (protocol 1). Moreover, as a more applied countermeasure, daily exercise training will also prevent the loss in plasma volume, maintain haemoglobin concentration at baseline values, and prevent the fall in cerebral blood flow (protocol 2). Finally, head elevation will lower arterial pressure at brain level, but will not affect the expected decrease in cerebral blood flow with bedrest (protocol 3). In addition to cerebral blood flow, ocular blood flow was estimated from the posterior ciliary artery.

METHODS

Each participant underwent bedrest three times, with three different experimental protocols: 1) 48 hours of bedrest where after participants received plasma volume expansion (PVE) via an isotonic crystalloid intravenous infusion to restore haemoglobin concentration to supine values; 2) 48 hours of bedrest whereby participants performed 40 minutes of steady-state moderate continous exercise twice a day; 3) 48 hours of bedrest with head only elevation.
Measurements were performed in the supine posture and after bedrest. Cerebral blood flow was measured using Duplex ultrasound of the internal carotid and vertebral artery alongside a supplementary estimate from transcranial Doppler of the middle cerebral artery. Blood flow to the eye was estimated via Doppler ultrasound of the posterior ciliary artery. Finally, haemoglobin concentration, heart rate (ECG), mean arterial pressure (Finometer), and end-tidal CO_2 were obtained at all time points.

PRELIMINARY DATA

Data collection is currently ongoing. Our preliminary data suggest that cerebral blood flow tended to decrease during bedrest, but it was restored (supine 895±99, vs PVE 934±230 ml/min) with the normalization of haemoglobin concentration (supine 13.8±0.0, post bedrest 15.1±0.2, vs PVE 13.7±0.6 g/dL). Moreover, daily exercise training seemed to prevent the fall in cerebral blood flow (supine 947±49, vs bed rest 1015±66 ml/min) associated with bedrest. In contrast, there was a tendency for cerebral blood flow to decrease (supine 977±188, vs bed rest 788±53 ml/min) during bedrest despite head elevation. Interestingly, posterior ciliary artery velocity showed variable results in this limited sample size, yet a uniform decrease was observed in the exercise countermeasure trial (supine 10.6±1.1, vs bedrest 9.0±2.1 cm/sec).

OUTLOOK

By exploring the underlying mechanisms associated with changes in cerebral and ocular perfusion with bedrest, this study aims to provide the science base for effective countermeasures. Therefore, this study is an important step towards ensuring astronauts' safety during future long-distance space missions. Potential differences in cerebral and ocular perfusion during bed rest may in addition shed light on the mechanism of SANS.

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GRAVITATIONAL DOSE-RESPONSE CURVES FOR CARDIOVASCULAR AND OCULAR VARIABLES AFTER 24H BEDREST OR DRUG-INDUCED HYPOVOLEMIA

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INTRODUCTION: Exposure to microgravity results in a permanent headward fluid shift, which might be related to spaceflight-induced neuro-ocular changes, and stagnant or retrograde blood flow, and venous thrombosis in the jugular vein. Thus, characterizing the physiological changes associated to fluid shifts are important to the development of potential countermeasures. Our previous work has quantified cardiovascular and ocular gravitational dose-responses to graded tilt on healthy individuals (Whittle R.S. et al, 2022; Whittle R.S. et al, 2023). In this study, we propose to quantify fluid shift, cardiovascular, and ocular gravitational dose-responses in deconditioned individuals.

METHOD: Thirty-two healthy subjects (16M/16F) will undergo 24h of 6° head-down bed rest (HDBR) and furosemide-induced rapid hypovolemia in a crossover design, as represented in **Figure 1**. Before and after the deconditioning interventions, subjects will be exposed to a graded tilt protocol (from 45° head-up tilt to 45° head-down tilt, in increments of 15º) to generate gravitational dose responses. Measurements include calf volume, segmental hydration state, plasma volume, continuous cardiovascular hemodynamic, superficial venous network, muscle and cerebral blood flow, characteristics of the common carotid artery and internal jugular veins (cross-sectional area and internal jugular vein pressure), optic nerve sheath diameter, intraocular pressure, and ophthalmic artery perfusion.

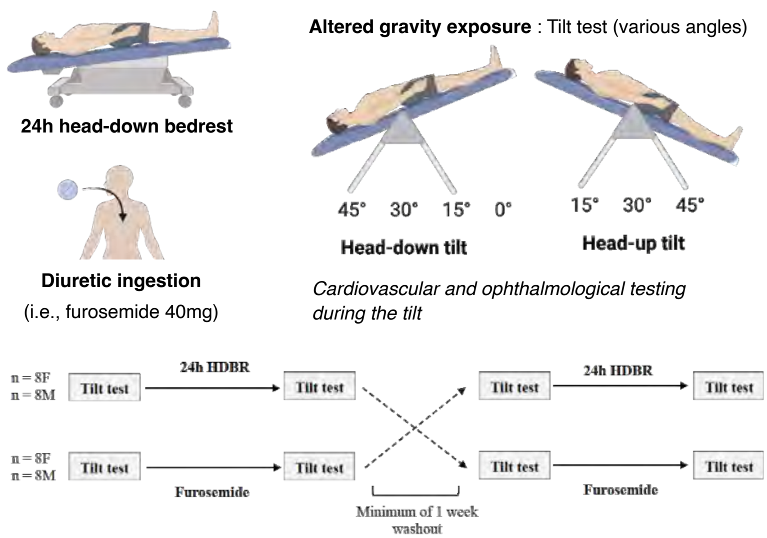

Figure 1: Schematic representation of the study design. Participants will be assigned to one of the two interventions: 6° HDBR or furosemide-induced rapid hypovolemia. A graded tilt test (from 45° HDT to 45° HUT, in increments of 15°) will be performed to generate gravitational dose-response curves of multiple fluid shift, cardiovascular, and ocular parameters. After a minimum of 1 week washout period, participants will repeat a similar protocol using the alternative intervention (counterbalanced order). HDBR: head-down bedrest.

HYPOTHESIS: We hypothesized that dose-response curves after the deconditioning interventions will differ from the "healthy" dose-response curves for most variables. We will also perform the experiments in both males and females to characterize sex differences in these responses. We aim to generate a comprehensive integrative framework for fluid-shift response prediction, constituting an important terrestrial model to reference spaceflight induced changes. It will contribute to the assessment of the headward fluid shift, the pathogenesis of SANS and spaceflight venous thromboembolism events, and inform the development of countermeasures and in-flight prescriptions.

ACKNOWLEDGEMENTS

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

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INTRODUCTION

Self-organized criticality (SOC) is a universal theory that governs the dynamics of natural systems, including the cardiovascular system (Muñoz, 2018). Cardiovascular SOC has been observed to correlate significantly with upright posture, particularly concerning incidents of vasovagal syncope that predominantly occur in this stance (Fortrat & Gharib, 2016; Fortrat & Ravé, 2023). The relevance of cardiovascular SOC extends notably to spaceflight simulation, where it sheds light on cardiovascular deconditioning and the associated heightened risk of orthostatic syncope (Navasiolava, 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 sole means of replicating the fluid shift experienced in the upright position during spaceflight is through the lower body negative pressure test (LBNP). This test, introduced on the Mir space station, 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 both in actual and simulated weightlessness scenarios.

METHODS

Actual Weightlessness: LBNP was applied to a cohort of 10 cosmonauts (1 female, aged 42 ± 1 years, weighing 73 ± 3 kg, with a mean height of 1.76 ± 0.02 m, reported as mean ± SEM) at various time points before, during, and after spaceflight aboard the Mir station. The duration of spaceflight ranged from 2 weeks to 12 months. LBNP tests before the flight were conducted at two time points: two months and one month (-60d and -30d, respectively). Additional testing occurred in the first three months of flight, and after the third month of flight (<120j and >120j, respectively), as well as 3 days and 7 days post-landing (3d and 7d, respectively). The LBNP protocol comprised 10 minutes of rest followed by 5 minutes at –25mmHg, 1 minute at -35mmHg, and finally, 5 minutes at –45mmHg. Beat-by-beat RR-interval was continuously monitored using Physiolab, a modified Portapres device, throughout the entire LBNP protocol. Recordings were subsequently split into three 5 min periods that included rest, -25mmHg and -45mmHg for analysis of cardiovascular SOC as explained below.

Simulated Weightlessness: Nine healthy men aged 25 to 43 years participated. They underwent a 5-day dry immersion (DI-5 CUFFS). Beat-by-beat RR interval was recorded during LBNP according to the standard ESA protocol (LBNP). The procedure included a 5 min rest followed by increment of -10 mmHg step applied during 3 min until -60 mmHg or the occurrence of orthostatic intolerance symptoms. Consecutive heartbeats were isolated for each LBNP step form rest to -40 mmHg for analysis of cardiovascular SOC as explained below.

RR-interval time series were manually filtered. Bradycardia episodes were identified and counted according to their length in number of beats as previously described and to draw the Zipf's plots (Fortrat et Ravé, 2023). The maximal length of bradycardia episodes was determined for each LBNP step. An index of criticality for a whole LBNP test was determined as the sum of the maximal length of bradycardia of each step.

Periods of spaceflight were compared by means of a mixed model analysis followed by a Dunett's test. Before and after dry immersion periods were compared by means of a paired t-test after check for normality by means of Kolmogorov Smirnov's test.

RESULTS

Details of both the experiments and results have been previously reported (Coupé et al., 2010; Robin et al., 2020). These previously reported results demonstrated the cardiovascular deconditioning through the resting tachycardia following both experiments. Only five cosmonauts from the ten performed inflight LBNP. SOC index was increased during spaceflight and remained increased until seven days after (Figure 1). This index assessed on standard ESA LBNP procedures during simulated weightlessness remained unchanged (Figure 2).

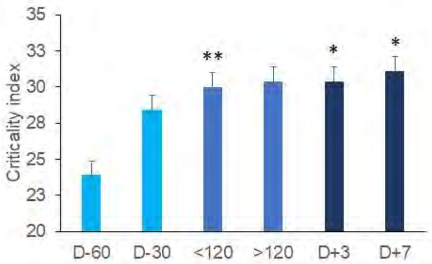

Figure 1: Cardiovascular criticality index during lower body negative pressure performed before, during, and after spaceflight (60 and 30 days before, less or more than 120 of flight, and 3 and 7 days after landing; * $p < 0.05$, ** $p < 0.01$ vs D-60

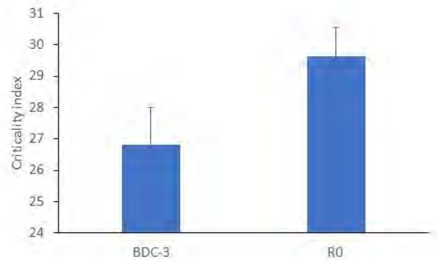

Figure 2: Cardiovascular criticality index during lower body negative pressure performed before and after a spaceflight simulation by dry immersion (BDC-3 and R0, respectively).

DISCUSSION

The main finding of our study is that cardiovascular self-organized criticality is altered during actual weightlessness. These findings confirmed the result of an altered cardiovascular SOC during spaceflight simulation by means of head-down bed rest (Navasiolava et al., 2023). The orthostatic test used during this later experiment was a stand test and contrast with the today commonly used standard procedure of LBNP recommended by ESA. Our experiment however showed that this procedure is not appropriate to study cardiovascular SOC probably because of the short duration of LBNP steps during this procedure.

CONCLUSION

Actual spaceflight alters cardiovascular self-organized criticality. Further studies to improve our understanding of this important topic must be performed during specific procedures that include long enough recording times to perform a reliable analysis.

ACKNOWLEDGMENTS

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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

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ABSTRACT

The amplitude parameters of blood pressure (BP) and heart rate (HR) oscillations in the low-frequency (LF, frequency of baroreflex waves, ~ 0.1 Hz) and high-frequency (HF, corresponding to the respiratory frequency, 0.2-0.25 Hz) ranges are widely used to assess the state of the cardiovascular system of astronauts and patients suffering from orthostatic instability. To increase the information content of the traditional approach we analyzed time (phase) relationships between blood pressure and heart rate oscillations. A passive tilt test was performed (transition from a lying position to an orthostatic position, 65 degrees), during which the person maintained a constant and comfortable breathing rate. Software has been developed to set the breathing rhythm, record and analyze experimental data. Narrow-band components extracted by digital filtering from the obtained time series of BP and HR were presented in the form of an analytic signal, that allowed us to determine their phases and then calculate phase difference and phase synchronization in different frequency ranges. It was shown that in healthy people the degree of synchronization of LF oscillations in blood pressure and heart rate increases during orthostasis, while at the same time there is a sharp decrease in the time lag (phase difference) of HF oscillations of blood pressure and heart rate. After a 1-2 week stay in “dry” immersion (the period of the most pronounced dysregulation of the cardiovascular system), the effects described above disappear, which indicates an impairment of the nervous regulation of heart rate. Thus, complex analysis of the time relationships of blood pressure and heart rate oscillations in the LF and HF ranges can be used to assess the nervous regulation of the heart during simulated gravitational unloading. This approach might be used in the development of countermeasures to prevent orthostatic intolerance.

FUNDING

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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 & 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.

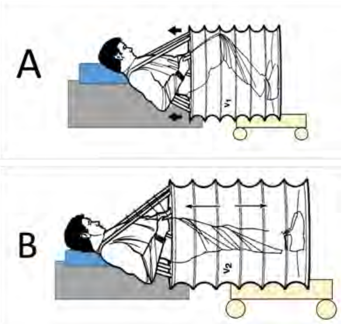

Figure 1. *SELF Device. A raised platform supports the user's upper body while a moveable platform underneath the bottom plate allows the chamber to expand and contract.*
A. *Squat maneuver with open valves.*

B*. Leg extension with closed valves. Expansion of the sealed chamber generates negative pressure.*

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 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 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.

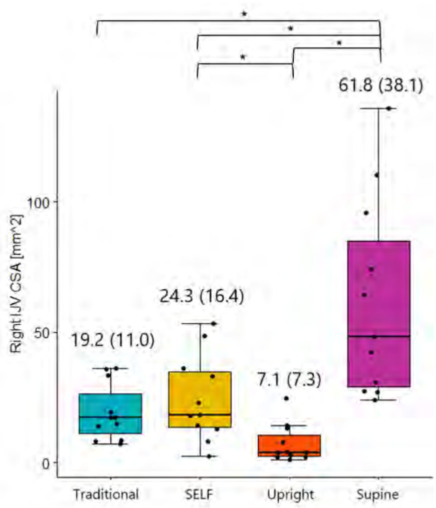

***Figure 2**. Right IJV CSA [mean (SD)] across different conditions.*

DISCUSSION

Our findings indicate that SELF LBNP may reduce IJV CSA, and by extension, 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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Exploring the Impact of Simulated Microgravity on Osteoarthritis Development: The Role of CD36 and Sex-Specific Responses in a Mouse Model

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BACKGROUND

Leveraging the unique conditions of space microgravity, our research offers groundbreaking insights into osteoarthritis (OA), a degenerative joint disease impacting over three million Canadians and predominantly affecting load-bearing joints such as the knee. With knee OA (KOA) known for its higher incidence and severity among females—a phenomenon not fully explained by existing risk factors—the application of simulated microgravity (SMG) presents a novel approach to understanding the disease's progression and the biological sex differences in response to it.

By employing rotating wall vessel (RWV) technology to simulate spaceflight conditions on Earth, our studies focus on the biomechanical unloading experienced in microgravity, which has been observed to induce OA-like changes in articular tissues. This innovative research strategy allows us to explore the potential mechanisms of OA development and progression in a controlled environment, mimicking the microgravity of space to provide new perspectives on the disease's molecular underpinnings.

Initial results from our investigations reveal that SMG significantly alters the molecular expression profiles in engineered meniscus constructs derived from non-KOA patients, notably influencing genes related to chondrocyte hypertrophy and OA progression while downregulating protective genes. Further analysis has illuminated sex-specific molecular responses to SMG, demonstrating pronounced differences between male and female specimens. This highlights the importance of considering biological sex in OA research, as it significantly influences disease development and response to microgravity conditions. Extended RNA sequencing under SMG conditions has identified distinct molecular signatures for different sex-based groups, pointing towards an OA-like transcriptome profile in females particularly responsive to SMG.

One of the most significant findings from our microgravity studies is the modulation of CD36, a transmembrane protein implicated in lipid metabolism and OA pathogenesis. Its altered expression under SMG conditions and known upregulation in KOA patients underscore the potential mechanisms by which microgravity-induced cellular responses can mimic and inform us about OA development, particularly in terms of sex-specific disparities.

METHOD

In this phase of our investigation, we employed age-matched young mice, encompassing both sexes, including CD36 knockout (ko) mice and their C57Bl/6 strain control counterparts, as our animal model. Meniscus tissues were extracted from these mice, including both medial and lateral sections, leading to the creation of four distinct experimental groups: 1.Female CD36 ko mice 2.Female C57Bl/6 control mice 3.Male CD36 ko mice 4.Male C57Bl/6 control mice. These isolated meniscus tissues underwent a 3-week culture in simulated microgravity (SMG) conditions, utilizing a rotating wall vessel bioreactor. A control group was concurrently maintained under standard gravity conditions for comparative analysis.

Following the culture period, tissue constructs from each group were randomly selected for a series of analyses, including RNA sequencing (RNAseq) and reverse transcription-quantitative polymerase chain reaction (RT-qPCR), bone mineral quantification, histology and immunofluorescence assessments, shotgun proteomics, and evaluations of DNA and cartilaginous glycosaminoglycan (GAG) content (Figure1).

RESULT

The groups exposed to SMG, regardless of their genotype, showed significant degradation of extracellular matrix (ECM) components and a marked reduction in GAG content compared to the control group. These changes suggest an enhanced process of local hypertrophic differentiation and calcification, underscoring the potent OA-inducing effects of the SMG environment. Notably, within the SMG-exposed female cohorts, the deletion of CD36 significantly mitigated the advancement of meniscus calcification. By day 21, the calcification region in the CD36 ko group was approximately 20% smaller in size than that observed in the wild-type (WT) counterparts. Ongoing RNA sequencing aims to elucidate the specific mechanisms by which CD36 influences.

SIGNIFICANCE

This investigation sheds light on the complex molecular interplay modulated by microgravity, enhancing our comprehension of OA's pathogenesis, particularly regarding sex-specific differences and the modulatory role of CD36. By simulating the conditions of spaceflight microgravity, our research unveils novel insights into the mechanisms driving KOA and delineates the protective function of CD36 within this context. The findings not only bridge a significant gap in our understanding of OA but also lay the groundwork for the development of innovative therapeutic strategies. The pivotal role of CD36 in OA progression under SMG conditions accentuates the potential for targeted interventions, both in space health management and terrestrial medical practice, opening new avenues for the prevention and treatment of OA.

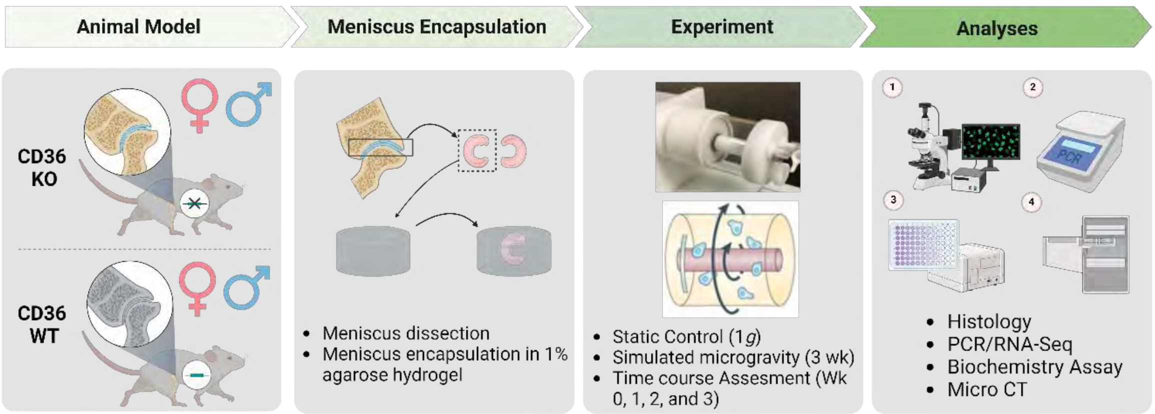

Figure 1: Experiment method overview

SpaceBike –
Preliminary Insights into Neuromuscular Adaptation through Bed Rest

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INTRODUCTION:

Learning is the key to progress in life, and lifelong learning is crucial for the development and improvement of humankind. From the first day of life, the brain changes through the process of learning, which is based on the emergence of well-developed pathways that are deeply embedded in the brain's structure and are called engrams. Walking, for example, is the most fundamental form of human movement and is based on motor engrams that are responsible for the execution of highly automated movements. Given the plans of international space agencies to travel deep into our solar system, the question arises as to how far it is possible to adapt motor engrams developed with and for the gravitational pulling of Earth to changing (i.e., reduced) gravitational conditions. Astronauts in space experience fluid redistribution due to microgravity, leading to increased intracranial pressure (ICP) which could affect the functionality of the engrams. Research has already shown that reduced gravity alters neural communication (Kohn et al, 2018). The influence of environmental changes, such as sustained elevated ICP, on neuronal activation patterns can be studied using a stable, automated movement pattern like cycling. Previous studies have reported that cortical activity during cycling can be localised using a combination of electroencephalography (EEG) and electromyography (EMG), with EEG and EMG showing stable oscillations and a high correlation (Schneider et al, 2013).
The SpaceBike project aims to expand the knowledge about the interaction between mechanical factors of muscle physiology and the corresponding neuromuscular regulation under changed environmental conditions, and thus offer novel information about potential motor engram adaptations in microgravity.

METHODS:

Data presented here were recorded on a supine bike during a 60-day head-down tilt bed rest study in Toulouse, France at baseline (BDC-3), once during the bed rest phase after 23 days (HDT23) and two times in the recovery phase (R+3 and R+28). The participants (n=12) were subdivided into three groups with training interventions six times per week: Cycling with artificial gravity (AG+EX), only cycling (EX) and a control group (CON) with no countermeasure. Every participant performed a submaximal incremental cycling task, starting with a load of 1 W/kg body weight and increasing by 1 W/kg body weight every two minutes until maximal exhaustion.
During exercise, brain activity was recorded with a 64-channel EEG cap and averaged cortical density was calculated for the lower limbs' representation in the motor cortex (MNI coordinates 0/0/60). Muscle activity of 14 muscles was continuously recorded with wireless EMG electrodes differentiating the central and peripheral adaptation processes. The data are currently descriptive, as the number of participants per group (n=4) is too small for adequate statistical analysis.

RESULTS:

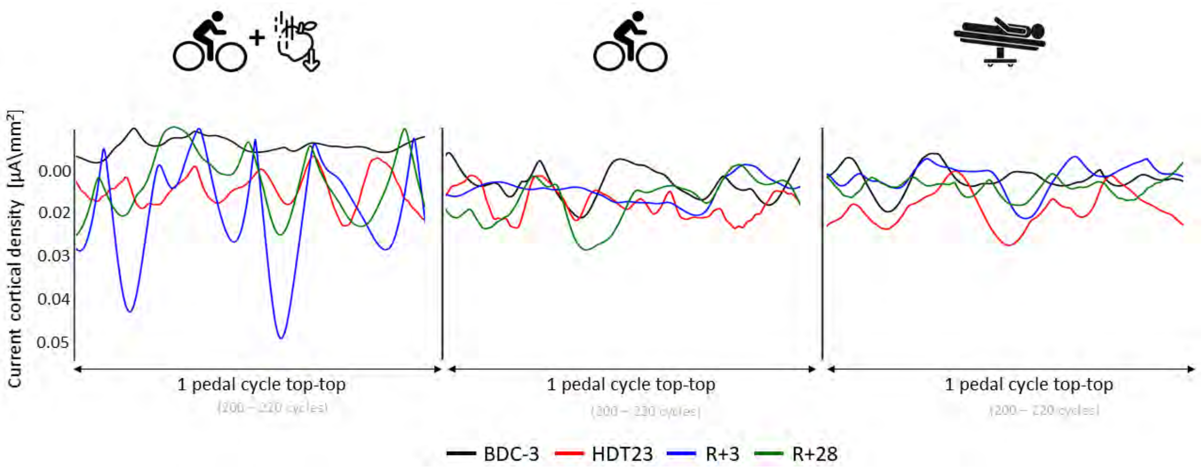

Figure 1: Current cortical density normalized to one pedal cycle top-top for the three different countermeasure groups (artificial gravity + cycling ergometer, cycling ergometer, and control group) of a 60-day head down tilt bed rest study at four different time points.

The participants of the AG+EX group showed more pronounced and stable oscillations in the motor cortex after bed rest, a pattern that can already be seen in HDT23, most pronounced at R+3 and normalized partly in R+28. Participants of the EX and CON groups did not show changes in the amplitudes of the motor cortex after the bed rest period (s. Figure 1). Regarding muscle activity, the standard deviation of the temporal occurrence of the peak of each muscle within a pedal cycle was calculated and showed a lower standard deviation in the AG+EX group compared to the EX and CON groups.

CONCLUSION:

Preliminary data suggest that the combination of artificially-induced gravity using a short-arm human centrifuge and a cycle ergometer task (AG+EX) leads to a possible development of a motor engram, as the use of a supine bike was new to the participants, which is reflected in more pronounced oscillations after the bed rest phase in the motor cortex. The partially reduced ICP during cycling in the AG+EX group, induced by the centrifugation, might support the hypothesis of the development of a new motor engram, which is also reflected in a more uniform movement in the form of a lower standard deviation of the temporal peak occurrence of the leg muscles after bed rest. This could indicate a more stable movement pattern and thus a more distinct neuronal activation pattern.
Understanding the general physiological process of learning new movements and how previously learnt movements adapt to changing conditions will enable successful space missions to be carried out in the future. The knowledge gained here can also be transferred to help patients with neurological diseases on Earth to re-learn movements and adapt to new situations.

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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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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 M., et al., 1988) and by phase synchronization index (PSI) (Negulyaev V.O., 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.

Aim

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.

METHODS

Two groups of healthy men where exposed to 21-day HDBR ("HDBR" group, n=9, age 31±5 yrs., BMI 24.2±2.8 kg/m^2) or 21-day DI ("DI" group, n=8, age 29±4 yrs., BMI 22.3±2.9 kg/m^2). 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 ($S_{RRi\text{-}LF}$) and SAP ($S_{SAP\text{-}LF}$) 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 $S_{RRi\text{-}LF}$ / $S_{SAP\text{-}LF}$ ratio and reported as cardiac baroreflex sensitivity (cBRS); mean PSI for SAP and RRi in LF band (PSI_{LF}) was calculated as previously described (Negulyaev V.O., 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. While $S_{SAP\text{-}LF}$ didn't change in horizontal position during modelled GU, $S_{RRi\text{-}LF}$ tended to decrease ($p<0.05$ – "Time" factor) with no observable difference between models; thus, cBRS in horizontal position also tended to decrease with GU exposure time ($p<0.05$ – "Time" factor). PSI_{LF} in horizontal position didn't change with exposure time and didn't differ between models.

Responses to HUT are shown in figure 1. $S_{SAP\text{-}LF}$ response increased with the exposure time without observable difference between models (Fig. 1 A), while $S_{RRi\text{-}LF}$ response decreased (Fig. 1 B). Notably, in DI the decrease of RRi LF power in HUT was quite dramatic (the decrease closing to -100%) and post-hoc tests revealed significantly higher decrease of $S_{SAP\text{-}LF}$ in DI compared to HDBR on day 14. Thus, cBRS reaction to HUT aggravated with GU exposition time and showed greater decrease in DI compared to HDBR ($p<0.05$ – “Time”x”Model” interaction) (Fig. 1 C). PSI_{LF} reaction to HUT didn’t change in HDBR while diminished completely in DI from day 14 (Fig. 1 D).

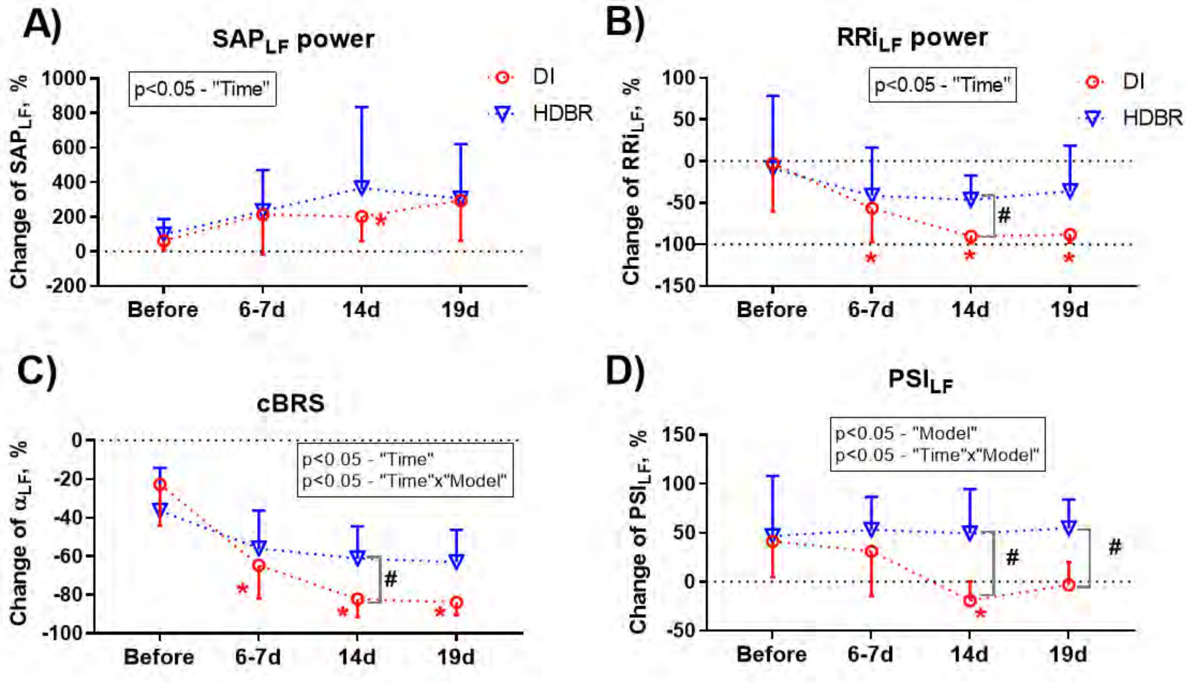

Fig.1 Response of $S_{SAP\text{-}LF}$ (A), $S_{RRi\text{-}LF}$ (B), cBRS (C) and PSI_{LF} (D) to HUT obtained at different stages of DI (red) and HDBR (blue). * - $p<0.05$ – compared to “Before”; # - $p<0.05$ – compared to same timepoint in other model (Sidak’s multiple comparisons test).

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 P.T., 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.

FUNDING

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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 S. 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 day, 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 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. 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.

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

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 A. et al, 2023). Analysis of the two VIVALDI studies will also provide insight into the physiological comparability between sexes in adaptation to weightlessness.

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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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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. 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).

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.

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. 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.

General tolerance to the study was good overall, with an increase in discomfort during the DI phase (Figure).

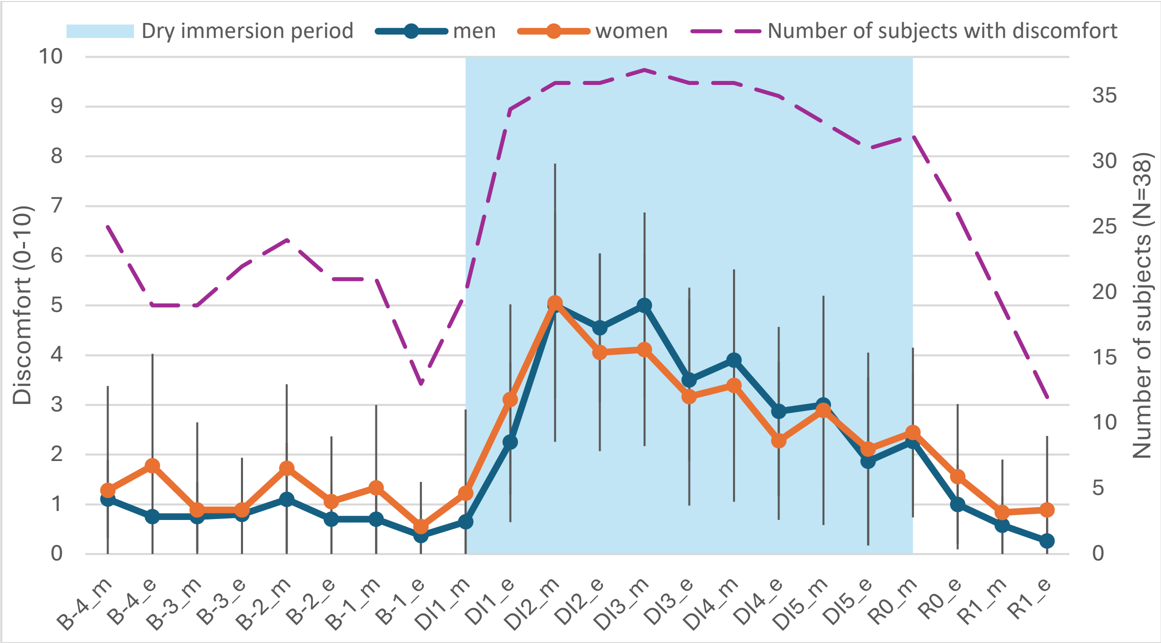

Figure: General discomfort (mean) during the study on a 0-10 scale. Survey was taken every day, morning (m) and evening (e) from BDC-4 (B-4) to R+1 (R1) for 20 men and 18 women.

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.

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.

DISCUSSION AND 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.

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

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Diurnal rhythms which influence numerous body processes are triggered by inner oscillators and modulated by environmental conditions. Dry immersion (DI) creates very specific "supportless" conditions characterized by a constant hydrostatic compression, constant ambient temperature, constant body position, constantly maintained physical inactivity, and accompanied by metabolic and hormonal shifts.

We aimed to study the effects of DI on circadian rhythms objectivized by continuous core temperature, movement counts, heart rate (HR), and blood pressure (BP) measurements. Moreover, we hypothesized that homeostatic set-point of core temperature itself might be modified by immersion.

Healthy male (M) and female (F) volunteers aged of 21-40 yrs were studied before (B4 to B1), during (D1 to D5), and after (R0 & R1) five-day thermoneutral DI with no raise allowed. Core body temperature monitoring (n=7M & 6F) was performed continuously from B3 to R1 using ingestible pill telemetry via e-Celsius Performance BodyCap system. Body movements were continuously quantified via ankle (n=19M & 17F) and wrist (n=19M & 16F) actigraph GT3X+ accelerometers from B4 to R1. Continuous monitoring of HR and BP (n=19M & 18F) was performed at B3, B1, D1, D3, D5 using cuffless SOMNOtouch NIBP system.

Under DI, mesor core body temperature lowered of about 0.18° in males and of 0.10°-0.15°C in females. At the first day of DI daytime temperature pattern was flattened; at D2-D5 circadian rhythms were globally preserved.

Day-night difference in activity counts was reduced from 40-fold difference for ankle and 20-fold difference for wrist to about 10-fold for both ankle and wrist.

HR slightly decreased under DI, while BP slightly increased at D3 and D5. Nocturnal dipping (difference between average for 9h-22h and average for 23h-6h) at D3 compared to B3 decreased for HR from 25% to 19% in F and from 30% to 18% in M, for SBP from 11% to 5% in F and from 12% to 6% in M, and for DBP from 14% to 6% in F and from 14% to 5% in M.

Thus, DI lowers core body temperature and dampens circadian variations of heart rate and blood pressure. We conclude by discussing these findings.

(VIVALDI) – Exploring Bone Adaptation and Energy Metabolism Between Males and Females Under Dry Immersion Conditions

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INTRODUCTION

Space physiology research continues to rely on microgravity analogues such as dry immersion (DI) to simulate the microgravity environment effectively, understand the underlying pathophysiology, and develop effective countermeasures. DI, a concept initially developed by Russian scientists, has grown in popularity among European researchers (Linossier et al., 2017; Linossier et al., 2022) due to its ability to effectively mimic the hypodynamic and hypokinesic environment whilst capturing the physiological changes observed in microgravity. However, DI studies to date have focused predominantly on male participants. Since the number of female astronaut recruits is on the rise, and given the significant differences in female physiology, it is essential to study how these differences might impact future missions as soon as possible. Therefore, this study (VIVALDI) aimed to evaluate differences in bone adaptation and differences in energy metabolism between males and females under (DI) conditions.

Figure 1:Participant under DI conditions. Image adapted from (Linossier et al.,2017)

METHODS

A total of 37 healthy participants (nineteen males 28yrs±4.32) and (eighteen females 29yrs±4.76) underwent five days of DI (Fig.1), which included a 48hr post-DI recovery period. All participants underwent a comprehensive medical assessment and a thorough physical examination which included a VO_{2max} assessment prior to enrolment. All participants were deemed medically fit by a certified physician and were free from acute and chronic illnesses. All female participants had regular menstrual cycles and were not on the combined oral contraceptive pill. All antecubital venous blood samples were collected at fasting and at the same time (approximately 07:30 AM) for the duration of the study. Samples were taken at baseline (BDC-24h), after 24h-(DI-24h), 48h-(DI-48h), 120h-(DI-120h) immersion, and finally 48h after the return to loading conditions (R+2)

Markers such as (Fig.2) C-terminal crosslinked telopeptide of type I collagen [CTx], procollagen type I N-terminal propeptide [P1NP], bone alkaline phosphatase [bAP], intact and N-mid osteocalcin fragment [OC] and insulin-like growth factor 1 [IGF1] were determined by automated chemiluminescence immunoassay (IDS-iSYS automated analyzer, Boldon, UK). Enzyme-immunoassay (EIA) kits measured the

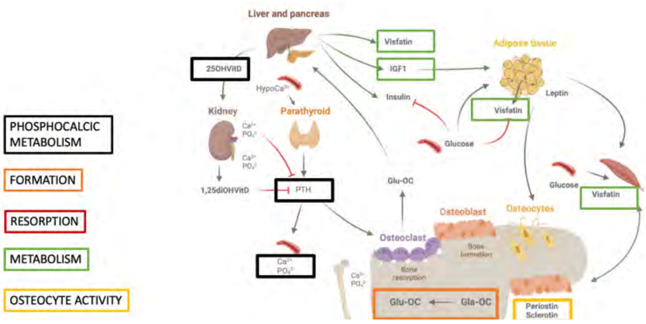

Figure 2: Core biological markers. Image adapted from Robin et al., 2023)

following parameters: tartrate-resistant acid phosphatase isoform 5b [TRAP5b] (Microvue Bone, Quidel Corporation, San Diego, CA, USA); undercarboxylated [Glu-OC] and carboxylated [Gla-OC] OC (Takara Bio, Inc., Otsu, Japan); a secretory form of nicotinamide phosphoribosyl-transferase [visfatin] (Adipogen AG, Liestal, Switzerland); periostin (Biomedica Medizinprodukte GmbH, Wien, Austria). Serum intact parathyroid hormone [1-84 PTH], calcium and phosphorus were measured using electrochemiluminescence immunoassay (Cobas®8000 modular analyzer, Roche Diagnostics Ltd., Rotkreuz, Switzerland).

RESULTS & CONCLUSION

Five days of DI highlighted differences in adaptation between groups, particularly in females. When looking at bone metabolism, bone formation markers exhibited marginally higher concentrations in females than males. Interestingly, higher amounts of undercarboxylated OC were observed in females throughout all timepoints, with peak concentrations as early as 48hrs in females. Furthermore, CTX (Fig.3) a resorption marker, was marginally higher in females and reached peak concentrations later than in males. In addition, osteocyte function was significantly more impaired in females throughout the DI period and recovery period (Fig.4). Taken together, the DI-induced changes in bone remodeling markers demonstrated an uncoupling effect favoring bone resorption during DI and the recovery phase in both groups with more pronounced changes in females.

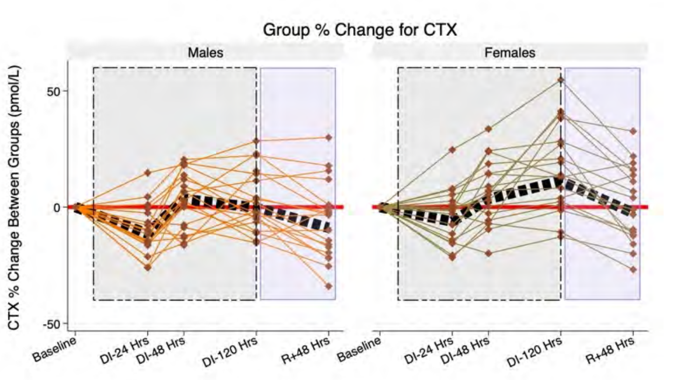

Figure 3: CTX concentrations between groups (n=19 males; n=18 females) during immersion (grey box) and recovery (purple box). Solid (black) line shows the mean change over time with horizontal (red) line as reference to baseline.

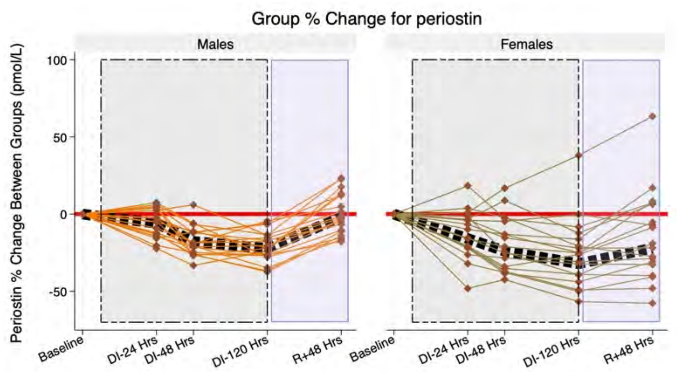

Figure 4: Periostin concentrations between groups (n=19 males; n=18 females) during immersion (grey box) and recovery (purple box). Solid (black) line shows the mean change over time with horizontal (red) line as reference to baseline.

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

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INTRODUCTION: Impaired orthostatic tolerance has been reported after spaceflight when returning to Earth gravity due to hypovolemia (Blomqvist C.G. et al, 1994; Coupé M. et al, 2011; Gharib C. & Hughson R.L., 1992; Jordan J. et al, 2022), changes in cardiac and baroreflex autonomic control (Fritsch J.M. et al, 1992; Hughson R.L. et al, 1994), and increased venous compliance of lower limbs (Fortrat J.O. et al, 2017). Altered venous filling has already been shown during bedrest (Louisy F. et al, 1997) and spaceflights (short-term: Louisy F. et al, 2001) (long-term: Fortrat J.O. et al, 2017) using lower limb plethysmography in men. The dry-immersion (DI) is another ground-based model to mimic microgravity exposure, and venous filling and emptying properties has never been assessed in women and directly compared with men using dry-immersion.

METHOD: We assessed the venous function on 18 women and 19 men before, during, and after 5 days of strict DI. A venous filling and emptying properties were assessed by air plethysmography (APG® 1000, ACI Corporation, San Marcos, CA, USA). A venous occlusion was performed using a manual pneumatic thigh cuff with changes in calf volume determined at 30, 40 and 50 mmHg as shown in **Fig.1**. Plasma volume changes and blood viscosity (derived from hematocrit and proteins count) have been measured. The calf volume changes were estimated from MRI images at day 5 compared to pre-DI.

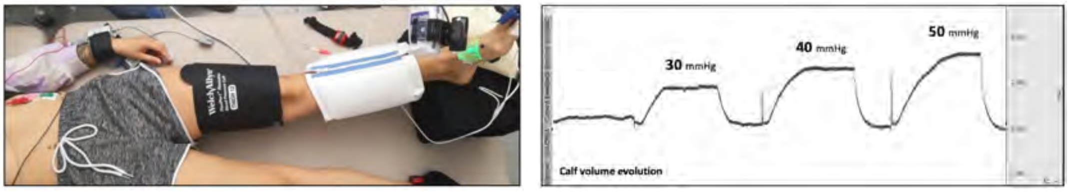

Figure 1: Venous occlusion cuff on the thigh, and air plethysmograph around the calf to record volume changes.

RESULTS: We assessed the integrative venous function before, during, and after DI in women and men. During DI, in both men and women, the arterial flow index decreases by 40%, the venous emptying rate decreases by 12% in women and the venous distensibility index decreases in men. The blood viscosity increases and the calf muscle volume decreases in both men and women during DI.

DISCUSSION: In our study, we observed a decrease in leg venous distensibility during DI which It could be due to the chronic hydrostatic compression effect of the water mass around the body. Long-term compression therapy is known to be an efficient conservative treatment of venous incompetence. The blood viscosity increases mainly due to the hypovolemia and the increased hematocrit. The decrease in calf muscle volume could be mainly attributed to the hypovolemia and fluid centralization during DI.

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Gender Related Differences On Dry Immersion-Induced Ophthalmological Changes

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INTRODUCTION

The neuro-ophthalmological changes called Spaceflight Associated Neuro-ocular Syndrome (SANS) and their effects on the optic nerve are an important medical issue. After long duration flights, some astronauts present ophthalmological modifications such as a globe flattening, an increase in peripapillary retinal nerve fibre layer thickness (pRNFLT) and total retinal thickness (TRT). However, even if exposure to microgravity does not pathologically elevate intracranial pressure (ICP), somehow it prevents the normal lowering of ICP when standing on Earth. Ground-based analogues such as dry immersion (DI) are essential models for determining the effects of spaceflight on astronauts' bodies. This study aimed to evaluate the ophthalmological modifications induced by a 5-day DI both in women and men.

MATERIALS AND METHODS

General protocol

Eighteen women and 19 men experienced 5 days of DI. The protocol was as follows: 4 days of ambulatory control period before DI (BDC-4 to BDC-1), 5 days of DI (DI 1 to DI 5) and 2 days of ambulatory recovery (R 0 to R+1). During the DI period, the subjects were covered with an elastic waterproof fabric and were freely suspended in thermoneutral water.

Ophthalmological measurements

All subjects underwent a complete bilateral ophthalmologic examination before (BDC) and a few hours (5–6 h) after (R) DI. The examination included pRNFLT measurements using optical coherence tomography (OCT). Ganglion cell complex (GCC) images were acquired on a 6 × 6 mm mapping square centred on the fovea. RNFLT measures were acquired with circular-scan centered on the optic disc, and segmentation was verified manually.

Statistical analysis

OCT data were expressed as mean ± SD. The normality of the distributions was assessed with the Shapiro-Wilk normality test. Two-way ANOVA (time x gender) with Sidak's method post-hoc was used to analyze data. Differences were considered statistically significant when $P \leq 0.05$.

RESULTS

PERIPAPILLARY RETINAL NERVE FIBRE LAYER THICKNESS

We noted a thickest basal global pRNFLT in TI quadrant in women compared to men. We observed a slight thickening pRNFLT in T and TI quadrants after DI only in women.

	Women (n = 18)		Men (n = 19)		ANOVA (Interaction x Time x Gender)
	BDC	*R*	*BDC*	*R*	
pRNFLT(μm)	103.0 ± 11.5	103.7 ± 11.5	99.9 ± 7.2	99.9 ± 7.1	P = 0.110; P = 0.087; P = 0.272
TS (μm)	142.9 ± 22.5	142.4 ± 22.2	139.7 ± 17.6	140.3 ± 17.7	P = 0.223; P = 0.980; P = 0.691
T (μm)	75.1 ± 11.0	76.9 ± 11.3*	70.2 ± 10.2	69.9 ± 10.1	**P = 0.019**; P = 0.070; P = 0.095
TI (μm)	153.8 ± 22.7	155.6 ± 21.9*	137.2 ± 17.7†	138.0 ± 16.6†	P = 0.201; **P = 0.002**; **P = 0.013**
NI (μm)	115.8 ± 21.1	116.9 ± 21.2	112.1 ± 20.1	112.5 ± 19.9	P = 0.307; **P = 0.031**; P = 0.555
N (μm)	77.3 ± 15.1	77.5 ± 15.0	77.1 ± 12.3	77.7 ± 12.4	P = 0.636; P = 0.247; P = 0.996
NS (μm)	105.3 ± 18.8	106.1 ± 19.2	114.8 ± 17.9	114.6 ± 17.3	P = 0.154; P = 0.432; P = 0.139

*N: nasal, NI: nasal-inferior, NS: nasal-superior, T: temporal, TI: temporal-inferior, TS: temporal-superior. *P < 0.05 vs. BDC, †P < 0.05 vs. Women.*

MACULAR TOTAL RETINAL THICKNESS

Macular TRT was not significantly modified after DI both in women and men.

GANGLION CELL COMPLEX

We denoted a slight thinning GCC in TI quadrant after DI both in women and men.

	Women (n = 16)		Men (n = 19)		ANOVA (Interaction x Time x Gender)
	BDC	*R*	*BDC*	*R*	
Global GCC (μm)	53.9 ± 3.9	53.4 ± 3.9	54.2 ± 4.2	53.8 ± 4.1	P = 0.728; **P = 0.005**; P = 0.827
S (μm)	54.8 ± 3.4	54.8 ± 3.7	55.4 ± 4.4	54.9 ± 3.9	P = 0.080; P = 0.217; P = 0.784
TS (μm)	51.1 ± 4.2	50.2 ± 4.4	50.6 ± 4.5	50.3 ± 5.0	P = 0.333; **P = 0.031**; P = 0.908
TI (μm)	53.5 ± 4.1	52.6 ± 4.6*	53.3 ± 4.3	52.5 ± 4.1*	P = 0.808; **P = 0.001**; P = 0.904
I (μm)	54.6 ± 3.7	54.2 ± 3.8	54.7 ± 4.4	54.5 ± 4.2	P = 0.631; P = 0.136; P = 0.888
NI (μm)	55.1 ± 4.5	54.7 ± 4.2	55.5 ± 4.6	55.3 ± 4.2	P = 0.502; P = 0.080; P = 0.729
NS (μm)	54.7 ± 4.4	54.5 ± 4.5	55.6 ± 4.4	55.3 ± 4.1	P = 0.878; P = 0.192; P = 0.571

*I: inferior, NI: nasal-inferior, NS: nasal-superior, S: superior, TI: temporal-inferior, TS: temporal-superior. *P < 0.05 vs. BDC.*

CONCLUSION

In all, DI induced slight ophthalmological changes, such as 1) a thickest RNFL in T and TI quadrants in women, and 2) a thinnest global GCC in the TI quadrant both in women and men. All these modifications were not clinically significant. Although these ocular changes did not reach thresholds like those observed during spaceflight, 5-day DI was sufficient to induce small but significant ocular changes.

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

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ABSTRACT

Spaceflight is an extreme environment that affects the immune system of approximately 50% of astronauts. With planned long-duration missions, such as the deployment of the Lunar Gateway and possible interplanetary missions, it is mandatory to determine how all components of the immune system are affected, which will allow the establishment of countermeasures to preserve astronaut health. However, despite being an important component of the immune system, antibody-mediated humoral immunity has rarely been investigated in the context of the effects of the space environment. It has previously been demonstrated that 30 days aboard the BION-M1 satellite and 21 days of hindlimb unloading (HU), a model classically used to mimic the effects of microgravity, decrease murine B lymphopoiesis. Furthermore, modifications in B lymphopoiesis reported in young mice subjected to 21 days of HU were shown to be similar to those observed in aged mice (18-22 months). Since the primary antibody repertoire composed of IgM is created by V(D)J recombination during B lymphopoiesis, we assessed the degree of similarity between changes in the bone marrow IgM repertoire and in the V(D)J recombination process in 2.5-month-old mice subjected to 21 days of HU and aged (18 months) mice. We found that in 21 days, HU induced changes in the IgM repertoire that were approximately 3-fold less than those in aged mice, which is a rapid effect. Bone remodeling and epigenetics likely mediate these changes. Indeed, we previously demonstrated a significant decrease in tibial morphometric parameters from day 6 of HU and a progressive reduction in these parameters until day 21 of HU, and it has been shown that age and microgravity induce epigenetic changes. These data reveal novel immune changes that are akin to advanced aging and underline the importance of studying the effects of spaceflight on antibody-mediated humoral immunity.

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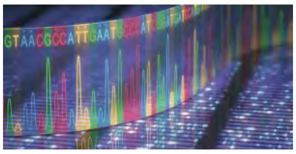
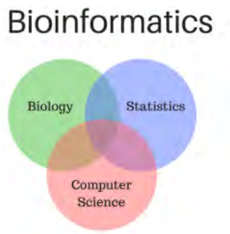
Mice subjected to 21 days of hindlimb unloading (HU)

18 months old for mice

- Construction of IgM heavy chain cDNA libraries from the bone marrow of HU, aged and control mice.

- NGS sequencing of libraries





- Bioinformatics: -Only functional sequences were considered.
  -Diversity analyses.

➔ **In 21 days, HU induced changes in the IgM repertoire that were approximately 3-fold less than those in aged mice, which is a rapid effect.**

Graphical abstract: It was previously shown that 21 days of hindlimb unloading (HU) leads to decreased B lymphopoiesis similar to aging. Since the primary antibody repertoire composed of IgM is created in the bone marrow during B lymphopoiesis, we assessed the degree of similarity between changes in the bone marrow IgM repertoire in 2.5-month-old mice subjected to 21 days of HU and aged mice.

Effects of Simulated Microgravity on Immune System Potency in 3D Microenvironment

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Human space travel and exploration are of interest to both the industrial and scientific community. However, there are many adverse effects of spaceflight on human physiology. In particular, there is a lack of mechanistic understanding of the extent to which microgravity affects the immune system. As of yet, most studies investigating the effects of microgravity on cells of the immune system are limited to peripheral blood or traditional 2D cell culture that recapitulates circulating blood. On Earth, to better mimic interstitial tissue, 3D cell culture has been well established for physiologically and pathologically relevant models. In our work, we utilize 2D cell culture and 3D collagen matrices to gain an understanding of how simulated microgravity, using a random positioning machine (RPM), affects different immune cells such as T cells and dendritic cells. We found that 3D cell culture attenuates the effects of simulated microgravity on the transcriptome and nuclear irregularities of immune cells compared to 2D cell culture. We also utilized simulated microgravity as a model to study an aging immune system. Our data indicate that aged or loose tissue and exposure to RPM-induced simulated microgravity both independently alter the immunogenicity of immature and mature dendritic cells. Our findings are a step forward to better facilitate healthier future space travel and enhance our understanding of the aging immune system on Earth.

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

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ABSTRACT

Space missions in the ISS, and future space exploration to the Moon and Mars, involve medium- to long-duration manned spaceflights. Deconditioning of the musculoskeletal system is a major consequence and impacts astronauts throughout their mission, from the very first days of flight (Vico and Hargens, 2017). These early deteriorations are also seen during dry immersion (an analog model on Earth) lasting 3 to 5 days, where bone remodeling blood markers (Linossier et al. 2017; 2022) and reduced muscle strength and mass are already significantly altered (Demangel et al. 2017; Fovet et al. 2021). Consequently, the maintenance of the musculoskeletal system during the mission or its recovery after the flight becomes a critical point and requires astronauts to follow intense rehabilitation programs to maintain or regain their physical abilities. Astronauts on the ISS perform both endurance and resistance exercises (with ARED, CEVIS, and TVIS devices) that induce activation of several pathways involved in bone and muscle plasticity. Despite these programs, recovery remains incomplete, particularly at bone level, even two years after return to Earth (Juhl et al. 2021). It is therefore crucial to better manage these physiological adaptations in order to protect the health and physical capacity of crew members. In order to prevent musculoskeletal deconditioning, numerous countermeasures have been tested, in particular on terrestrial models as dry immersion and bed rest. For now, the best means of prevention remains physical activity. However, even if astronauts are in great physical form before flying, and exercise up to 2h per day on the ISS (Petersen et al. 2016), their current training programs are still not fully efficient in the prevention of bone and muscle loss.

It is with this aim that we set up the NEBULA (Nutrition and Exercise Biology for UnLoaded Astronauts) research project. The first part of this program was focused on the early musculoskeletal changes during the first days of microgravity and test the hypothesis that improving pre-flight fitness will delay the harmful effects of microgravity. Accordingly, Brocca et al. (2022) already investigated the idea that a moderate intensity endurance training performed before a spaceflight analog protocol could postpone the onset of unloading-induced muscle waste by promoting muscle remodeling. Our study aimed to go further and evaluate the impact of a specific training program characterized by a progressive high intensity endurance and resistance training to be performed during the 3 weeks preceding the unloading. Sixty 14-week old males C57B6J mice were split into 6 groups (n=10) performing or not the physical training for 3 weeks, followed or not by a 7 or 21-day of hindlimb unloading (HU7 and HU21) period. Femur and soleus and gastrocnemius muscles were examined after the pre-conditioning period (preflight), HU7 and HU21 (Figure 1).

For the bone system, micro-computed tomography (µCT) of the femur distal metaphysis showed a greater trabecular bone volume (BV/TV, +25%; $p<0.001$) in preconditioned groups (PC) compared to control (Ctrl) at the end of the preconditioning. At HU7, trabecular BV/TV was preserved in PC while a decrease was already seen in Ctrl. However, at HU21, BV/TV was similarly decreased in PC and Ctrl (Figure 2A). The delay of trabecular bone wasting at the distal metaphysis in PC at HU7 was confirmed using histomorphometric analysis. In addition, histomorphometry revealed a slower increase in bone osteoclastic resorption activity and a higher bone formation activity in PC in comparison to Ctrl at HU7. Similar adaptation was observed in gastrocnemius and soleus muscle tissues through histological analyses. Gastrocnemius muscle fibers cross sectional area (CSA) was higher in PC than in Ctrl at preflight time point demonstrating the hypertrophic effects of the pre-conditioning protocol in motor muscles. As for trabecular bone, soleus and gastrocnemius muscle mass decreased in Ctrl while it was preserved in PC in HU7. However, PC and Ctrl muscle mass then significantly decreased at HU21 (Figure 2B). Taken together, these results exhibit the beneficial effects of a combination of endurance and resistance physical exercises on bone and muscle functions especially in terms of mass and architecture. They also validate the hypothesis that a pre-flight training has protective effects in the first days of HU exposure by postponing microgravity-induced musculoskeletal deterioration.

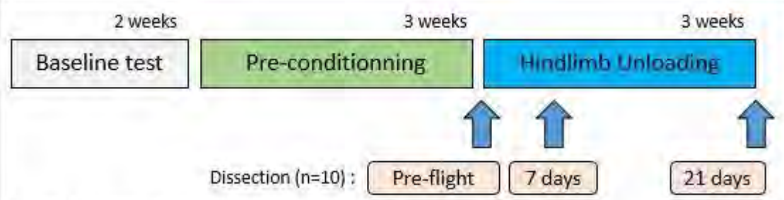

Figure 1 : organisation chart

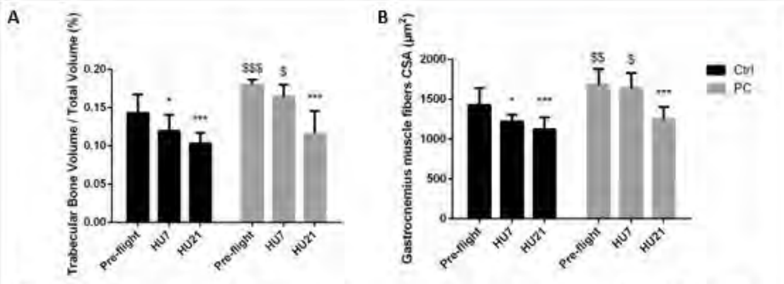

Figure 2 : A. Femur trabecular BV/TV (%). B. Gastrocnemius muscle fibers CSA (µm²). * : different from pre-flight measurements of the same group. $: different from pre-flight measurements of Ctrl group. * : p<0,05 ; ** : p<0,01; *** : p<0,001

In conclusion, pre-conditioning protocols could be added to existing prevention programs in order to alleviate the early deterioration of microgravity, a period of acute changes where in the next future, crewmembers will be confined in a spacecraft with no possibility to do physical activity before reaching a space station as planned in the Gateway program.

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

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Abstract
In a prior experiment conducted in the European Biorack facility for biological research on the Space Shuttle/Spacelab (IML-1 on STS-42), we presented groundbreaking findings on the direct responses of fetal mouse long bones to near-weightlessness. The current study aimed to validate these earlier results and investigate the impact of varied durations of daily 1×g exposure on the growth and mineralization of isolated fetal mouse long bones during microgravity.

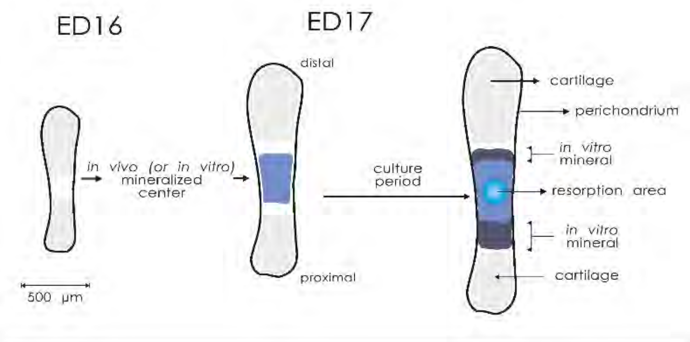

Figure: Graphical display of an Embryonic Day 16 (ED16) and ED17 fetal mouse metatarsal long bones. For the IML-2 and Bion-10 experiments we used 17 days old bones at the time of dissection. The ED17 rudiments are about 1.5 to 2 mm long at dissection. Before culture, the central mineralized zone (the diaphysis) has developed in vivo. It is flanked by zones of cartilage (the epiphysis). The whole matrix is surrounded by a thin layer of perichondrium. During culture there is an increase in total length as well as an increase in length of the diaphysis (in vitro mineral). In some bones a more translucent resorption area can be seen after some time.

This paper details the outcomes of two distinct experiments involving fetal mouse metatarsals. One was conducted on an American Space Shuttle mission (IML-2 on STS-65), and the other on a Russian Bio-Cosmos flight (Bion-10 on Cosmos-2229). While the hardware and experimental conditions differed, the biological material remained consistent:

17-day-old fetal mouse metatarsal long bones cultured for 4 days. In both experiments, microgravity cultures were compared with those in an on-board 1×g centrifuge. Ultrastructural analyses revealed a reduction in proteoglycan numbers in the microgravity-exposed groups compared to the 1×g conditions. However, no differences were observed in the nuclear/cytoplasmic ratio, cell divisions, glycogen granules, or the size and orientation of collagen fibrils. Overall metatarsal length increase remained unaffected by microgravity, but the growth in the length of the mineralized diaphysis was significantly reduced under microgravity conditions. These findings align with a previous microgravity experiment (IML-1 on STS-42) involving 16-day-old mouse fetal metatarsals.
Furthermore, we demonstrated for the first time that the microgravity-induced reduction in cartilage mineralization is completely nullified by daily exposure of long bones to 1×g for at least 6 hours, with no discernible effects after 3 hours of 1×g exposure. Intermittent 1×g exposure did not impact overall growth. These in vitro results suggest that prolonged exposure may be necessary when considering on-board centrifugation as a multisystem countermeasure for spaceflight-related near-weightlessness pathologies.

SPINAL MECHANISMS TRIGGERING THE SPONTANEOUS TONIC ACTIVITY OF THE POSTURAL SOLEUS MUSCLE UNDER HINDLIMB UNLOADING

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A gradual increase in rat soleus muscle electromyographic (EMG) activity is known to occur after 3-4 days of hindlimb unloading (HU), a well-known model simulating microgravity. The physiological significance and mechanisms of such spontaneous soleus activity under unloading conditions are currently unclear. Since hyperactivity of motoneurons and muscle spasticity after spinal cord injury are associated with (K+)/chloride (Cl−) co-transporter 2 KCC2 downregulation, we hypothesized that a decrease in KCC2 in motoneurons would be responsible for an increase in soleus muscle EMG activity during HU. We aimed to investigate the effect of two KCC2 agonists (prochlorperazine and CLP290) on the electrical activity and intracellular signaling pathways of rat soleus muscle under HU conditions. Wistar rats were divided into the following groups: 1) control (C), 2) control + daily i.p. injections of prochlorperazine (10 mg/kg) or CLP290 (100 mg/kg) (C+KCC2 activator), 3) 7-day HU group (7HU) + placebo and 4) 7-day HU + daily i.p. injections of prochlorperazine (10 mg/kg) or CLP290 (100 mg/kg) (7HU+KCC2 activator). Protein expression and phosphorylation of KCC2 in motoneurons of the lumbar spinal cord was determined by Western blotting. An electromyogram of the rat soleus muscle was recorded using intramuscular electrodes. Protein and mRNA expression of signaling molecules in rat soleus muscle was assessed by Western blotting and RT-PCR.

KCC2 content in the lumbar spinal cord after 7-day HU significantly decreased by 34% relative to the control group. HU-induced decrease in KCC2 protein content was prevented by administration of both prochlorperazine and CLP290. In parallel with the restoration of KCC2 content, both prochlorperazine and CLP290 administration during 7-day HU prevented a gradual increase in the soleus muscle EMG activity from day 3 to day 7 of HU. Thus, it can be stated that spontaneous neuromuscular activity of the soleus muscle is reduced by prevention of KCC2 reduction in the lumbar spinal cord regardless of the nature of the drug used.

In addition, we compared the effects of CLP-290 and prochlorperazine on morphological and signaling parameters of the soleus muscle under 7-day HU. The use of both drugs during HU aggravated HU-induced decreases in the markers of mitochondrial biogenesis (PGC1alpha, COXI, COXII). Unlike CLP290, treatment of rats with prochlorperazine resulted in some positive changes in the unloaded soleus muscle: prevention of 1) the atrophy of slow-type fibers, 2) slow-to-fast transformation of muscle fibers and 3) an increased expression of E3-ubiquitin ligases. The use of prochlorperazine during HU also maintained the parameters of translational capacity (ribosome content) in the unloaded rat soleus muscle. We speculate that these positive effects of prochlorperazine on the soleus muscle might be associated with some effects of this drug on myoplasmic calcium content via blocking alpha-2 adrenoreceptors (one of the known side effects of this drug).

In conclusion, our study suggests that there is a causal relationship between the emergence of the spontaneous tonic activity of the soleus muscle occurring during the course of HU and the content of KCC2 in the lumbar spinal cord. The study was supported by the Russian Science Foundation (RSF) grant no. 22-15-00151.

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

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BACKGROUND

Studies investigating the effects of microgravity on bone tissues and gene expression regulation have shed light on the intricate mechanisms underlying bone health in space environments. However, only a few studies have addressed the opposite condition, e.g. the effects of increased gravity on mammal organisms and on bone tissues in particular. The goal of the present study was to assess the deregulation of gene expression and to establish the most affected pathways in femurs of mice subjected to a 27-day exposure to 3g by centrifugation (see Campioli et al. abstract "Adaptation to 3g Hypergravity: A Multidisciplinary Tissue Sharing Program from a 15- and 27Day Mouse Experiments" for general information)

MATERIALS AND METHODS

Briefly, the experiment involved male C57BL/J6 mice, aged 19 weeks (at the end of the experiment). After 27 days, 3g exposed and relative unexposed control mice in MDS payload were sacrificed and femurs were isolated. Femurs were cut in the two epiphysis and flushed to remove the bone marrow. Total RNA was extract and analyzed with Tape Station 2200 (Agilent) and sent for RNA seq analyses to IGATech (Udine, Italy).

RESULTS

To investigate the molecular basis underlining the effect of gravity on mice femur bone we performed mRNA sequencing. Preliminary analyses were performed: raw data were normalized, filtered and then analyzed by calculating the ratio between controls and 3g samples. We chose an arbitrary 2.0 fold change cutoff to consider a given mRNA as differentially regulated (DER). To identify gene exhibiting significant changes in expression between the two experimental conditions, we performed a volcano plot analysis. In Fig. 1A we provide both statistical significance and the magnitude of deregulation. In particular, we found 402 upregulated and 27 downregulated transcripts with an adjusted P-value<0.05 in the 3g condition. To gain a mechanistic understanding of the biological processes affected by exposure to 3g, the ShinyGO 0.80 tools were used to identify the respective Gene Ontology (GO) Biological Process terms that were associated with 3g DERs and significantly over-represented. The results are shown in Fig. 1B. Furthermore, we found that DER were mainly associated with the KEGG pathways Extracellular Matrix-receptor interaction and Protein digestion and absorption and at a lesser extent with pathways associated with growth and pluripotency signaling (Fig. 1C, D).

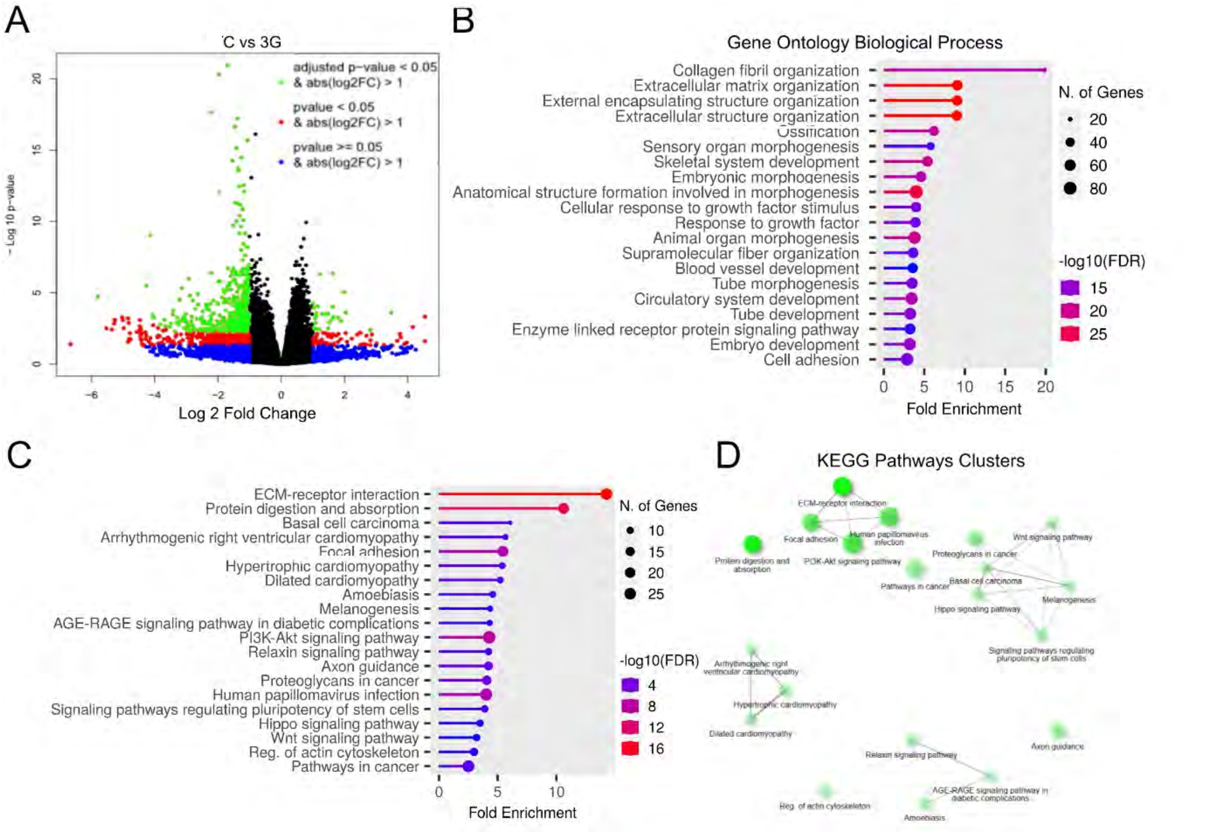

Figure 1. A) Volcano plot showing deregulated mRNAs (DER) in femurs of mice exposed to 3g vs MDS control (TC). B) Gene Ontology Biological Process term enrichment associated with DER. C) KEGG pathways associated with DER. D) KEGG pathways clustering.

CONCLUSIONS

This study suggests novel insights into the genomic response of mouse femur to increased gravity, as indicated by the significant deregulation of gene expression after exposure to 3g conditions. Our results confirm that mammalian bones are highly sensitive to mechanical stimuli, and the differential expression of 402 upregulated and 27 downregulated genes suggests that hypergravity induces a broad genomic response, with a particular emphasis on upregulation of genes.

The upregulated genes are significantly associated with the extracellular matrix (ECM)-receptor interaction and protein digestion and absorption pathways, as revealed by KEGG pathway analysis. Similarly, the enrichment of Gene Ontology (GO) terms related to biological processes indicates involvement of ECM organization and ossification. These findings may indicate an adaptive response aimed at enhancing bone matrix production and turnover, as well as nutrient absorption processes that could be pivotal in maintaining bone integrity under stress conditions that mimic high gravity. Interestingly, the downregulated genes did not show a strong association with specific biological pathways, which might suggest a more targeted suppression of certain genes rather than a general downregulation response. These adaptations seem to correlate also with KEGG pathways involved in growth and pluripotency signaling, albeit to a lesser extent. This could be indicative of a cellular strategy to bolster developmental processes in response to the enhanced mechanical demands imposed by hypergravity.

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

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All living organisms have evolved and adapted to live under Earth's gravitational force. To sustain safe human space exploration, it is important to understand how the different effectors, including gravitational force, influence organisms. Altered levels of gravity affect the physiological function of multiple tissues, cells, and organs in living organisms.
Many adverse conditions present in Space, such as hypoxia, hypothermia, and microgravity, cause integrated alterations in the lipid membrane composition, inducing greater sensitivity to oxidative stress. Indeed, previous studies, also from our laboratory, suggested that microgravity modifies the permeability of the plasma membrane and cellular metabolism in erythrocyte, modifying cholesterol and phospholipid levels. In addition, hypergravity also affects the physiological functions of tissues and organs; furthermore, the evaluation of the effects of the hypergravity is a fundamental step towards complete knowledge of the physiological response to altered gravity. Aim of this study was to investigate in vivo the effects of hypergravity on lipid phenotype and metabolism in mice erythrocytes.

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. Vivarium animals and MDS-like cage animals were compared as controls.
After 30 days of experiment a tissue sharing protocol was performed among international researchers, to analyze all the 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. Finally, to analyze the impact on oxidative homeostasis, the hemolyzed fractions were used to test antioxidant enzyme activities.

Our results show that the exposure of mice to an altered gravity induced a modification in the fatty acid composition of 3xg mice compared to control mice, indicating a direct effect of the increased level of gravity. The cholesterol content in membranes was significantly increased. To evaluate the effect of hyper-gravity conditions on the animal's inflammatory and metabolic processes, the ratio between inflammatory eicosanoids and anti-inflammatory eicosanoid precursors was calculated, and a slight reduction in the inflammation index given by arachidonic acid/eicosapentaenoic acid ratio was observed. These findings could be due to a process of metabolic compensation during long-term exposure that leads to a resolution of inflammation. To evaluate the impact of fatty acid composition on the potential level of oxidative stress, we calculated the peroxidability index (PI), which measures the sensitivity of fatty acids to peroxidation; PI was significantly increased under 3xg conditions. Furthermore, we have analyzed the endogenous antioxidant activity in the hemolysate of scavenging enzymes, and the amount of glutathione content in the red blood cells. The enzyme activity of GSH peroxidase shows a significant increase in 3xg mice compared to control mice.

This study demonstrates that hypergravity induces changes in both lipid composition and antioxidant system of erythrocytes. Our results will be integrated with other tissue and metabolic data obtained by other researchers of the team. Further studies will be necessary to identify possible countermeasures to ensure an adequate level of crew health and safety during long-duration space missions.

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.

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.

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. The ESA bedrest standards are followed, and subjects were selected accordingly.

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

After a 14-day period of baseline data collection (BDC), the volunteers are 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 are horizontal. This is followed by 14 days of recovery. The ESA bedrest standards are applied for the duration of the study, with a strict control of nutrition, sleep schedule, activity, and an absence of visits. The first campaign was carried out in Spring 2023 for the first 12 subjects, and the second campaign started in February 2024.

Countermeasure protocol

While the control group has no exercise, both other groups have a 30-minute daily routine of exercise on a bike ergometer. Load is based on their individual VO_2max, as measured in BDC on a supine ergometer. There is an alternation of 2-minute intervals from 40%VO_2max to up to 80%VO_2max. In the AG + bike group, this same exercise is 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.

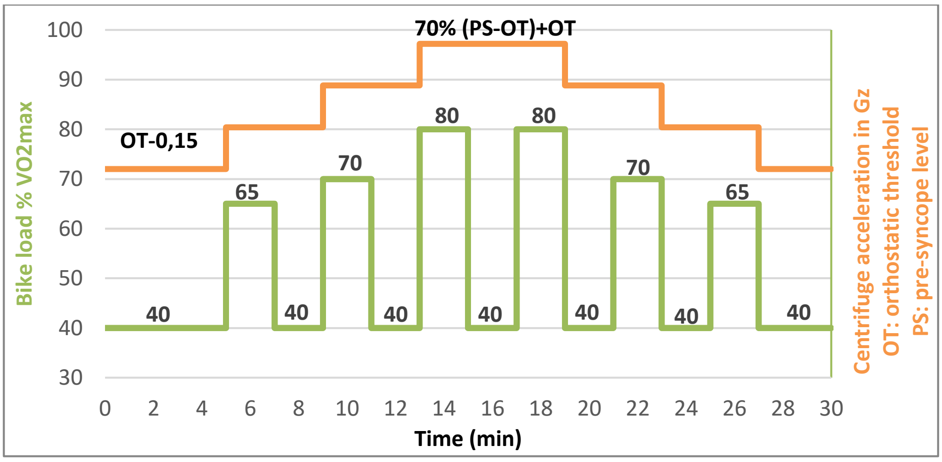

Figure 2: daily countermeasure protocol for the bike + artificial gravity group.

Measurements

A set of tests is 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 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 will be 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.

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.

Does Artificial Gravity Tolerance Change Across seasons?

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Background: Future space explorers will encounter physiological challenges due to prolonged exposure to a hypogravity environment. Artificial gravity (AG) is a potential countermeasure against spaceflight deconditioning and orthostatic intolerance, generated by centripetal acceleration through a short-arm centrifuge. While studies have shown positive effects and adaptations of human physiology to AG, limited attention has been given to seasonal variations affecting AG tolerance.

Aims and Study: This study aims to analyze differences in AG tolerance across different seasons and their potential impacts on cardiovascular parameters.

Hypothesis: We hypothesized that AG tolerance would be greater during colder seasons due to known influences of seasonal changes on the cardiovascular system.

Methods: AF from 0.6G to 1.7G were used, AG were increased by 0.1G at each step. Each step from 0.6G to 1.7G were lasted for 3 minutes. Orthostatic tolerance time determined based on the occurrence of presyncope symptoms.

Results: Two healthy young male participants were involved. Significant variations were observed in the rate of change of blood pressure (BP) across seasons during the AG tolerance test ($p < 0.05$). Artificial gravity tolerance was higher during winter sessions. The rate of change of BP during AG runs was higher in summer sessions and more consistent in winter sessions.

Conclusions: Seasonal variations in AG tolerance were found, with higher tolerance and more consistent blood pressure responses observed during winter. These findings suggest that environmental factors, such as temperature, may influence human physiological responses to AG, with implications for future space mission planning.

Significance: Understanding AG tolerance variations is crucial for optimizing crew health and performance in space missions. Additionally, insights gained may contribute to understanding cardiovascular changes in aging, with implications for medicine.

Future Directions: Further investigation is needed to explore gender-specific cardiovascular and autonomic regulations to optimize spaceflight challenges for both male and female astronauts.

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COMPREHENSIVE EXPLORATION OF ARTIFICIAL GRAVITY SOLUTIONS FOR OPTIMIZING LONG-TERM SPACE EXPLORATION MISSIONS

Maryam Almarzooqi

The detrimental impact of extended microgravity exposure on astronaut health necessitates innovative solutions for long-term space exploration. This study meticulously investigates artificial gravity interventions as viable strategies to counteract the physiological challenges arising from weightlessness. A detailed analysis is initiated by examining the adverse effects of microgravity on bone density, muscle mass, and cardiovascular health, emphasizing the critical need for effective countermeasures to ensure astronaut well-being during extended missions (Chen, 2021).

Our primary research focus encompasses an exhaustive evaluation of various artificial gravity technologies, including rotating spacecraft and centrifugal force mechanisms. This examination aims to assess the practicality, effectiveness, and potential applications of these technologies in diverse space environments (Schmidt, 2016). Simultaneously, we explore the engineering challenges associated with integrating artificial gravity solutions into spacecraft design, addressing crucial factors such as energy consumption and logistical considerations for successful deployment in space missions (Brown et al., 2024).

Moreover, in our comprehensive literature review, we synthesize current research findings on artificial gravity, encompassing both established and emerging technologies. This synthesis provides a nuanced understanding of the advantages and limitations of each solution, offering valuable insights for informed decision-making in the integration of artificial gravity into future space missions (Clément, 2015) .The practical implications of these technologies are considered, contributing to the ongoing discourse on enhancing astronaut health, psychological well-being, and overall mission success rates in the context of long-term space exploration. The maximum gravitational acceleration level also is a factor if short-radius intermittent artificial gravity is used. Levels up to 2 G at the feet are probably useful, especially if combined with exercise. Passive, 100% G-gradient levels as high as 3 to 4 G at the feet are tolerable for more than 90 minutes in most subjects (Kalita, 2020). Active (bi-cycling) exercise on the Space Cycle is well tolerated from a hemodynamic perspective at G levels up to 3 G at the feet.

Fig:1 Space cycling

This abstract serves as a foundational resource, offering accessible insights into artificial gravity solutions for the broader scientific community, space agencies, and researchers involved in optimizing astronaut health and performance during extended space missions. The referenced research provides a solid basis for understanding the intricacies of artificial gravity technologies, their potential applications, and the challenges associated with their implementation in real-world space exploration scenarios.

The exploration of artificial gravity solutions for optimizing long-term space exploration missions is not without its challenges. One primary challenge involves the intricacies of integrating artificial gravity technologies into spacecraft design (Kalita, 2020). Engineering challenges, highlighted by factors such as energy consumption and logistical considerations, pose hurdles that demand careful navigation. The need to balance the effectiveness of artificial gravity solutions with the practical constraints of space missions introduces complexities that require innovative solutions. Additionally, as this study delves into both established and emerging technologies, staying abreast of the rapidly evolving field and ensuring a clear understanding of the advantages and limitations of each solution can be challenging. The risk of unintentional replication of existing research findings, given the expansive nature of the literature on artificial gravity, adds another layer of complexity.

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

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INTRODUCTION

Short-radius centrifugation (SRC) is a promising countermeasure against the adverse effects of space flight. The main difficulty of using this method is the potential occurrence of adverse orthostatic and vestibular reactions – syncope, cross-coupled illusion, and associated motion sickness (Kotovskaya A.R. et al., 1980; Clément G. and Pavy-Le Traon A., 2004). Recent studies using SRC and Human Centric Rotator Device suggest a potential for any individual to tolerably vestibular acclimate (Bretl K.N. and Clark T. K., 2022). There is also evidence that artificial gravity training may improve orthostatic tolerance (Stenger M.B et al., 2007). Our previous research demonstrated a significant improvement in postural tolerance after 2 repeated SRC sessions in the interval training mode with 4 intervals from 22 to 28 rpm (Saveko A.A. et al., 2023; preprint). In this study we decreased the number of intervals in the SRC mode (up to 3) and expanded the quantity of measured parameters to assess the possibilities of reducing and exploring the adverse effects of the SRC interval training mode.

METHODS

Twelve healthy male volunteers aged 34 to 46 were observed before and immediately after each of the 3 consecutive SRC (radius of 235 cm). The interval between SRC sessions was at last 3 days. Six volunteers have been exposed to the SRC training with 4 intervals from 22 to 28 rpm, and six other volunteers – the SRC training with 3 intervals. Gravity gradient (ΔG) was 74.5%. The subject's head was at a distance of 60 cm from the axis of rotation. See more details in Fig. 1.

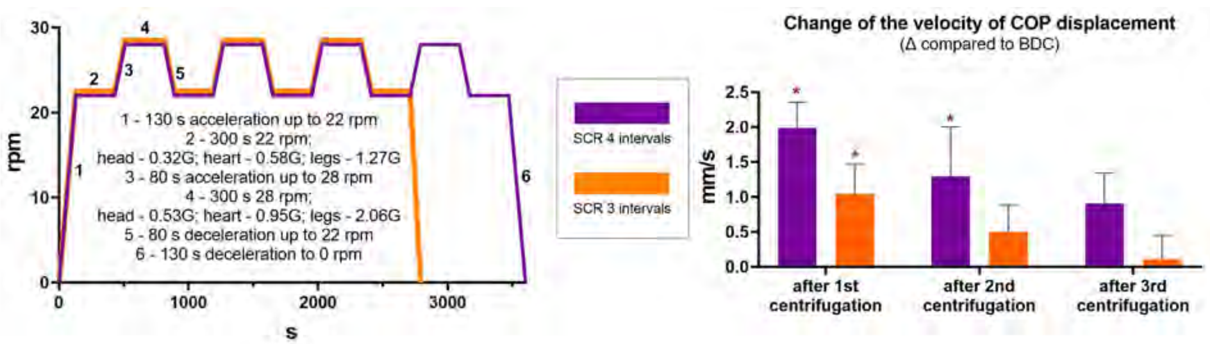

Figure 1. On the left - scheme of SRC modes; on the right – change of the velocity of COP displacement while maintaining a vertical posture (20 s) without visual feedback after each of three consecutive SRC sessions in different training modes (with 4 intervals – purple; with 3 intervals – orange). * - significant difference from the initial data ($p \leq 0.05$).

The Footscan system 0.5 m (RSscan, Belgium) was used for the registration of postural characteristics. The Muscle Lab 4000e system (Ergotest Innovation a.s., Norway) was used to register EMG data of mm. soleus, gastrocnemius lat. and med., tibialis. The heart rate (HR) was recorded using the Polar H10 chest sensor (Polar Electro, Finland). In the previous research, 6 men were asked to fix the center of pressure (COP) at a given point in a standing position using visual feedback. Then, on command, they closed their eyes and kept standing for 20 s, trying to remain in the same position. In the recent data collection, the same test had a longer duration - 120 s. We registered only postural characteristics in the group exposed to the SRC mode with 4 intervals (previous). In addition to the postural study, we synchronously recorded EMG and heart rate in the group exposed to the SRC mode at 3 intervals (recent). All data were analyzed using repeated measures ANOVA and Pearson’s correlation (GraphPad Prism version 8).

RESULTS

The shortened SRC mode caused fewer adverse effects on vertical stability. For example, changes in the velocity of COP displacement were less expressed, and postural tolerance occurred faster (Fig.1). A longer synchronous recording of postural characteristics with EMG and HR data allowed us to record several interesting phenomena that require clarification in the following studies. For example, initially, there was a significant negative correlation between HR dynamic and COP displacement in the sagittal plane - the decrease in HR was accompanied by the forward shift of COP Y. After the first rotation, this correlation disappeared, but another remarkable correlation appeared: at the moment of the forward shift of COP, an immediate increase in the EMG activity of the gastrocnemius muscle was recorded. These phenomena indicate the inclusion of specific regulatory mechanisms against the vertical destabilization after SRC (Fig. 2).

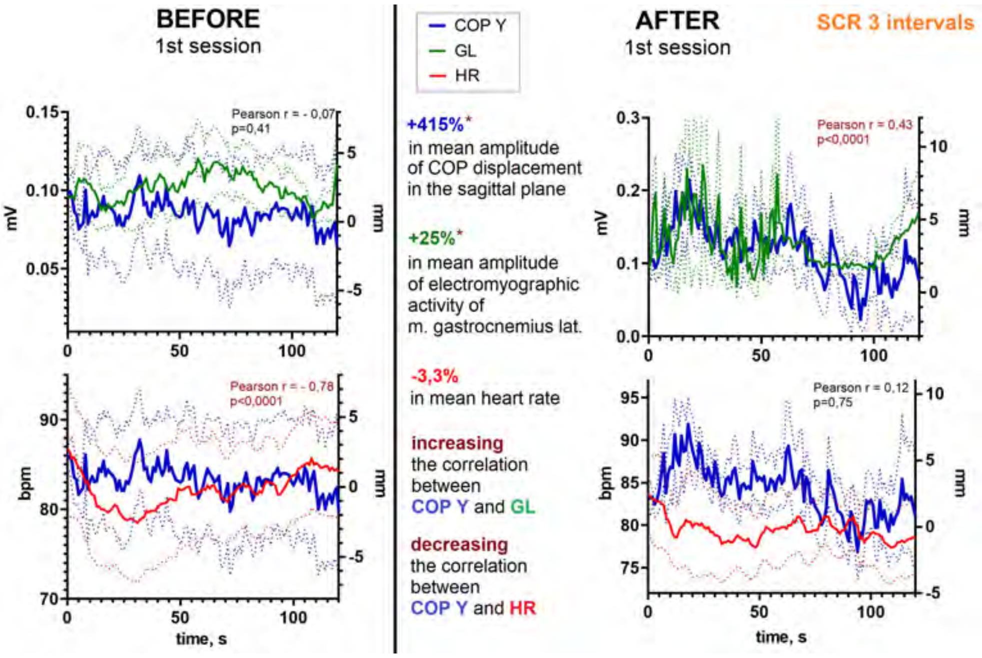

Figure 2. Dynamics of the velocity of the center of pressure displacement in the sagittal plane (COP Y; blue), EMG activity of m. gastrocnemius lat. (GL; green) and heart rate (HR; red) while maintaining a vertical posture (120 s) without visual feedback before (left) and immediately after the first SRC mode with 3 intervals (right). * - significant difference from the initial data ($p \leq 0.05$). The registration frequency was 60 Hz, n= 6. The dotted lines indicate SEM.

FUNDING

The study was supported by the Ministry of Science and Higher Education of the Russian Federation under agreement № 075-15-2022-298 from 18 April 2022 about the grant in the form of subsidy from the federal budget to provide government support for the creation and development of a world-class research center, the "Pavlov Center for Integrative Physiology to Medicine, High-tech Healthcare and Stress Tolerance Technologies".

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

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CONTEXT

The prospect of colonizing the Moon within this decade, followed by human missions to Mars before the end of the next decade, has for several years been driving an increase in research into space medicine and physiology, as well as extreme environments, and thus a growing need for training future researchers (in both the public and private sectors). In addition, fewer than 150 astronauts are currently certified for spaceflight. As human exploration of space grows, especially with the rapid expansion of private space missions, more astronauts will be needed, especially commercial astronauts. The Federal Aviation Administration has issued regulations on the qualification and training of commercial astronauts [1] and some companies are already planning to offer selection and training to provide professionals with the knowledge and skills required to perform work in space [2]. The selection, training and medical monitoring of these professionals will require new experts in this field.

THE OBJECTIVES OF SPACEMED MASTER'S DEGREE

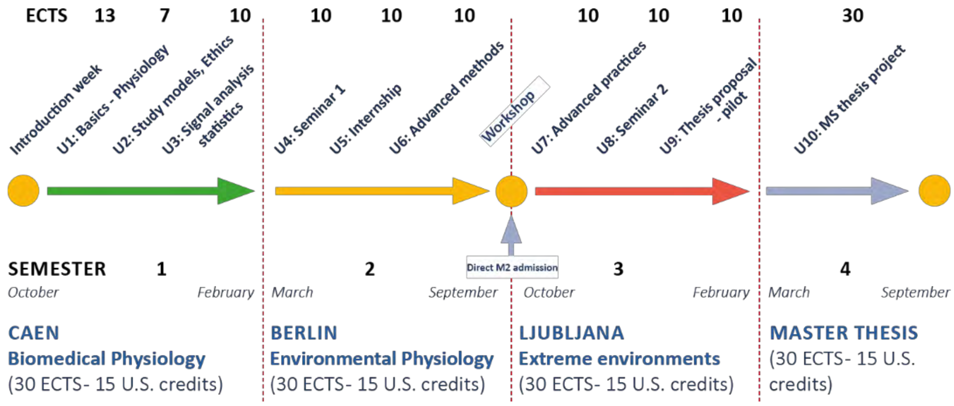
The objective of the SpaceMed Master's degree is to provide training for those interested in pursuing a research career in space life sciences and extreme environments, as well as for medical doctors who would be able to offer medical support for future commercial space flights. The course also provides valuable training to biomedical engineers who will be able to design, optimise, operate, and validate aerospace life support devices.

In order to develop this new, innovative and high-level Master's degree, the three partners (Charité – Universitätsmedizin Berlin, Germany; Mednarodna podiplomska šola Jožefa Stefana, Slovenia; Université de Caen Normandie, France) have joined forces, pooled resources, and developed common mechanisms related to quality assurance, accreditation and recognition of degrees and credits. Almost 40 International Associated Partners (research institutions, space agencies, industrial partners) have been progressively incorporated into the project depending on their specific expertise.

This new master's program has been selected and funded by the European Union as an Erasmus Mundus Joint Master. This funding enables 20 scholarships, worth 33,600€ each (*i.e.* €1,400 per month for 24 months), to be awarded each year.

PROGRAM OUTLINES

SPACEMED is a 2-year full-time European Master's program offering a unique integrated inter- and multidisciplinary study on the effects of extreme environments, particularly space flight, on humans. The curriculum places a strong emphasis on hands-on activities in state-of-the-art laboratories and on field studies, including space flight analogues (i.e., parabolic flights, Antarctic habitats, experimental bed rest studies, etc.), and extreme environments, such as those experienced at high altitude, underwater, and in hot and cold, dry and humid environments.



WHO CAN APPLY?

Student with a Bachelor's degree in natural sciences or kinesiology (180 ECTS / 90 U.S. credits), a Master degree in engineering, or a medical degree.

REFERENCES

[1] Code of Federal Regulations, Title 14, Chapter III, Subchapter C, Part 4607. https://www.ecfr.gov/current/title-14/chapter-III/subchapter-C/part-460

[2] https://www.spaceflightinstitute.com/

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

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ABSTRACT

ESA's vision of the future for human spaceflight and robotic exploration (HRE) is a sustainable and international endeavour to visit new places and make novel discoveries. The ESA HRE strategy includes three destinations where humans will work with robots to gather new knowledge: low-Earth orbit on the International Space Station, the Moon and Mars.

To sustain and enable human exploration missions, ESA HRE has an ambitious and dedicated research programme to better understand the hazards of space, to understand how astronauts react and adapt to the space environment and to focus on countermeasures to mitigate unwanted effects from space hazards. In addition, particular research is also focusing on how to allow humans to embark on exploration class missions. The main research topics focus on human health and performance (physiology and behavioural health), radiation, habitation and habitability (incl. microbiology) and medical capabilities. Furthermore, space and space analogues also offer unique possibilities to study health problems related to diseases, ageing and immobility which then might yield benefits for terrestrial medicine.

This presentation will focus on ESA's Human Exploration activities, including both human research and biology, across the variety of platforms and destinations within the ESA HRE portfolio. Here, the team aims to outline recent highlights stemming from ESA Life Sciences research, the research priorities for the future, and how the (international) science community can get involved with ESA's HRE science programme.

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

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ABSTRACT

Using rotors to expose animals to different levels of hypergravity is an efficient means of understanding how altered gravity affects physiological functions, interactions between physiological systems and animal development. Furthermore, rotors can be used to prepare space experiments, e.g., conducting hypergravity experiments to demonstrate the feasibility of a study before its implementation and to complement inflight experiments by comparing the effects of micro- and hypergravity. Here, we present a new platform called the Gravitational Experimental Platform for Animal Models (GEPAM), which is part of European Space Agency (ESA)'s portfolio of ground-based facilities since 2020, to study the effects of altered gravity on aquatic animal models and rodents. This platform comprises rotors for hypergravity exposure (three aquatic rotors and one rodent rotor) and models to simulate microgravity (cages for mouse hindlimb unloading and a random positioning machine (RPM)). Four species of amphibians can be used at present. All murine strains can be used and are maintained in a specific pathogen-free area. This platform is surrounded by numerous facilities for sample preparation and analysis using state-of-the-art techniques. Finally, we illustrate how GEPAM can contribute to the understanding of molecular and cellular mechanisms and the identification of countermeasures.

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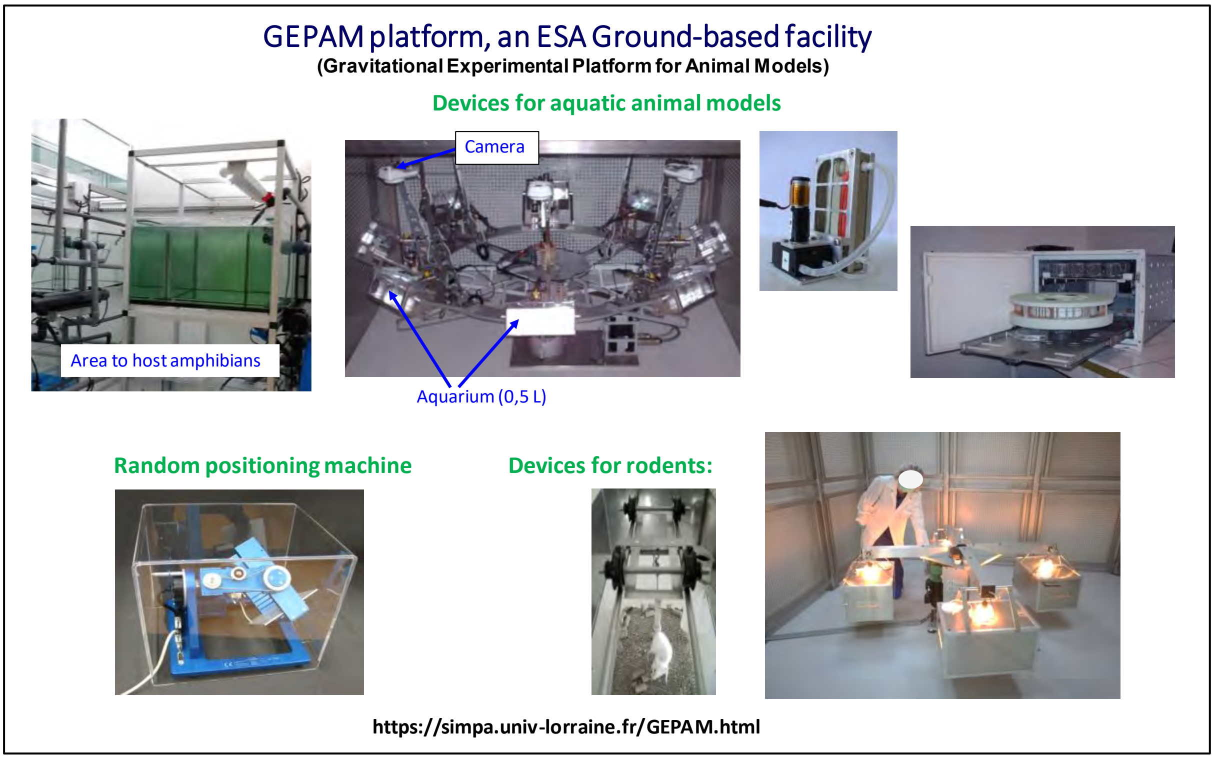
Bonnefoy J., Ghislin S., Beyrend J., Coste F., Calcagno G., Lartaud I., Gauquelin-Koch G., Poussier S. and Frippiat J.-P., 2021, *International Journal of Molecular Sciences*, 22, 2961.



Graphical abstract: Facilities present in the GEPAM platform to subject animals to altered gravity. This platform comprises different aquatic rotors, space-qualified mini-aquaria, a random positioning machine (RPM) to expose embryos, tadpoles or cells to simulated microgravity, cages to subject rodents to hindlimb unloading, as well as one rotor for rodents. GEPAM is located inside a brand-new animal house and surrounded by numerous facilities to prepare and analyze samples

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

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ABSTRACT

ESA parabolic flights provide an accessible, low-cost, reduced gravity environment for scientific research, technology development, and astronaut training. The human-accessible nature of the *Airbus A310 Zero-G* aircraft platform from Novespace allows experimenters to operate their experiments in-person, enabling real-time optimisation of scientific and technical return, and human biomedical experimentation with statistically significant *N* values. A high cadence of the reduced gravity periods (ninety-three times 22s per flight week) provides for redundancy and repeatability, whilst variation of the reduced gravity level, in addition to high quality weightlessness (average absolute deviation <0.007g), provides a gravitational analogue of any planetary body with $0<g<1$, including Lunar and Martian gravity.

In addition to ESA parabolic flights' long-standing service to the scientific community via the *SciSpacE* programme, two new application routes opened in January 2024: The first invites technology research proposals from academic institutions in ESA member states, offering platform access in a similar manner to the scientific community. The second invites proposals from commercial entities via an adaptation of the existing ESA process for evaluation commercial utilisation activities, offering access to ESA parabolic flights on a *pro-rata* basis according to the fraction of resources utilised. Together, these access routes represent the commitment of ESA's Directorate of Human and Robotic Exploration (HRE) to support research enabled by our platforms, and that which will enable our further exploration of LEO, the Moon and Mars.

This presentation will provide an overview of ESA parabolic flight activities, an introduction to the capabilities of the Novespace *A310 Zero-G*, examples of previous and ongoing parabolic flight research, and details of how the scientific, educational and engineering communities can submit research proposals via ongoing and recently developed application routes to join ESA parabolic flights.

NAVIGATING THE NASA IRB AND HUMAN RESEARCH MULTILATERAL REVIEW BOARD (HRMRB): AN ETHICS PERSPECTIVE

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The purpose of the NASA Institutional Review Board (IRB) is to review research activities involving human subjects to ensure that ethical standards for the care and protection of human subjects have been met and that the proposed research activities comply with all pertinent federal regulations and NASA policies. Protection of human participants in research is the shared responsibility of NASA, the IRB, and the scientific investigators designing and conducting the research. This talk aims to educate attendees about the long history of misconduct involving human subject research through the examination of historical case studies that were pivotal in developing the policies governing research today. In addition, we will provide information on the process of doing international human subject research on the ISS and how to get approval through the Human Research Multilateral Review Board (HRMRB).

ClinicalTrials.gov: Understanding the Clinical Trials Requirements at NASA

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ClinicalTrials.gov is a public registry designed to fulfill ethical obligations by providing information about clinical research studies to the general public, patients, medical practitioners and the research community. In the United States, recent revisions to human subject's regulations have prompted new criteria for what constitutes a clinical trial along with requirements not typically requested for other types of human subject's research. Identification of investigational clinical trials is the shared responsibility of NASA, the IRB, and the scientific investigators designing and conducting the research. This talk aims to educate attendees on the history of the development of Clinicaltrials.gov, discussion of why these requirements are important to both participants and researchers, and information on how to identify clinical trials research. In addition, we will provide information on the different requirements for clinical trials conducted at NASA or aboard the International Space Station.

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

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INTRODUCTION: Muscle unloading results in the increased accumulation of calcium ions in skeletal muscle fibers [1] [2]. 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. The throughput of IP3Rs is regulated by Ca^{2+} and IP3, and depending on the concentration of IP3, IP3Rs can induce different intensities of calcium signaling [3].

HYPOTHESES: The current study tested a hypothesis that IP3R activation regulates soleus muscle unloading-induced atrophy by the effect on gene expression via an increase in nuclear calcium ion concentrations [4]. 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 proteolytic processes.

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),

RESULTS: This study for the first time showed that relative soleus muscle weight was improved by 2-APB treatment during 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. The percentage of fast muscle fibers similarly increased in both the 7HS and 7HSA groups. This correlated well with the increased mRNA expression of MyHC IId/x and IIb in the 7HS and 7HSA groups.

The content of phosphorylated CaMKII beta in muscle nuclei was significantly increased in the 3HS group vs control, while in the 3HSA group it was similar to the control values. According to the previous publications [5], the CaMKII phosphorylation can be used as a marker of increased calcium concentration. Similar to previous observations [6] [7] eEF2 phosphorylation was increased in the 7HS group. Phosphorylation 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 suggesting improved protein synthesis. At 7 days of unloading the content of 18S and 28S RNA was decreased in both 7HS and 7HSA groups when compared with the control. The content of phospho-S6 ribosomal protein was decreased in the 3HS and 3HSA groups. Previously, a decrease in the content of S6 protein was found during the development of atrophic muscle processes [8].

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. It is known that IP3R is an activator of ULK1 expression [9]. 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. Similar results were observed for the mRNA expression of IL6. Blocking IL6 receptors prevents the development of muscle atrophy [10], and the introduction of IL6 into the muscle, on the contrary, leads to its atrophy [11].

Calcineurin (CaN) and IP3R are well known calcium sensors. The content of CaN and IP3R was increased in the 3HS group, but it was not different from the control in the 3HSA group.

CONCLUSIONS: It can be suggested that maintenance of muscle mass during unloading after treatment with 2-APB is associated with the prevention of the decrease of ribosomal biogenesis and protein synthesis. At 7 days of unloading, it was also affected by the downregulation of ULK1 and IL6 expression in the unloaded 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.

This study was supported by the Russian Foundation for Fundamental Investigations (RFFI; project No. 20-015-00138; TLN).

KEYWORDS: muscle unloading; muscle atrophy; IP3 receptors.

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

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Activating the sympathetic outflow with mental challenge provides a possibility to counteract orthostatic intolerance and syncope (Goswami N. et al, 2012). As orthostatic intolerance and syncope are common post-spaceflight and in older adults upon change of posture from supine to standing up, there is an urgent need to search for countermeasures that alleviate orthostatic intolerance. Cognitive tasks, such as motor imagery (MI) were shown to increase the autonomic response (Collet C. et al, 2013). MI is defined as explicit mental execution of one's own body movements or a task in order to simulate or plan future motor action (Stevens J.A., 2005). MI involves similar mechanisms underlying motor preparation and execution and is thus likely to elicit changes in the sympathetic activation to insure the necessary cardiorespiratory responses to the upcoming expected energy expenditure. Despite the existing evidence of increased sympathetic tone in response to MI, it is still unclear whether MI has the potential to influence cardiovascular, autonomic and microvascular responses to an orthostatic challenge. In this study, we investigated whether imagery training of leg antigravity movements can influence the orthostatic responses. We hypothesised that by activating sympathetic activity, MI application primes the cardiovascular system for the upcoming body verticalization by increasing heart rate or blood pressure.

Twenty-seven healthy volunteers (n=14 males), mean age of 24 ± 3.76 participated in the study protocol consisting of: 15 min supine laying at baseline (BL), followed by 5 min of standing (Stand I), 5 min recovery laying (R-I), and 15 minutes of MI, after which a second standing period of 5 min (Stand II) was performed. The session ended with a 5 min supine recovery period. The MI task started from a supine position at rest (BL-II), and contained the imagery of lifting up both legs in slightly knee flexion to a vertical position, and to move legs down to the starting position. In order to avoid mental fatigue, the MI was performed in blocks of 60 sec MI alternated by 30 sec of rest for a duration of 15 min, resulting in a total of 10 min MI training time. Hemodynamic monitoring included measurement of blood pressure (upper arm oscillometry and finger plethysmography), heart rate (3-lead ECG) and thoracic impedance using a Task Force Monitor® (TFM, CNSystems, Graz, Austria). Changes in the peripheral vascular control and calf venous volume were monitored by near infra-red spectroscopy (NIRS, Omegawave BOM-L1 W) during the Supine-to Stand test.

During MI, heart rate, diastolic and mean arterial blood pressure as well as the total peripheral resistance index were significantly higher as compared to the breaks in-between MI as well as compared to baseline measures at rest. During standing, following either ten minutes of rest (Stand I) or MI (Stand –II), no significant differences in hemodynamic parameters were observed. OxyHB (and correspondingly oxy/deoxyHB) was significantly higher following MI, especially at the beginning of stand.

Motor imagery improved the hemodynamic parameters in preparation for standing up, and showed a priming effect with regard to peripheral tissue circulation during standing. Performing MI may be considered as orthostatic priming, which, if carried out before standing up, may have a clinical potential in the prevention of orthostatic intolerance, especially in returning astronauts and in senior citizens.

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Novel GPR120 agonist modulates systemic and neuroinflammation

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INTRODUCTION:

Our aim was to target the systemic and central inflammation in obese mice by using a newly synthetized GPR120 analogue.

MATERIALS & METHODS:

We synthesized GPR120 agonist, called NKS5. We determined its molecular dynamic interaction with the receptor. In C57Bl/6 male mice, fed a high-fat diet, we elucidated its effects on obesity and its related parameters like systemic and neuroinflammation, by immunofluorescence staining, targeting microglia-specific calcium-binding proteins (IBA-1). Neuroinflammation severity was assessed in multiple hypothalamic, cortical and subcortical regions.

RESULTS:

Daily intake of NKS-5 *via* feeding bottles modulated gut microbiota, decreased food intake and progressive weight gain in obese, but not in control, mice. Interestingly, the NKS5 decreased circulatory proinflammatory factors such as lipopolysaccharide (LPS), tumor necrosis factor (TNF) alpha, and interleukin (IL) 6 in obese mice. Moreover, in intraperitoneal glucose tolerance test (IPGTT), the NKS5 accelerated the glucose clearance in obese mice. The NKS5 also decreased the expression of IBA-1 expression in different regions of the brain.

CONCLUSIONS:

Our results show that novel chemical GPR120 agonists might represent a new strategy to reduce systemic and neuroinflammation.

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

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ABSTRACT

Human spaceflight is known to cause a reduction in both muscle mass and muscle strength, as well as an increased risk of back pain in astronauts. In fact, up to 68% of astronauts report experiencing back pain, with 4% experiencing severe symptoms. The objective of this review is to assess the efficacy of far infrared emitting suit treated with Zinc Oxide in mitigating low back pain and improving paraspinal muscle strength during spaceflight. Our hypothesis is that using far-infrared-garments using nanotechnology has the potential to decrease back pain during spaceflight. In vitro results showed the suit has the potential to improve tissue perfusion, with FIR-emitting particles that deliver targeted pain relief to the muscle spasm of the back. This allows the particles to penetrate the skin up to 1.5 inches (almost 4 cm) decreasing pain during activity and providing overall comfort. Additionally, the product includes separate arm bands and supportive socks, infused with FIR-emitting particles to alleviate pain in the arms and legs. Particularly, in the range of 8–14 mm, FIR is proven to have many biological effects. The emitted heat from FIR garments influences cell membrane, mitochondrial metabolism and could increase blood circulation, tissue regeneration, induce upregulation of calcium-dependent nitric oxide (NO), and calmodulin in different cell lines, having positive effects on antioxidative, anti-inflammatory, and analgesic effects.

KEYWORDS: Back Pain, Paraspinal Muscles, Spine, Spaceflight.

***Figure 1:** Introducing the Space Fit Suit, an innovative far-infrared radiating suit specifically designed by Sigma Fit LLC for applications in Intravehicular Activity (IAV).*

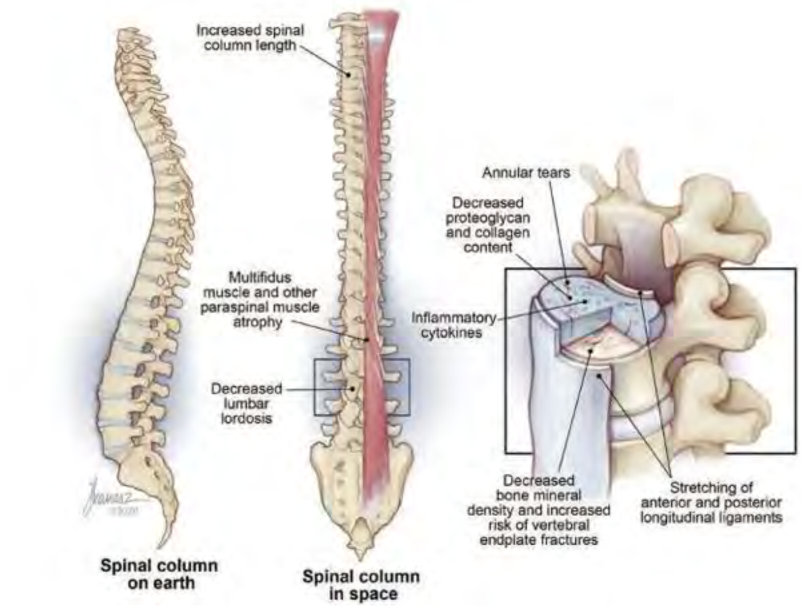

***Figure 1**: Spinal column length on Earth and in space. Credit: Johns Hopkins Medicine.*

***Figure 3:** Results showed that ZnO optimize the functionality and overall fabric properties that led to significant results in fabric compressibility of the Space Fit suit compared to the control group (non-coated fabric).*

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

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Recent interest in long-term space missions, such as those proposed for Mars exploration, underscores the necessity of investigating how the space environment impacts the musculoskeletal system. Microgravity-induced decreased loading poses a significant risk for bone and muscle mass loss and increases susceptibility to injuries (Deymier et al., 2020). Previous research has shown that exposure to microgravity for 28 days reduces the latency and amplitude of the Achilles tendon reflex, indicating alterations in neuromuscular function (Fujii and Jaweed, 1992).

Tendons, ligaments, and connective tissue serve as crucial links between bone and muscle, supporting joint stability and force transmission. While animal studies have provided insights into the effects of unloading on these tissues, the cellular and molecular impacts of short- and long-term microgravity exposure on tendons in humans remain inadequately explored. Existing data suggest that unloading decreases the stiffness of human Achilles and patellar tendons, primarily due to changes in material properties, although mechanisms such as a reduction in collagen cross-links may contribute to the observed effects (Liphardt et al., 2023). Moreover, tendons and enthesis play a critical role in joint movement by transmitting forces generated by muscles to the bone with minimal energy dissipation and deformation (Franchi et al., 2007; Magnusson and Kjaer, 2019; Roffino, 2021).

Understanding the alterations occurring in muscle, tendon, and bone under chronic mechanical load reduction is essential for mitigating its deleterious effects. Numerous reviews have documented the impact of reduced mechanical loading on muscle and bone, although with less emphasis on tendon integrity (Franchi et al., 2007; Magnusson & Kjaer, 2019). Studies employing earthbound analogs have examined the effects of disuse on the musculoskeletal system, revealing decreased muscle mass, tendon thickness, bone loss, and compromised strength and stiffness in both tendon and bone (Deymier et al., 2020).

Exploring the mechanical dynamics of the Achilles tendon is crucial for addressing various musculoskeletal conditions and optimizing athletic performance. This study presents a myotonometric approach of MusTone device (Nistorescu et al., 2018, 2019, 2022) to assess Achilles tendon properties and evolution during 21 days in Dry Immersion environment. The MusTone device employs advanced sensor technology to measure mechanical perturbations in the tendon, providing valuable insights into its behavior.

The 21-day Dry Immersion (DI) experiment conducted at Institute of Biomedical Problems (IBMP) of the Russian Academy of Sciences in 2018 (Tomilovskaya et al., 2021) involved the MusTone device in measurements over a sample of 6 male subjects. The subjects were all right-dominant with an average age of 29 years ($M = 29$, $SD = 3.68$), average height of 176.38 cm ($M = 176.38$, $SD = 4.38$), average body weight of 77.03 kg ($M = 77.03$, $SD = 8.97$) and average Body Mass Index (BMI) of 24.7 kg/m² ($M = 24.7$, $SD = 2.07$), which was within the normal acceptance range. Ethical approval for the experiment was granted by the bioethical commission of the IBMP and informed consent was obtained from all subjects. Measurements were performed at 5 specific timeframes: Preparation period - P (Day 5 before DI), during the immersion period (i.e., DI-A: Day 5-10, DI-B: Day 13-17, DI-C: Day 21) and Recovery period - R (Day 2-4 after DI).

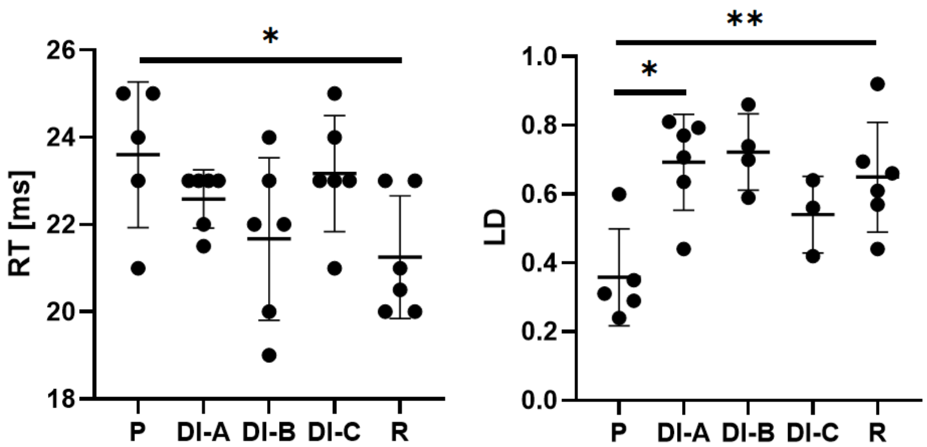

Figure 1. Achilles tendon evaluated in terms of mechanical stress Relaxation Time (RT) and Logarithmic Decrement (LD); statistical significance of results: * $p < 0.05$, ** $p < 0.01$.

The data analysis for Achilles tendon mechanics with MusTone device showed that mechanical stress relax time (RT) decreases and Logarithmic Decrement (LD) increases during DI experiment in a time dependent manner. The average value of the RT in preparation period (P) was 23.6 ± 1.673 (ms). After DI, in recovery period (R), the average value of the RT was 21.25 ± 1.405 (ms), the RT values were statistically significant ($p < 0.05$). This 11% early decrease verify the previous findings about reduced muscle elastic properties, especially for antigravity muscles, but also for tendon structure and elastic properties changes, including involvement of the fibrocartilaginous enthesis. The average value of the LD before DI was 0.358 ± 0.141 (P). In the first days of immersion (DI-A), LD increases significantly to 0.6925 ± 0.1395, $p < 0.05$ and after DI exposure, at recovery period (R), the average value of the LD was also increased at 0.6492 ± 0.1594, $p < 0.01$. The 60% increase of LD reveal even better the reduction in Achile tendon thickness and viscoelastic properties.

Briefly, the results obtained with MusTone device accurately evaluate the Achile tendon behavior and can be used in the rehabilitation programme shaping and follow-up. These findings contribute to our understanding of the effects of prolonged inactivity on human health and may inform the development of countermeasures for astronauts during space missions. Additionally, insights gained from this study have implications for terrestrial applications, such as bed rest studies and rehabilitation protocols for individuals with limited mobility.

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Exploring the Therapeutic Potential of Gravitational Psychology in Disease Understanding

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ABSTRACT:

Gravitational psychology, a burgeoning field at the intersection of gravitational biology and psychology, offers a novel perspective on understanding the intricate relationship between gravitational forces and human health. This abstract delves into the potential of gravitational psychology as a tool for comprehending disease pathology and advancing therapeutic interventions. Gravity, a fundamental force shaping the dynamics of life on Earth, exerts profound influences on biological systems, including cellular function, organ development, and even behavior. Gravitational psychology seeks to unravel how these gravitational cues influence neural processes, cognitive functions, and emotional states, thereby impacting overall well-being. By integrating principles from physics, neuroscience, and psychology, gravitational psychology provides a framework for investigating how altered gravitational conditions, such as microgravity or hypergravity, may contribute to the onset or progression of various diseases. Understanding these mechanisms is particularly pertinent in space exploration, where astronauts face unique physiological challenges in microgravity environments. Furthermore, gravitational psychology offers insights into terrestrial health issues, shedding light on conditions influenced by gravitational imbalances, such as vestibular disorders, postural instability, and mood disorders. By elucidating the interplay between gravitational stimuli and neurological responses, researchers can develop targeted interventions for mitigating these disorders and improving quality of life. Moreover, gravitational psychology holds promise for elucidating the pathophysiology of neurodegenerative diseases, such as Parkinson's and Alzheimer's disease, which are characterized by disturbances in motor function and cognitive decline. By elucidating how gravitational cues modulate neuronal circuits and synaptic plasticity, researchers can identify novel therapeutic targets for these debilitating conditions. In conclusion, gravitational psychology represents a multifaceted approach to understanding the intricate interplay between gravitational forces and human physiology, with profound implications for disease understanding and therapeutic innovation. By leveraging.

HEADING 1:

Unraveling the Influence of Gravity on Human Health.

Heading 2:

Bridging Neuroscience and Gravitational Biology: Insights into Disease Mechanisms.

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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

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ABSTRACT

The identification of safe and easily-determined-inflight biomarkers to monitor the immune system of astronauts is mandatory to ensure their well-being and the success of the missions. Here, we evaluated the relevance of two biomarkers whose determination could be easily implemented in a spacecraft in the near future by using bedridden volunteers as a ground-based model of the microgravity of spaceflight. Our data confirm the relevance of the neutrophil to lymphocyte ratio (NLR) and suggest platelet to lymphocyte ratio (PLR) monitoring to assess long-lasting immune diseases. We recommend coupling these ratios to other biomarkers, such as the quantification of cytokines and viral load measurements, to efficiently detect immune dysfunction, determine when countermeasures should be applied to promote immune recovery, prevent the development of disease, and track responses to treatment.

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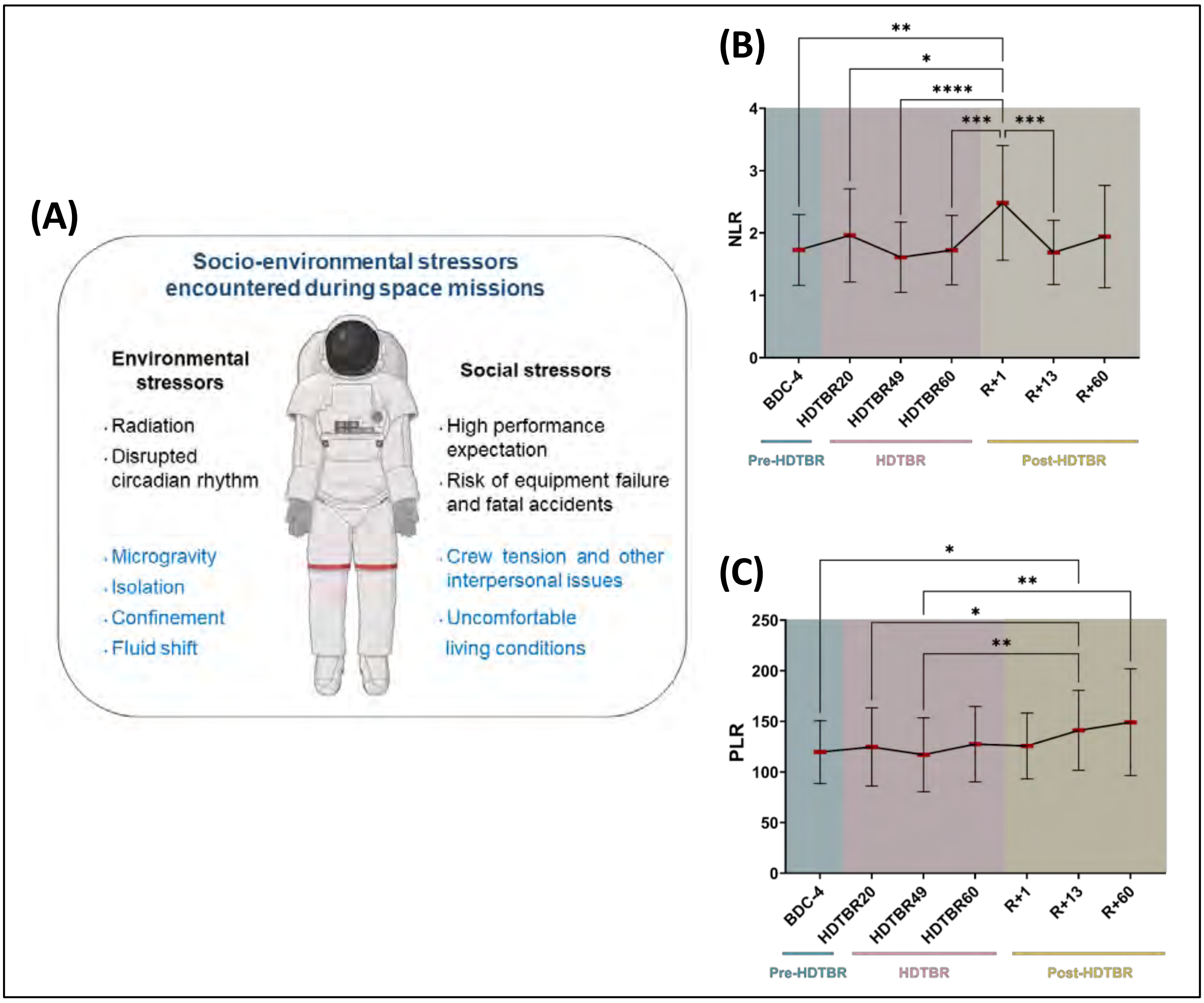

Graphical abstract: (**A**) Socio-environmental stressors encountered during space missions. Those encountered during head-down tilt bed rest exposure appear in blue. (**B**, **C**) Evolution of NLR and PLR ratios before, during and after two months of head-down tilt bed rest. n=20, except at R+60, where n=17. Horizontal bars indicate the mean. Statistically significant differences were revealed using either one-way ANOVA or a linear mixed effects model analysis. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. BDC, baseline data collection (blue); HDTBR, head-down tilt bed rest (pink); R, recovery (yellow).

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

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ABSTRACT

INTRODUCTION: Isolation and confinement impose significant stressors during space travel, affecting the physical and mental well-being of crewmembers. Research has shown that space travel accelerates vascular aging and increases the susceptibility to cardiovascular and cerebrovascular disorders. However, the impact of prolonged isolation and confinement on (micro-)vascular function remains insufficiently explored.

METHODS: Retinal vascular imaging was conducted on five crewmembers during and after an 8-month SIRIUS-21 space analog mission. Measurements of central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE), arteriovenous ratio (AVR), and pulse wave velocity (PWV) as an indicator of arterial stiffness were obtained.

RESULTS: The analysis of data from the isolation period and post-isolation period revealed an increasing trend in the average CRVE (Pearson's $r = 0.784$, R-square = 0.62), indicating dilation of retinal venules. Conversely, the AVR demonstrated a decreasing trend (Pearson's $r = -0.238$, R-square = 0.057), suggesting a higher risk of cardiovascular and cerebrovascular dysfunctions. However, these trends did not reach statistical significance. Furthermore, the average PWV exhibited an upward trend both during and after isolation for all crew members.

CONCLUSION: Isolation and confinement during space travel appear to contribute to the retinal vascular damage and arterial stiffness, implying an elevated risk of cardiovascular and cerebrovascular disorders. Further investigations are necessary to validate and expand upon these findings, particularly as we prepare for future manned missions to the Moon and Mars.

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Figure 1: AVR downward trend (Pearson's r -0.238, R-square 0.057) predicting a higher risk of cardiovascular and cerebrovascular dysfunctions, but neither trend were statistically significant.

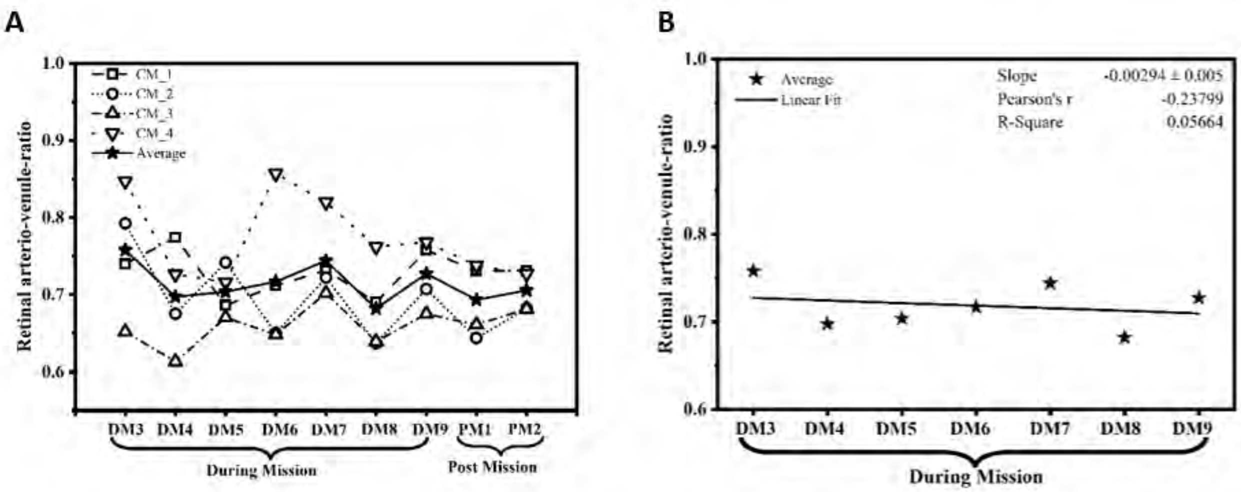
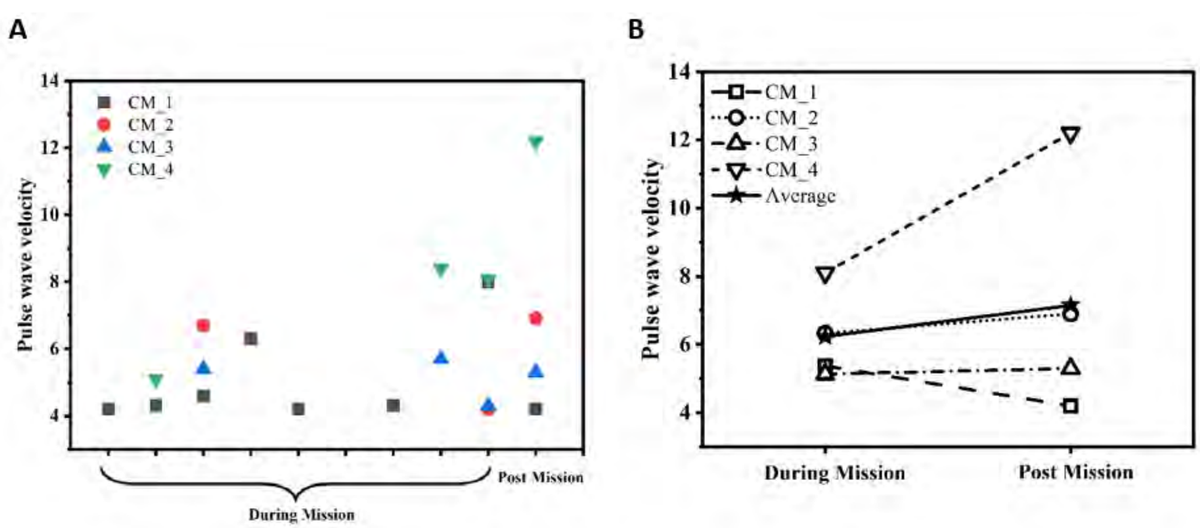


Figure 2: PWV shows an upward trend indicates a potential increase of vascular stiffness during and post isolation.



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

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Orthostatic intolerance (dizziness upon standing up) is a multifaceted condition with serious consequences due to the high risk of falls and falls-related injuries. Orthostatic intolerance disproportionally affects older and female populations and it is often seen following prolonged bedrest confinement, but also in returning astronauts. Sinusoidal (side-alternating) vibration/whole body vibration has been reported to be effective against a plethora of major health conditions, in particular against muscle atrophy and bone mineral density loss, which are seen in both, older adults and astronauts. However, its effects on the cardiovascular system - and its potential as a countermeasure for orthostatic intolerance- remain largely unexplored. Therefore, we investigated the effects of varying sinusoidal vibration frequencies and body weight loading on hemodynamic and autonomic responses during standing up. Specifically, the effects of four protocols (vibration frequencies of 13 and 25 Hz, as well as 15° and 30° head up tilt, HUT, corresponding to different body weight loading) on hemodynamic and autonomic responses to a supine to stand test were assessed. We hypothesized that significant differences in heart rate, systolic blood pressure and autonomic responses will be seen with different frequencies and weight loading.

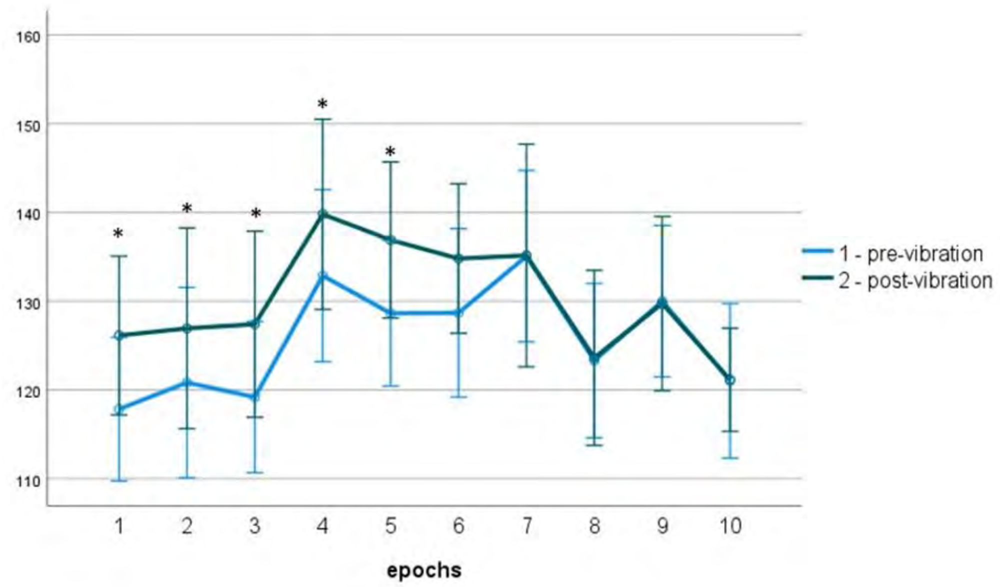
Twenty-nine healthy, young volunteers (n = 15 males) participated in the vibration study. Each participant visited the laboratory four times, each visit separated by one week, applying the four different protocols in a randomized order. During each visit, either 15° or 30° HUT in combination with 13 Hz or 25 Hz vibration frequency was applied. Continuous hemodynamic and autonomic responses were measured using the Task Force Monitor (TFM, CNSystems, Graz, Austria). The study protocol consisted of 15 minutes on the tilt-bed for the baseline recording, followed by 5 minutes of stand (stand I) and further 5 minutes supine again. In the supine position, the participant's feet placement on the vibration platform was checked to ensure it was flat between two marker points and the Galileo vibration platform was turned on for 15 minutes (according to one of the four protocols). Immediately after completion of the vibration protocol, the participant carried out another 5 minutes of unassisted standing (stand II), followed by another 10 minutes lying supine again (recovery, see figure 1).

Figure 1: Study protocol (* head up tilt)

Baseline	Stand I	Rest	Intervention: vibration + HUT*	Stand II	Recovery
15 minutes	5 minutes	3 minutes	15 minutes	5 minutes	10 minutes

We observed significant differences in heart rate and systolic blood pressure responses during the supine to stand test when different frequencies or weight loading were used. Systolic and diastolic blood pressure were significantly higher for the 25 Hz, 30° HUT protocol, and heart rate – for the 13 Hz, 30° HUT; the latter resulted in a tendency for lower heart rate during the vibration, and in higher systolic blood pressure during the stand test.

Figure 2: Changes in systolic blood pressure during stand and recovery for the 25 Hz, 30° HUT intervention.



The results of this study are important as the application of whole-body vibration during bed rest can provide cardiovascular training and prevent orthostatic intolerance. In particular older persons and chronically bedridden patients as well as astronauts could benefit from this passive form of exercise.

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Performing the bedrest study for the space medicine educational programs

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

The bed rest experiment provides an opportunity to comprehensively study the effects on the human body during space missions by simulating the muscle and bone fragility, fluid shift changes, and their impact on the nervous and cardiovascular systems. This is achieved by having subjects undergo a long-term head-down tilt of 6 degrees, mimicking the conditions experienced during space travel.

Using the muscle electromyography (FreeEMG1000), we measured the electromyographic activity of the soleus, gastrocnemius, tibialis anterior, peroneus longus, vastus lateralis, rectus femoris, biceps femoris, and gluteus medius muscles of the bed rest subjects during walking before and after bed rest. The physical movements we perform casually are the result of highly complex motor control. Due to the presence of a vast number of muscles in the human body, the central nervous system faces a tremendous amount of information processing, as it needs to control each individual muscle. To address this issue, the concept of "muscle synergy," which is a neurocontrol mechanism that groups functionally similar muscles together, has been proposed as a strategy for simplifying the vast amount of information processed by the central nervous system. Using the electromyographic data, we calculated the muscle synergies (weighting vectors from muscle synergies to each muscle) and their activations (motor commands from the central nervous system to muscle synergies) through non-negative matrix factorization.

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1 Deep Space Initiative

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

Long-term exposure to microgravity has proven to cause biomechanical side effects to our human physiology. With the onset of commercial spaceflight and space tourism following the drive of multiple national space agencies and corporations around the world, the importance to avoid and mitigate these effects is more prevalent than ever. It is necessary to 1) identify all internal-external factors causing the side effects; 2) identify existing counter-interventions; and 3) develop new countermeasure strategies studied to be effective.

There is still much to be elucidated regarding the physiological effects from being exposed to various gravitational stresses across the scale (μg < partial gravity <1 g). In this review, we describe these fundamental physiological changes that occur in the human skeleton and highlight the unique importance of exercise in space. We discuss the interplay between the musculoskeletal, neuromuscular, and the cardiovascular system, noting how each system responds to changing gravitational environments. While investigating the effect of microgravity on bone density, muscle atrophy, and joint stability, simulations of hypogravity and reduced gravity provided insight into the potential effects of acceleration and deceleration forces on the body. Also discussed are the clinical and preclinical interventions that have been used to investigate biomechanical stress during movement. Compared to 1G, studies in microgravity and in particular, partial gravity showcase significant reduction in several aspects including mechanical work and ground reaction forces, resulting in a need for sufficient exercise and countermeasures in order to compensate for this inadequate in mechanical stimuli.

This review presents an in-depth discussion of the biomechanical stress on the human body during functional movement under varying gravity conditions, and summarizes findings and data collected from existing studies in the last 10 years. The insights from this review not only remain important, but also hold promise for improving human performance, reducing injury risks, and optimizing physical training regimens in both space exploration and terrestrial applications.
Overall, it summarizes our knowledge of the effects of biomechanical stress in space, and will help provide more constructive understanding on how to best conduct exercise programmes that will be critical to overall health, ensuring the safety of our future astronauts and future missions.

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

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Under conditions of microgravity or simulated gravitational unloading (so-called functional unloading), the skeletal muscles of mammals undergo a number of negative changes. Already on the first day of simulated gravitational unloading, the level of expression of genes that regulate the biogenesis and functions of mitochondria significantly decreases, and the expression pattern of myosin genes changes. In the case of postural (postural-tonic) muscles, such as the soleus muscle, which in humans ensures the vertical position of the body, these changes lead to a decrease in the proportion of muscle fibers of the "slow", oxidative and fatigue-resistant type, which ultimately leads to increased muscle fatigue and the disturbance of its normal functioning. In parallel with these changes, there is an activation of proteolysis and a decrease in protein synthesis, which leads to atrophy of both "slow" and "fast" muscle fibers and also contributes to a decrease in muscle function. The described changes are observed not only during space flight, but also during prolonged bed rest, during limb immobilization, and even during temporary restriction of activity. As part of many years of research conducted at our Institute, a theory was developed that explains the development of negative changes occurring in postural (postural-tonic) muscles by the absence of a support stimulus, which leads to a decrease in support afferentation and inactivation of muscle fibers (Shenkman and Kozlovskaya 2019).

Electrical muscle stimulation technique can directly restore the activity of slow or fast muscle fibers depending on the frequency of stimulation (50-60 Hz for fast fibers and 10 Hz for slow fibers). High-frequency electrical stimulation of skeletal muscles is used in sports medicine and leads to increased muscle hypertrophy in athletes; it is often used in addition to training or as a replacement for training for athletes who are forced for some reason to stop training. Low-frequency electrical stimulation of rodent muscles leads to an increase in the content of mitochondrial enzymes and the proportion of "slow" muscle fibers, and also leads to a decrease in muscle fatigue in humans (Scott, Vrbova et al. 1985).

The goal of the ongoing project was to study the effect of electrical stimulation on a number of molecular changes recorded after functional unloading (using the "dry immersion" model), and to compare the results obtained after "dry" immersion with electrical stimulation and after "dry" immersion without preventive measures. In the first experiment, ten healthy men were exposed to 6 days of dry immersion, as described in previous studies. 10-8 days before the start of dry immersion, biopsy samples of the soleus muscle were taken from the left leg of each participant (pre-DI samples), the second series of samples was taken after 6 days of the experiment (post-DI samples). In the second experiment, there were 8 participants, who underwent the two sessions of hips and shins electrical stimulation, 40 min morning session of 25 Hz stimulation, and afternoon session of 10 min 2500 Hz modulated to 50 Hz stimulation. The biopsy samples from soleus muscles were taken similarly as in the first experiment. The study was approved by the Committee of Biomedicine Ethics of the Institute of Biomedical Problems of the Russian Academy of Science (Protocols № 594 from 6 September, 6th, 2021 and № 620 from July, 12, 2022).

After the experiments, the biopsy samples were immediately frozen in liquid nitrogen and then used for total protein fraction preparation, total RNA extraction and frozen transverse cryosection preparation. The proteins content and posttranslational protein modifications were determined by Western blot. Gene expression levels were determined by RT-PCR. Muscle fibers cross-sectional area were determined by immunohistochemical analysis.

6-day dry immersion (DI) did not lead to any significant changes in soleus muscle slow-to-fast fiber-type ratio or myofiber cross-sectional area, while after the DI with electrical stimulation (ES-DI) fast-type soleus myofibers were significantly lower than before the ES-DI, and the percent of fast-type fibers decreased vs pre-ES-DI values. The lack of effect of 6-day DI on slow myofiber CSA and slow-to-fast fiber-type ratio is in variance with the previous results in a 7-day DI experiments, where slow-type fibers CSA was downregulated (Moukhina, Shenkman et al. 2004). The causes of this discrepancy remain obscure. 6-day DI lead to downregulation of myosin isoform IIa, while did not affect other myosin isoforms expression vs pre-DI samples. ES-DI lead to downregulation of IId/x myosin and upregulation of IIa myosin isoforms vs pre-DI samples.

At the same time, 6-day DI lead to significant downregulation of mitochondrial-related markers genes expression vs pre-DI samples, such as COXI, COXII, COXIV, OPA1 and mitofusin-1, while after the ES-DI these parameters did not change vs pre-ES-DI values. However, PGC1alpha dene expression declined in the post-DI samples vs pre-DI in the both experiments. It also should be mentioned, that gene expression of several mitophagy regulators genes expression (such as BNIP3, LC3B, Fundc, OPTN) were either downregulated or did not change after the DI, while being significantly upregulated after the ES-DI. This situation may indicate the autophagy and/or mitophagy upregulation in the ES-DI sample group, which may explain the downregulation of fast-type fibers in the ES-DI sample group.

The study was supported by the Ministry of Science and Higher Education of the Russian Federation under agreement № 075-15-2022-298 from 18 April 2022 about the grant in the form of subsidy from the federal budget to provide government support for the creation and development of a world-class research center, the “Pavlov Center for Integrative Physiology to Medicine, High-tech Healthcare and Stress Tolerance Technologies”.

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TIME-COURSE OF ALTERATIONS IN THE EXPRESSION OF MECHANOSENSITIVE ION CHANNELS IN RAT SOLEUS MUSCLE UNDER SIMULATED MICROGRAVITY

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Mechanosensitive cation channels represent one of the key membrane-imbedded mechanosensors involved in the mediation of rapid electrochemical responses to changes in mechanical forces. These channels play an important physiological role in the regulation of various Ca^{2+}-related processes such as nitric oxide synthesis, protein synthesis and degradation, cytoskeleton formation, as well as differentiation and fusion of myoblasts and muscle regeneration. Under prolonged real or simulated microgravity postural muscles (such as slow-twitch soleus muscle) undergo severe atrophy characterized by decreases in muscle mass, myofiber cross-sectional area (CSA), and slow-to-fast fiber-type transformation. It is not excluded that Ca^{2+}-permeable mechanosensitive channels may be involved in the processes underlying skeletal muscle atrophy in response to reduced mechanical loading/microgravity. Hence, the aim of the present study was to elucidate time-course changes in the expression of the key mechanosensitive channels (Piezo1, TRPC1, TRPC3, TMEM63B) in rat postural muscle during simulated microgravity. To this end, Wistar male rats were subjected to hindlimb unloading (HU) for 1, 3, 7 and 14 days. Relative changes in gene expression of Piezo1, TRPC1, TRPC3 and TMEM63B were determined by real-time quantitative PCR analysis. Protein levels of Piezo1 were assessed by Western-blot analysis.

One-day HU resulted in a slight increase (+24%, $p<0.05$) in Piezo1 mRNA expression without any changes at the protein level. However, at the later stages of HU we observed significant reductions in the Piezo1 protein content in rat soleus muscle: – 64% ($p<0.05$) after 3-day HU, – 61% ($p<0.05$) after 7 -day HU, and – 82% ($p<0.05$) after 14-day HU. At the early stages of HU (1 and 3 days) there was a significant increase in the mRNA expression of both TRPC3 and TMEM63B compared to the control weight-bearing animals. Expression levels of TRPC1 were upregulated after 3-day HU (+ 44%, $p<0.05$) but downregulated following 14-day HU (– 38%, $p<0.05$). Seven-day unloading did not induce any changes in the gene expression of TRPC1, TRPC3 and TMEM63B. Mechanical unloading for 14 days resulted in a significant downregulation of TMEM63B mRNA expression (– 33%, $p<0.05$) compared to the control rats. Thus, our study revealed different patterns of gene expression of the key mechanosensitive cation channels in rat soleus muscle during the course of unloading. While at the early phase of mechanical unloading (1-3 days) there was a transient increase in the mRNA expression of Piezo1, TRPC1, TRPC3 and TMEM63B, at the later stage of unloading (14 days) a significant downregulation of TRPC1 and TMEM63B at the mRNA level and Piezo1 at the protein level was observed.

The study was supported by the Russian Science Foundation (RSF) grant no. 22-75-10046.

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

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ABSTRACT

Objective: To investigate the effects of simulated microgravity on sperm functions and the genes that regulate sperm motility, viability, and acrosome reaction.

Methods: Semen samples were collected from five healthy donors. Following an initial neat semen analysis, each sample was divided into three different groups: control, 2-dimensional (2-D) and simulated microgravity (s-MG). Evaluation of sperm functional parameters such as total motility (TM), progressive motility (PM), viability, and acrosome reaction (AR), along with the assessment of genes specific to sperm motility (*SPATA6, SPATA20*) and AR (*CABS1*) was performed at different time points (0, 1, 3 and 6 hours (Hr)). Data were analyzed using GraphPad Prism 9.03.

Results: At 3Hr and 6Hr, there was a significant reduction in TM in the 2-D and s-MG groups compared to the control ($p<0.05$) (Fig. 1A). Similarly, there was a significant decrease in TM in the s-MG group over time ($p<0.05$) (Fig. 1A), while there was no significant difference in PM (Fig. 1B). Viability was significantly reduced after exposure to s-MG for 1Hr ($p=0.02$), 3Hr ($p=0.02$) and 6Hr ($p=0.01$) (Fig. 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 ($p<0.05$) (Fig. 3). While there was no statistically significant difference observed in PM and mRNA expression of genes of interest (Fig. 4), the analysis reveals a discernible trend indicating the potential effects of s-MG.

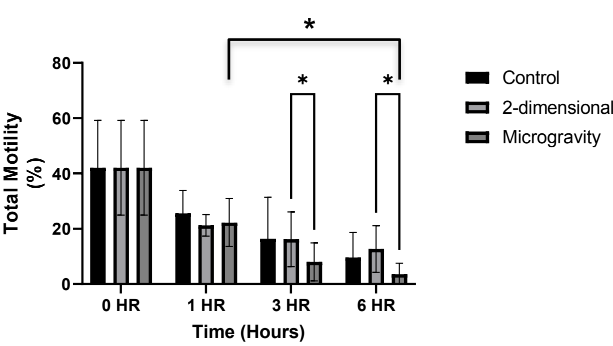

*Figure 1A: Effect of microgravity on sperm total motility. Total motility (TM) of each individual group plotted against time. There is an 18.66% difference in TM of the simulated microgravity from 1 hour to 6 hours, while there was a Significant 8.152% reduction in TM between the 2-Dimensional and simulated microgravity at 3 hours and a 9.076% reduction at 6 hours. *p<0.05, n=5.*

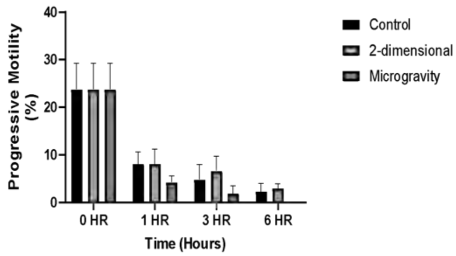

Figure 1B: Effect of microgravity on sperm progressive motility. Progressive motility of each individual group of control, 2-dimensional and simulated microgravity plotted against time. No significance. N=5

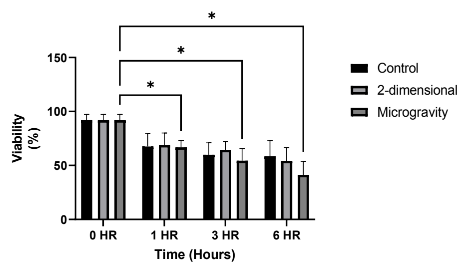

*Figure 2: Effect of microgravity on viability. Viability of each individual group of control, 2-dimensional and simulated microgravity (s-MG) plotted against time. The data shows significance of the s-MG from 0 hour to 3 hours and 6 hours. * p<0.05. N=5*

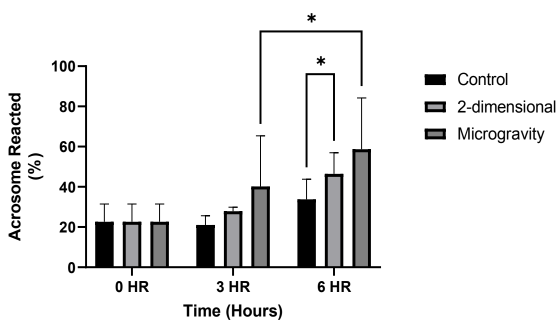

*Figure 3: Effect of microgravity on acrosome reaction. Acrosome reacted spermatozoa of each individual group of control, 2-Dimensional (2-D) and simulated microgravity (s-MG) plotted against time (n=2). The data shows significance of the s-MG from 0 hour to 3 hours and significance at 6 hours. Significance is seen in the time dependent effect of control to 2-D in the 6 hours time point. * p<0.05*

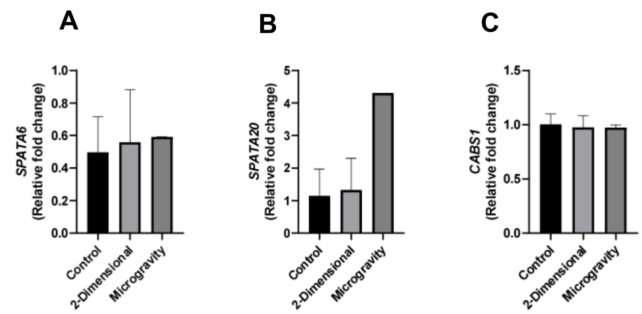

Figure 4: Effect of microgravity on the genes of interest. Following qPCR, there were no statistical significant difference in the mRNA expression SPATA6 and SPATA20. However, there was an observable trend increase in the expression of SPATA6 and SPATA20 in the s-MG group compared to control and 2-D (Figure 4A-B). No observable trends or significance were showcased in the mRNA expression of CABS1 (Figure 4C).

Conclusion: s-MG negatively affected TM, viability and AR, while PM and function-specific genes were not significantly impacted. Nevertheless, noteworthy effects were observed, suggesting the importance of further investigation. 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.

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

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INTRODUCTION & AIMS

Since its inception, researchers have shown that spaceflight induces adaptations that lead to a state of physiological deconditioning, such as loss of muscle and the cardiovascular system's inability to meet challenges as observed in both exercise intolerance and orthostatic intolerance (LeBlanc et al., 1995; Tank et al., 2011). These troubles are more pronounced upon their return to Earth's gravity. However, the recent incidental discovery of a blood clot in the left internal jugular vein of an astronaut aboard the International Space Station (ISS) revealed that there were more pressing health implications in-flight for these astronauts (Marshall-Goebel et al., 2019). The aim of this study was to examine the role of hypercoagulability and its potential influence in the development of these blood clots by analyzing standard coagulation tests data in females—an understudied group in space research—exposed to a dry immersion analogue.

METHODS

Participants

This investigation is a secondary-use study using previously collected data. These data are from a dry immersion (DI) project that took place at the MEDES Space Clinic in Toulouse, France. The participants were females aged between 20-40 years, had a body mass index (BMI) between 20-26 kg/m^2 and a height between 158-180 cm.

Dry immersion setup and blood collection

Participants lay on a highly elastic waterproof sheet in a specially designed dry immersion bath filled with thermoneutral tap water at 32-34.5°C. Dedicated medical crew (doctors, nurses, etc.) provided around-the-clock supervision. To perform showering and bathroom activities, participants left the tanks once per day while remaining at 6° head-down tilt on a stretcher. Of the 373 mL total blood volume collected from each participant during the study, coagulation analysis used 34 mL at BDC-1 (baseline, one day before DI), DI2 (DI day 2), DI5 (DI day 5), and R+1 (recovery).

Thrombin/antithrombin (TAT) complexes test

Test kit "EnzygnostR TAT micro" for determination of plasma levels of thrombin/antithrombin complexes was obtained from Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany. Before determination, the plasma samples were diluted 1:2 with 0.9% saline solution, then the TAT concentrations were determined according to the manufacturer's instructions.

ROTEM coagulation tests

The clot formation process was monitored using the TEM coagulation analyser (ROTEM®05) from Matel Medizintechnik (Graz, Austria). *Clotting time* (CT) is the time interval from adding trigger to initial fibrin formation; *maximum clot firmness* (MCF) reflects clot stability, and the *alpha angle* shows the velocity of fibrin built-up and cross-linking; *clot formation time* (CFT) reflects the time it takes from the beginning of clotting until the clot reaches 20 mm in firmness. The final sample volume was 340 µL. Clot formation was initiated by the addition of 40 µL of trigger solution containing, as described by Sorensen et al. (2003), tissue factor (InnovinR, recombinant human thromboplastin: Dade Behring Marburg GmbH, Marburg, Germany) and $CaCl_2$ (0.35 pM and 3 mM final concentration, respectively) to 300 µL of citrated plasma. The lyophilized product was dissolved in 4 mL of distilled water and subsequently diluted at a ratio of 1:250 in 0.9% saline solution (stock solution).

Statistical analysis

A repeated-measures ANOVA with Tukey-HSD post hoc test was used to determine statistical differences between the means for each of the measurement days for each of the five coagulation tests.

RESULTS

The ROTEM and TAT data showed mixed results following the statistical analysis of the five coagulation tests for 17 females reported as means with standard error. Clotting time increased significantly from BDC-1 (70.8±4.4s) to DI5 (83.2±4.4s) but declined by R+1 (75.7±4.4s). MCF showed a significant increase in clot stability at DI5 (20.9±0.7mm) when compared to BDC-1 (19.5±0.7mm), followed by a significant decrease in stability by R+1 (18.5±0.7mm). Due to incomplete data sets, only 8 participants had CFT data. The log transformed CFT data showed significantly decreased clot formation time on both days of exposure, DI2 (6.1±0.3s) and DI5 (6.1±0.3s), when compared to BDC-1 (6.7±0.3s) and R+1 (6.8±0.3s).

CONCLUSION

Exposure to simulated microgravity invokes changes in hemostatic balance reflected in coagulation tests for females after 5 days of exposure to DI. The participants showed increased time to clot while in dry immersion which, once started, formed more rapidly with increased stability.

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Sphingolipids as regulators of skeletal muscle phenotype at gravitational unloading

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Plasticity is a key feature of skeletal muscles that allows them to adapt to different types of activity/inactivity. Changes in the phenotype of postural muscles, such as the soleus muscle, are observed after prolonged spaceflight, hypokinesia, bed rest and other conditions leading to a decrease in specific functional load. In microgravity, a phenotype of a postural muscle like soleus, which normally expresses high percentage of slow myosin heavy chain isoform, changes towards increased expression of fast isoforms. The realization of this phenomenon depends on a number of intracellular signaling pathways. To date, the role of calcineurin, calsarcin, nuclear factor of activated T-cells (NFAT), glycogen synthase kinase 3 (GSK3) and other proteins in the remodeling of muscle phenotype has been proven (Schiaffino S. and Reggiani K., 2011, Sharlo K.A. et al., 2018).

Sphingolipids are the class of lipids tightly associated with many intracellular signaling processes. Ceramide, the backbone molecule of sphingolipids, can affect a number of functions in skeletal muscle (Bruni P. and Donati C., 2008). There is practically no data about the role of sphingolipids in regulation of muscle phenotype in normal and pathological conditions. Given that ceramide and acid sphingomyelinase (aSMase) are up-regulated in the unloaded muscle (Bryndina I.G. et al., 2018) we hypothesized that it can be involved in microgravity-dependent transition of soleus muscle phenotype from slow to fast myosin heavy chain (MYH) expression. Indeed, we have shown earlier that the change in the expression of slow and fast myosin isoforms can be partially prevented by the inhibitor of aSMase clomipramine (Sekunov A.V. et al., 2021).

The present work was aimed to study the role of catabolic sphingomyelinase pathway of ceramide formation in the expression of fast and slow MYH isoforms in the unloaded rat soleus muscle. The experiments were carried out on the adult male albino rats (n = 24, m = 180-230 g). Hindlimb suspension (HS) was used to simulate the effects of microgravity. The animals were divided into 4 groups: control, 7-day HS, 7-day HS with administration of the inhibitor of aSMase amitriptyline (per os, 250 mg/l dissolved in the drinking water, for 14 days - 7 days before and 7 days during the experiment), and treatment with AMI without HS. After the end of the experiments, under common anesthesia with zolazepam/tiletamine (Zoletil, Vibrac), soleus muscles were removed, weighed, frozen in liquid nitrogen and were kept at -80°C. Muscle phenotype and regulatory factors were studied using Western blotting and immunofluorescence microscopy of muscle cryo-sections, stained with antibodies to fast slow and fast MYHs.

The results of our experiments indicated that 7-day HS led to the atrophy of rat soleus muscle, which was characterized by the decreased wet muscle mass, cross-sectional area (CSA) and Feret diameter (FD) of muscle fibers (Fig. 1). As expected, immunofluorescence study of muscle cross-sections showed an increased aSMase and ceramide level. Amitriptyline treatment effectively attenuated both aSMase/ceramide and CSA/FD changes.

Immunofluorescence study also demonstrated the increased expression of fast MYH isoforms (Fig. 2). The changes in MYH expression were partially prevented in soleus muscles of suspended animals treated with the aSMase inhibitor amitriptyline. Staining of muscle sections with antibodies to the fast myosin isoform MYH IIdx demonstrated its up-regulation in the unloaded muscle and effectiveness of amitriptyline in preventing this effect. Western blotting confirmed an increase in the level of MYH IIdx protein after 7-day HS.

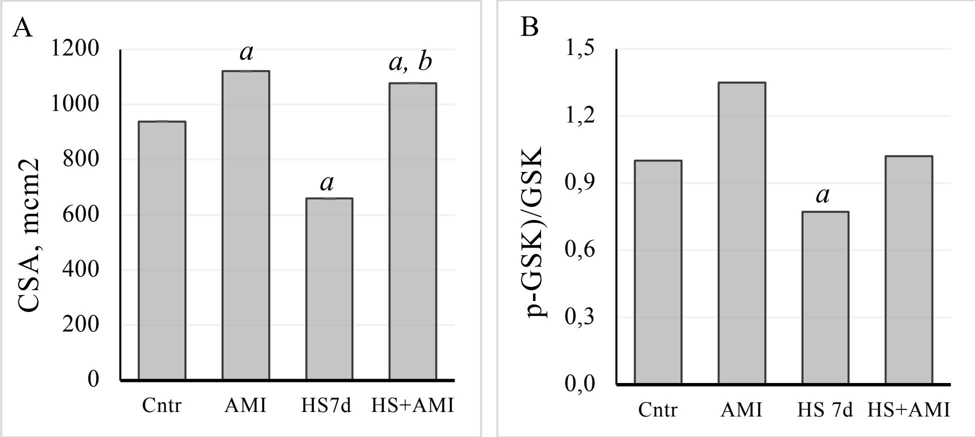

Fig. 1A-B. A. Cross sectional area (CSA) of soleus muscle fibers and p-GSK3β(Ser9)/GSK3β after 7-day HS and amitriptyline treatment. a –changes are significant in comparison with control values ($p<0.05$), b – with 7d HS

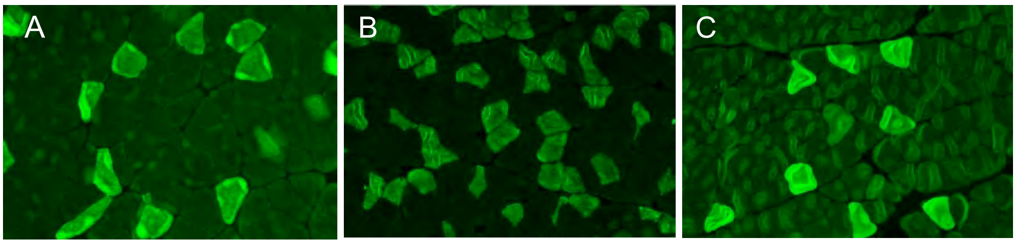

Fig. 2. Immunofluorescence study of soleus muscle cross sections after 7-day HS. Amitriptyline prevents an increase in fast myosin heavy chains isoforms caused by HS. Muscle sections were stained with primary antibodies to fast myosin and Alexa Fluor® 647. A – control, B – 7-day HS, C – 7-day HS with amitriptyline treatment

We found also that amitriptyline attenuates the decrease in negative phosphorylation of GSK3β caused by HS (Fig. 1B). It is known that GSK3 plays an important role in the regulation of phenotype transition in slow-twitch soleus muscle during microgravity. GSK3 is a serine/threonine kinase that enables to phosphorylate NFAT and therefore prevents its entry into the mionuclei; it results in a decrease of slow MYH expression. The increase in phosphorylation of GSK3β at Ser 9 under amitriptyline treatment was accompanied by the decrease in NFAT phosphorylation at Ser326 (by 14.7%, $p<0.05$). Therefore, up-regulation of fast MYH isoforms expression in soleus muscle caused by unloading was prevented.

The results of our experiments indicate that sphingolipid mechanisms play an important role in regulation of muscle plasticity under the functional unloading of postural muscles.

This work was supported by Russian Science Foundation (grant #23-25-00420)

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

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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 16-30-day orbital experiments (Gulimova et al., 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. 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. 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 (Jonasson et al., 2012). 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. 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.

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 using XPCT at the ID17 beamline of ESRF synchrotron

(Grenoble, France), as well as traditional histological methods. Detailed information about animals and experimental conditions, logistics, methods of sample preparation, XPCT experiment, data processing, statistical analysis and results of the histological study are described in (Bukreeva et al., 2023). For image segmentation, we used specialized software Ilastik, which implements the supervised learning method - “random forest” and is built on the process of interactive image marking.

Results. Using XPCT data, a quantitative assessment of the morphometric parameters of TG's spine and notochord 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) 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.

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 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-dimentional slices and volume processing modes.

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.

Financing. Our study was performed within the state assignment of Avtsyn Research Institute of Human Morphology of Federal state budgetary scientific institution "Petrovsky National Research Centre of Surgery"; in part of tomographic data processing - within the state assignment of the NRC "Kurchatov Institute".

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Exploring Cardio-postural Interactions in relation to Prolonged Space Missions

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INTRODUCTION

Spaceflight produces observable physiological changes in humans, leading to time dependent adaptation processes with subsequent effects upon re-entry to gravitational environments. Post-flight orthostatic intolerance remains a health and safety concern for astronauts both upon landing and during the days of recovery (Blaber A.P. et al., 2011). Baroreflex plays an essential role in blood pressure regulation under orthostatic challenge. Reduction of arterial baroreflex response has been observed after both short-term (Gisolf J. et al., 2005) and long-term (Hughson R.L. et al., 2012) spaceflights. Upon standing, skeletal muscle contractions in the legs also play an important role in maintaining blood pressure equilibrium by propelling pooled blood in the veins back to the heart (muscle-pump). Xu et al. (2017) validated the cardio-postural model (Fig. 1) where the muscle-pump is activated by blood pressure changes through baroreflex-mediated events (i.e., muscle-pump baroreflex). In their study, healthy participants who underwent prolonged (60-day bed rest) had severely impaired muscle-pump baroreflex. However, the effects of spaceflight on muscle-pump baroreflex have never been investigated.

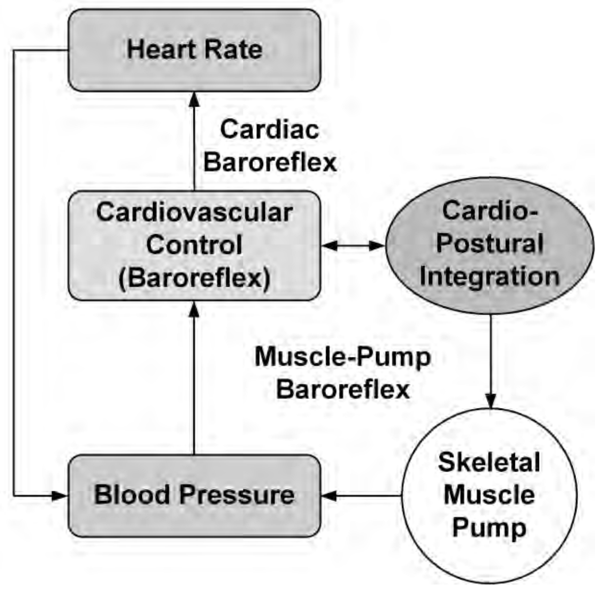

Figure 1: Cardio-postural model associated with blood pressure regulation under orthostatic challenge.

We aim to address this research topic by analyzing data from the Causal Analysis of Respiratory Dynamic Interactions in Orbit: Breathing, Rest, Exercise And The Heart (CARDIOBREATH) (Canadian Space Agency: 20EXPLSRS1) study through a special collaboration between Simon Fraser University (SFU) and Mohammed Bin Rashid University (MBRU) (MBRSC-FIN-2021-001). Data from four astronauts who spent 6 months on the ISS, including one astronaut from the United Arab Emirates (UAE), were used for analysis. In this collaborative study, the research team at MBRU analyzed the data of the UAE astronaut and the results were combined with the other three astronauts' data analyzed at SFU for presentation. Our hypothesis is that both cardiac baroreflex and muscle-pump baroreflex upon standing are impaired after a 6-month stay on the ISS.

METHODS

A supine to stand (StS) test (5-min supine followed by 6-min quiet stand) before (L-100 to L-60, BDC) and after (R+6 to R+8, POST) spaceflight was conducted for each astronaut to invoke orthostatic challenge. During each test, cardiovascular and postural data were collected including ECG and systolic blood pressure (SBP) using the Bio-M garment (Carré Technologies Inc., Montreal, QC, Canada), and surface EMG of lower leg muscles (tibialis anterior, lateral soleus, medial gastrocnemius, and lateral gastrocnemius) through the Trigno Wireless EMG System (Delsys Inc., Natick, MA, USA).

Data during quiet standing were used for analysis. RR intervals were extracted from the ECG for each data segment, based on which the beat-by-beat EMG impulse (EMG_{imp}) was calculated from the aggregate EMG data to represent the muscle contraction strength at each heartbeat. To characterize the regulatory coupling between two measurements, the active interaction time (fraction time active, FTA) between them was derived using the wavelet transform coherence analysis (Xu D. et al., 2020) for the following signal pairs: SBP→RR (cardiac baroreflex), SBP→EMG_{imp} (muscle-pump baroreflex), and EMG_{imp}→RR (mechanical muscle-pump + cardiac baroreflex). To further describe the relationship among the above regulatory mechanisms (Fig. 2), Venn diagrams were constructed to represent overlapped regions where two or more mechanisms were active at the same time (Garg A. et al., 2014). A repeated measures one-way ANOVA (Test Day) was used on FTA values obtained pre- and post-flight. Significance was assessed post hoc with Tukey's HSD with significance set at ($p<0.05$).

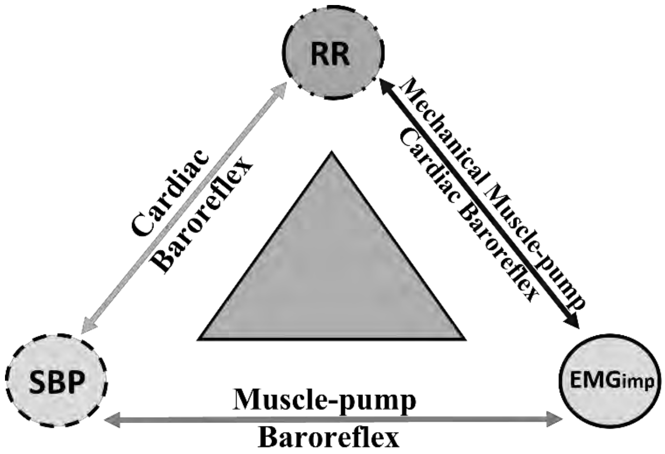

Figure 2: Regulatory mechanisms between cardio-postural systems. Arrows: mechanisms between two measurements; Circles: two mechanisms were active at the same time; Triangle: all three mechanisms were active at the same time.

The data analysis approaches were developed by the SFU team and research personnel in the MBRU team were trained to perform the same data analysis to ensure consistency in results.

RESULTS AND DISCUSSION

The statistical analysis found no significant changes in cardio-postural interactions post-flight based on data from four astronauts. Due to the small sample size (n=4), these results are likely inconclusive. With ongoing participant recruitment and data collection for CARDIOBREATH study, we expect that a final sample size of 14 and the higher statistical power is to reveal significant post-flight alterations in cardio-postural interactions, especially the muscle-pump baroreflex.

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

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During future long-duration missions, astronauts will be confronted with a variety of environmental and stressors that can impair their performance, including cognitive abilities and lead to hemodynamic changes (central and regional). However, they must maintain a high level of concentration to carry out missions despite environmental exposures. That's why the MooNIRS study evaluated the consequences of mental load in a context of microgravity.

The objective of this poster was to measure the effects of environmental conditions on healthy subjects, the plateau effect (simulated microgravity by HDT at different degrees) and the cognitive spot effect, at the level of central and peripheral cardiovascular parameters. The protocol was to make progressive angle steps from 0°, -10° to -20° for 20 minutes, with Toulouse N-back Task calculation tests for each step. The following central and peripheral cardiovascular measurements were taken throughout the study: for central hemodynamics, heart rate, systolic and diastolic blood pressure, stroke volume, pulse oxygen saturation, and total peripheral resistance; and for regional flows, cutaneous flow, muscle hemoglobin concentration, and calf volume change using strain gauge.

Our results showed an effect of tasks and plateaus on vascular systems:

- Plateau Effects: an expected fluid transfer was observed, evidenced by decrease in calf volume, a slight decrease in heart rate, a decrease in toe pulse wave amplitude, and a decrease in muscle blood volume in the calves as measured by NIRS, in parallel an increase in muscle blood volume in the deltoid.
- Cognitive Task Effects: independently of plateaus, an increase in heart rate, a decrease in stroke volume, as well as an increase in diastolic blood pressure were noted.

These data will help explore the effects of the space mission environment to improve the performance and well-being of the crew during long-duration missions.

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

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BACKGROUND

The profession of commercial airline pilots demands repeated manual handling tasks in the cockpit, significantly increasing the risk of musculoskeletal disorders (MSDs) due to high workload. Pilots frequently perform movements such as operating the overhead flight-deck panel, which involves repeated flexion, abduction, and rotation of the upper extremity, leading to torque on the rotator cuff muscles (Hohmann and Pieterse, 2022). Additionally, ultra long-haul flights (ULH), lasting up to 16 hours, contribute to musculoskeletal issues due to prolonged sedentary positions and exposure to various stressors (Rathi, 2022).

OBJECTIVES

This study aims to:

1. Identify common musculoskeletal disorders among commercial airline pilots in the UAE operating ULH flights.
2. Analyse the occurrence of symptoms during different stages of ULH flights.
3. Investigate the impact of repetitive movements in the cockpit on the development of MSDs.

METHODS

An observational cross-sectional study was conducted using The McGill Pain Questionnaire and the Modified Nordic Musculoskeletal Questionnaire (MNMQ). Data were collected electronically from 242 commercial airline pilots in Dubai, UAE. Inclusion criteria included age (30-60 years), years of experience, and exposure to ULH flights. Statistical analyses, including chi-square tests and logistic regression, were employed to determine prevalence and risk factors associated with MSDs.

RESULTS

Preliminary findings indicate a high prevalence of shoulder and neck pain among pilots, exacerbated during ULH flights. Repetitive movements, such as reaching for overhead controls and adjusting seats, were significant predictors of MSDs. The study also found that fatigue, vibration, and prolonged sedentary positions during ULH flights contributed to the severity of symptoms (Lawson et al., 2014; Lee and Kim, 2018).

CONCLUSION

This study underscores the importance of understanding repetitive movement patterns and their impact on MSDs among commercial airline pilots. The findings suggest the need for targeted interventions, including ergonomic adjustments and specific physiotherapy protocols, to mitigate the risk of MSDs and enhance pilot

safety and well-being. Additionally, the insights gained from this study could be beneficial for astronauts, who are similarly exposed to prolonged sedentary positions and repetitive tasks in microgravity environments, helping to develop conditioning programs to prevent musculoskeletal issues in space missions (Minoretti and Emanuele, 2022).

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

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ABSTRACT:

Type 2 diabetic patients experience dysregulated hemodynamic and autonomic systems that could potentially lead to orthostatic intolerance and falls. The objective of the study was to investigate the variations in hemodynamic and autonomic responses in male and female patients while performing a 5-min sit-to-stand test. Twenty-six type 2 diabetic patients (thirteen males and thirteen females) from a Dubai tertiary hospital participated in the study. Electrocardiogram, systolic blood pressure (SBP), cardiac output (CO) and stroke volume (SV) were acquired using the Task Force Monitor (CNSystems, Graz, Austria). Parameters such as SBP, CO, R-R interval, low frequency (LF), high frequency (HF), and LF/HF ratio demonstrated statistical differences when comparing the standing versus baseline values ($p<0.05$). As compared to males, only the females showed a drop in LF values in middle of the standing phase thus suggesting a decreased sympathetic activity during that period. In addition, males demonstrated higher LF values compared to females during the recovery phase. Our results suggest significant variations in both hemodynamic and autonomic responses in type 2 diabetic patients during sit to stand tests as well as during recovery. Finally, differences in the autonomic responses were seen across the sexes during standing.

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

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BACKGROUND

Upon entering microgravity humans lose water due to enhanced excretion and reduced water intake. Water-salt metabolism has marked sex differences, particularly, the vasopressinergic system is a classic example of sex differences in the brain, with more vasopressinergic neurons, higher vasopressin production and more vasopressin receptors in diverse regions of the male brain. The aim of this study was to investigate sex differences in vasopressin regulation of water intake, vasopressin expression and blood copeptin levels in hindlimb unloaded mice, and the role of sex steroids in these differences.

METHODS

Male and female C57BL/6 mice were subject to hindlimb unloading for 14 days followed by 10-day recovery. In order to evaluate the impact of sex steroids, mice were gonadectomized and subject to chronic hormone replacement (200 µg/kg testosterone propionate in males and 20 µg/kg estradiol benzoate in females, every other day) or vehicle (50 µl/mouse propylene glycol). Thus, a total of 4 experimental groups were created (N=7-10 per group). The effectiveness of hindlimb unloading was validated by the m. soleus weight, and the effectiveness of hormonal manipulations via sex steroid dependent organ weights (accessory glands in males, uterus in females). Water consumption was monitored daily. Hypothalamic vasopressin expression, plasma copeptin and aldosterone levels were measured before unloading, on unloading days 1, 3, 7 and 14, and on days 3 and 10 of recovery.

RESULTS

Water intake was reduced on the first days of unloading in all the mice disregarding sex or hormonal status. After adaptation to unloading euhormonal female mice consumed more water compared to gonadectomized females or males (euhormonal or gonadectomized, (Fig 1A). Copeptin levels dropped markedly in euhormonal males, but not in subhormonal males or females (Fig. 1B). The decrease in blood copeptin was governed by decreased preprovasopressin expression on the hypothalami of the euhormonal males, but not the subhormonal males or females (fig. 1C). Of note, no apparent recovery was found for copeptin levels or preprovasopressin expression upon reloading. Aldosterone rose during the initial days of unloading, fell below baseline during further unloading period and returned to baseline after reloading (fig. 1D).

CONCLUSION

Hindlimb unloading leads to a decrease in vasopressin expression and secretion that are most marked in euhormonal males but do not explain the changes of water intake during simulated microgravity.

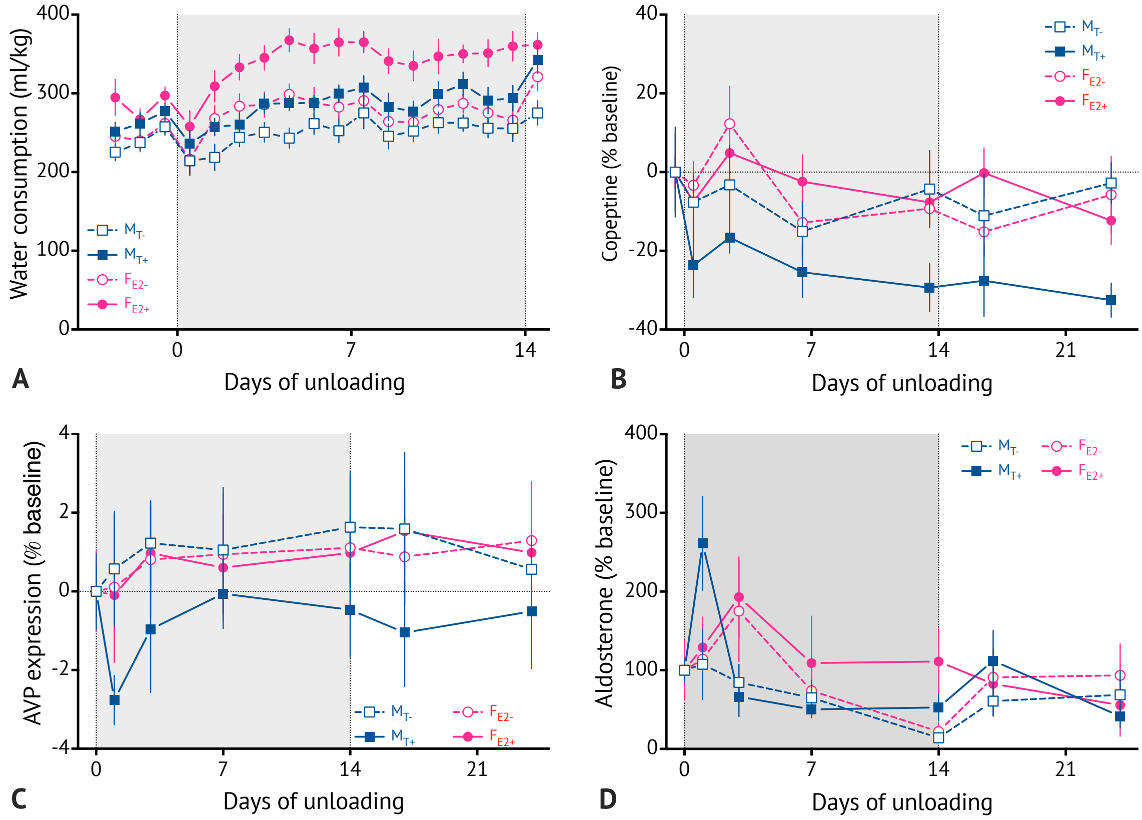

Figure 1 – Water consumption (A), plasma copeptine (B), AVP expression (C) and aldosterone levels (D) in male or female mice, eu– (T+, E2+) or subhormonal (T-, E2-) over 14 days of hindlimb unloading and 10-day recovery.

Adaptation to 3g Hypergravity: A Multidisciplinary Tissue Sharing Program from a 14- and 27-Day Mouse Experiments

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Life on Earth evolved under a consistent gravitational force of 9.81m/s². Changes to this force can significantly affect biological functions, particularly with increasing interest in space exploration. The Microgravity Development System (MDS), originally designed by the Italian Space Agency (ASI) for mice in space, was repurposed to study organism responses to hypergravity. This multidisciplinary effort involved eight Italian labs and four international partners, coordinated by the University of Genova, Italy. The experiments compared mouse adaptability in 3g conditions to vivarium and MDS controls, maximizing scientific rigor and yield.

MATERIALS AND METHODS

The experiment involved 35 male (C57BL/6J) mice, aged 19 weeks, exposed to 3g hypergravity for 27 days, from February 9th to March 8th, 2023. Key physiological responses were evaluated in three test groups: vivarium control, MDS cages control (MDS-Ctrl), and mice exposed to hypergravity (MDS-3g).

Mouse Adaptation to 3g: Protocol Summary

Following enhancements of MDS, mice were training for 14 days before the exposure to 3g. Habituation involved acclimating to MDS-specific feeding and watering systems, with consistent diet across all groups. Afterwards, mice in MDS were moved to 3g for durations of 14 and 27 days in large diameter centrifuge (LDC) (Fig.1. A, B&C). In the14-day of experiment only 6 mice per condition were included, as it was a test to check MDS functionality, and we took the opportunity to consider them in some analyses.

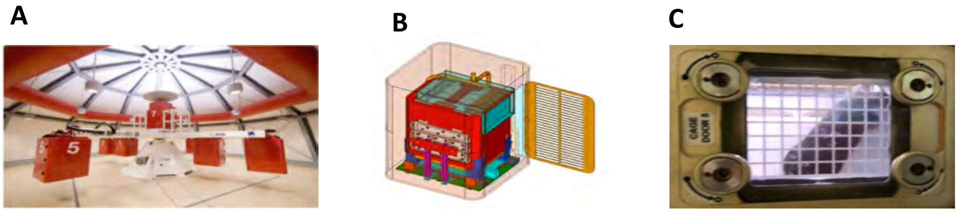

Figure 1. A) Large Diameter Centrifuge (LDC) at ESTEC, Noordwijk. MDS were inserted in the external gondolas. B) accommodation of MDS inside the gondola with an external grid in order to provide sufficient air from the ambient. C) Mouse inside the MDS cage drinking at the lixit. At the bottom a food bar is visible

Mice adaptive responses in MDS to 3g hypergravity

The 27-day of 3g exposure demonstrated an initial decrease in water intake and a significant body weight reduction in the MDS-3g group, followed by an adaptive recovery phase (Fig.2. A&B). During the first week in hypergravity, experimental animals adapted their eating and drinking behaviors without difficulty and exhibited mainly conservative, species-specific behaviors with slight adjustments to the new environment. By the second week, although they continued to feed and drink normally, individual variations in consumption emerged, alongside limited locomotor activities and initial observations of vertical behaviors. In the third and fourth weeks, no significant changes in consumption or behavior were noted, suggesting adjustment to hypergravity.

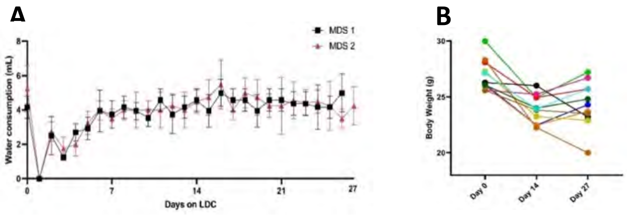

Figure 2. A) Daily water consumption(mL), and B) Body weight changes of mice within MDS-1 and MDS-2 payloads over 27 days of experiment, n=11, ± SD.

Hematological parameters

Compared to MDS-Ctrl, the blood analysis revealed a significant reduction ($p < 0.01$) in the white blood cell (WBC) count of MDS-3g (Fig.3.D). Interestingly, there was a notable decrease ($p < 0.01$) in WBC when comparing MDS-Ctrl to the vivarium group. Additionally, we analyzed the neutrophil-lymphocyte ratio (NLR) as a clinical biomarker of inflammation. An increased variance in this ratio reflects the response to chronic (long term) stress. The variance ratio test showed a significant increase ($p < 0.05$) when comparing the MDS-Ctrl group to the vivarium group (Fig.3.C). However, there was no significant change observed in MDS-3g comparing to MDS-Ctrl.

Moreover, hematocrit (HCT) and red blood cell (RBC) levels increased significantly ($p < 0.05$) in the MDS-Ctrl group compared with the vivarium group, while no significant changes were observed in MDS-3g mice (Fig.3.E&F). The elevated HCT and RBC levels in MDS-Ctrl group could be indicative of adaptive reactions, probably driven on by some dehydration status of the animal.

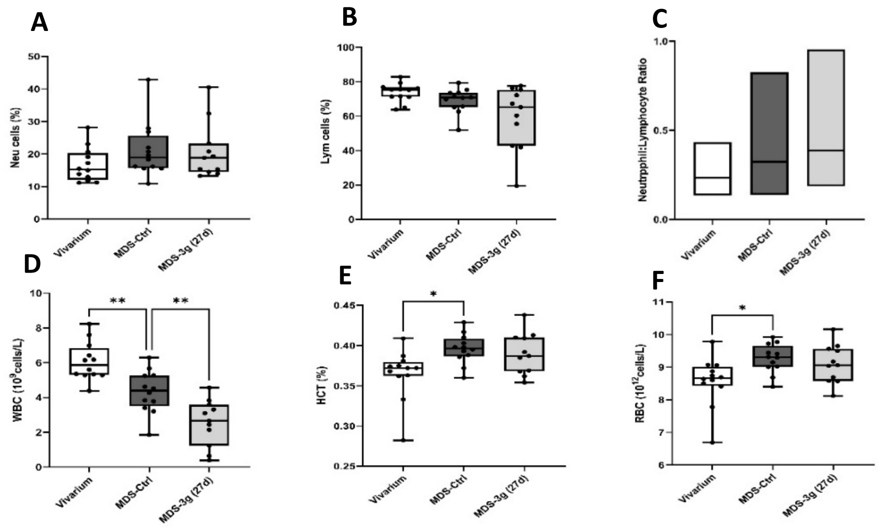

Figure 3. A) Neutrophil, B) Lymphocyte, C) Neutrophil to lymphocyte ratio (NLR), D) White Blood cell counts (WBC), E) Hematocrit, F) Red blood cell counts, and according to different conditions. Vivarium and MDS-Ctrl mice were n= 12, and 3g mice were n= 11. Data are expressed as the mean ± SD. A significant difference between the two groups was indicated: $*p<0.05$, and $**p<0.01$.

CONCLUSIONS

In conclusion, our findings indicate that mice, when subjected to the MDS enclosure and hypergravity, preserve their fundamental abilities to feed and hydrate, showcasing behavioral resilience as they adapt to environmental changes. The adaptation period, however, is characterized by a general stress condition in the animals, evidenced by initial weight loss and decreased water intake. This stress is also mirrored in certain hematological parameters. It will be intriguing to ascertain whether the stress experienced during the adjustment period can be converted into a reactive and positive stimulus for the various bodily systems, potentially contributing to a comprehensive understanding of organismal resilience under altered gravity conditions.

Lower Body Negative Pressure: Application and Methodology

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This plenary talk presents lower body negative pressure (LBNP) as a unique tool to investigate the physiology of integrated systemic compensatory responses to altered hemodynamic patterns during conditions of central hypovolemia in humans. LBNP has been used extensively to investigate complex physiological responses to a variety of challenges including orthostasis, hemorrhage, and other important stressors seen in humans such as microgravity encountered during spaceflight.

The LBNP stimulus has provided novel insights into the physiology underlying areas such as intolerance to reduced central blood volume, sex differences concerning blood pressure regulation, autonomic dysfunctions, adaptations to exercise training, and effects of space flight. Given the diverse applications of LBNP, it is to be expected that new and innovative applications of LBNP will be developed to explore the complex physiological mechanisms that underline health and disease.

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

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Background: Estrogen and progesterone levels undergo changes throughout the menstrual cycle. Existing literature regarding the effect of menstrual phases on cardiovascular and autonomic regulation during central hypovolemia is contradictory.

Aims and study: This study aims to explore the influence of menstrual phases on cardiovascular and autonomic responses in both resting and during the central hypovolemia induced by lower body negative pressure (LBNP).

Methods: The study protocol consisted of three phases: 1) 30 minutes of supine rest; 2) 16 minutes of four LBNP levels; and 3) 5 minutes of supine recovery. Hemodynamic and autonomic responses (assessed via heart rate variability, HRV) were measured before-, during-, and after-LBNP application using Task Force Monitor® (CNSystems, Graz, Austria). Blood was also collected to measure estrogen and progesterone levels.

Results: We have exclusively assessed 14 females: 8 in the follicular phase of the menstrual cycle (mean age 23.38 ± 3.58 years, height 166.00 ± 5.78 cm, weight 57.63 ± 5.39 kg and BMI of 20.92 ± 1.96 25 kg/m^2) and 6 in the luteal phase (mean age 22.17 ± 1.33 years, height 169.83 ± 5.53 cm, weight 62.00 ± 7.54 kg and BMI of 21.45±2.63 kg/m^2). Baseline estrogen levels were significantly different from the follicular phase as compared to the luteal phase: (33.59 pg/mL, 108.02 pg/mL, respectively, $p<0.01$). Resting hemodynamic variables showed no difference across the menstrual phases. However, females in the follicular phase showed significantly lower resting values of low-frequency (LF) band power (41.38 ± 11.75 n.u. and 58.47 ± 14.37 n.u., $p=0.01$), but higher resting values of high frequency (HF) band power (58.62 ± 11.75 n.u. and 41.53 ± 14.37 n.u., $p=0.01$), as compared to females in the luteal phase. During hypovolemia, the LF and HF band powers changed only in the follicular phase $F(1, 7) = 77.34$, $p<0.0001$ and $F(1,7) = 520.06$, $p<0.0001$, respectively.

Conclusions: The menstrual phase had an influence on resting autonomic variables, with higher sympathetic activity being observed during the luteal phase. Central hypovolemia leads to increased cardiovascular and autonomic responses, particularly during the luteal phase of the menstrual cycle, likely due to higher estrogen levels and increased sympathetic activity.

Disclosure statement: Parts of this abstract have been published as a full paper in *Frontiers in Cardiovascular Medicine* (Shankhwar 2024, article in press).

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Does Gender Influence Cardiovascular and Autonomic Responses to Central Hypovolemia?

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Introduction: Lower body negative pressure (LBNP) eliminates the impact of weight-bearing muscles on venous return, as well as the vestibular component of cardiovascular and autonomic responses. We evaluated the hemodynamic and autonomic responses to central hypovolemia, induced by LBNP in both males and females.

Methodology: A total of 44 participants recruited in the study. However, 9 participants did not completed the study protocol. Data from the remaining 35 participants were analysed, 18 males (25.28 ± 3.61 years, 181.50 ± 7.43 cm height, 74.22 ± 9.16 kg weight) and 17 females (22.41 ± 2.73 years, 167.41 ± 6.29 cm height, 59.06 ± 6.91 kg weight). During the experimental protocol, participants underwent three phases, which included 30 minutes of supine rest, four 4-minute intervals of stepwise increases in LBNP from -10 mmHg to -40 mmHg, and 5 minutes of supine recovery. Throughout the protocol, hemodynamic variables such as blood pressure, heart rate, stroke index, cardiac index, and total peripheral resistance index were continuously monitored. Autonomic variables were calculated from heart rate variability measures, using low and high-frequency spectra, as indicators of sympathetic and parasympathetic activity, respectively.

Results: At rest, males exhibited higher systolic (118.56 ± 9.59 and 110.03 ± 10.88, $p<0.05$) and mean arterial (89.70 ± 6.86 and 82.65 ± 9.78, $p<0.05$) blood pressure as compared to females. Different levels of LBNP altered hemodynamic variables in both males and females: heart rate ($F(1,16)=677.46$, $p<0.001$), ($F(1,16)=550.87$, $p<0.001$); systolic blood pressures ($F(1,14)=3186.77$, $p<0.001$), ($F(1,17)=1345.61$, $p<0.001$); diastolic blood pressure ($F(1,16)=1669.458$, $p<0.001$), ($F(1,16)=1127.656$, $p<0.001$); mean arterial pressures ($F(1,16)=2330.44$, $p<0.001$), ($F(1,16)=1815.68$, $p<0.001$), respectively. The increment in heart rates during LBNP was significantly different between both males and females ($p=0.025$). The low and high-frequency powers were significantly different for males and females ($p=0.002$ and $p=0.001$, respectively), with the females having a higher increase in low-frequency spectral power.

Conclusions and future directions: Cardiovascular activity and autonomic function at rest are influenced by gender. During LBNP application, hemodynamic and autonomic responses differed between genders. These gender-based differences in responses during central hypovolemia could potentially be attributed to the lower sympathetic activity in females. With an increasing number of female crew members in space missions, it is important to understand the role sex-steroid hormones play in the regulation of cardiovascular and autonomic activity, at rest and during LBNP.

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