ISGP & ESA LIFE SCIENCES MEETING 2018

Proceedings of the 39th Meeting

ESA-ESTEC - Noordwijk, The Netherlands - June 2018

INTERNATIONAL SOCIETY FOR GRAVITATIONAL PHYSIOLOGY
ISGP
1991

frontiers Event Abstracts

39TH ISGP & ESA LIFE SCIENCES MEETING

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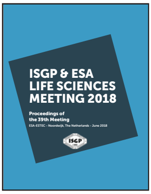

June 2018, ESA-ESTEC- Noordwijk, The Netherlands

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Welcome to the 39th ISGP Meeting & ESA Life Sciences Meeting

ISGP, the International Society of Gravitational Physiology holds his annual meeting that allows the presentation of original experimental research and review of current topics. The broad scientific spectrum of ISGP emphasizes gravity, life and physiology as its anchors.

The 39th ISGP annual meeting was organized for the very first time in the Netherlands in 2018.

Since the Netherlands hosts the largest center of the European Space Agency (ESA), the ESA technology center ESTEC, it was obvious to combine efforts and host the meeting at ESTEC in Noordwijk. This also provided the opportunity to combine the ISGP meeting the with ESA effect to explore and apply space related activities in the field of Health.

THE LOCAL ORGANIZING TEAM

Jack Van Loon, Veronica La Regina, Jason Hatton, Leonardo Surdo & Julia Eckert

LIST OF ORGANIZERS

Jack Van Loon, VU University, Amsterdam, Netherlands.
Martina Heer, Universität Bonn, Bonn, Germany.
Charles Fuller, University of California, Davis, United States.
Marc-Antoine Custaud, University of Angers, Angers, France.

ISGP 2019 Announcement

We are hosting the 40th Annual Meeting of ISGP and "Space Life Science and Medicine at Nagoya University Noyori Conference Hall on May 26 (Sunday) to 31 (Friday) in 2019. This conference will be integrated with the Japan Association of Physiology, Japan Association of Aerospace and Environmental Medicine, and Japan Aerospace Biological Society. The International Space Station now provides a platform for long-term microgravity exposure, allowing study of responses to long-term exposure to the spaceflight environment. Among other topics, this conference will examine the effects of long-term residence on the International Space Station, including orthostatic hypotension, osteoporosis, muscle atrophy, the influence of cosmic radiation on living systems, and the influence of the closed environment on the psychological situation of humans. Countermeasures to these responses and their usefulness in ground medicine will also be discussed. This proposal medical interventions and biological responses to establishment of a Lunar base and exploration class missions to Mars will also be topics.

The ISGP invites researchers from all over the world to participate in this meeting.

Satoshi Iwase, MD & PhD

Professor of Physiology, Aichi Medical University School of Medicine

CONFERENCE MAIN TOPICS

Autonomic Nervous System in Microgravity
Effects of Gravity on Human and Animal Body
Immunology and Nutrition
Crew performance and behavior
Is maintaining bone mass and strength really a done deal?
Space Radiation
Lunar Base and Martian Expedition
Countermeasures
Isolation and Confinement
Telemedicine
Artificial Gravity
Plant Physiology

EVENT TIMETABLE

March 1: Abstract deadline
April 2: Notification to the authors
April 15: Final Program
May 26-31: Conference

For more information, go to our society website www.isgp-space.org

History of the Annual Meeting of the International Society for Gravitational Physiology

The International Society for Gravitational Physiology (ISGP) was established in 1991. It evolved from the prior International Union of Physiological Sciences (IUPS) Commission on Gravitational Physiology, created in 1973. The annual meeting in Gravitational Physiology was established by the Commission with a meeting, held jointly with the Fall Meeting of the American Physiological Society (APS), in 1979 in New Orleans, Louisiana USA. This was an exciting and well-attended meeting, which presaged our annual meetings thereafter (Table 1). Further, with funding from NASA, the Proceedings of the meeting were published for the Commission by the APS in a special supplement of their journal, *The Physiologist*. From 1979 through 1993, the annual Gravitational Meeting was sponsored by the IUPS, frequently jointly with other societies. The meeting structure evolved to a four-day scientific meeting with four half-day morning invited symposia, on topics previously identified by the Commission, and four half-day sessions on voluntary papers. In addition, there was a separate day reserved for non-scientific interactions among the meeting registrants. This allowed for further interactions of the participants and a great deal of scientific and cultural exchange occurred regularly during this time. The meeting location was also purposely rotated in order to meet with both scientists and students who may have had difficulty traveling internationally. The typical rotation included Western Europe, Eastern Europe, North America and Asia.

In 1991, the Commission was instrumental in establishing an independent international society, the ISGP. Historically our membership has included approximately 1,000 members, representing over twenty countries. The ISGP is the annual sponsor of the Gravitational Meeting, in concert with local host institutions and funding agencies. This meeting still utilizes the same five-day format and rotates location regularly. The ISGP Council of Trustees now manages the agenda and format of the meeting. In 1994, the ISGP also established the *Journal of Gravitational Physiology*, which, in addition to publishing peer-reviewed articles in the field, also publishes the annual meeting Proceedings. Starting in 2018 we are now moving from our inhouse journal to an open-access journal, *Frontiers in Physiology* in the Section of *Environmental*, Aviation and Space *Physiology*. This Proceedings represents our first publication in that effort.

Table 1: Annual gravitational physiology meetings

1.	1979	New Orleans, LA, USA
2.	1980	Budapest, Hungary
3.	1981	Innsbruck, Austria
4.	1982	San Diego, CA, USA
5.	1983	Moscow, USSR
6.	1984	Lausanne, Switzerland
7.	1985	Niagara Falls, USA
8.	1986	Tokyo, Japan
9.	1987	Nitra, Czechoslovakia
10.	1988	Montreal, Canada
11.	1989	Lyon, France
12.	1990	Leningrad, USSR
13.	1991	San Antonio, TX USA
14.	1992	Berlin, Germany
15.	1993	Barcelona, Spain
16.	1995	Reno, NV, USA
17.	1996	Warsaw, Poland
18.	1997	Copenhagen, Denmark
19.	1998	Rome, Italy
20.	1999	Orlando, FL, USA
21.	2000	Nagoya, Japan
22.	2001	Budapest, Hungary
23.	2002	Stockholm, Sweden (w/ ESA)

24.	2003	Santa Monica, CA, USA
25.	2004	Moscow, Russia
26.	2005	Cologne, Germany (w/ ESA)
27.	2006	Osaka, Japan
28.	2007	San Antonio, TX, USA
29.	2008	Angers, France (w/ ESA)
30.	2009	Xi'an, China
31.	2010	Trieste, Italy (w/ ESA)
32.	2011	San Jose, California USA (w/ ASGSB in Fall)
33.	2012	Aberdeen, United Kingdom (w/ ESA)
34.	2013	Toyohashi, Japan
35.	2014	Waterloo, Canada (w/ CSA/ESA)
36.	2015	Ljubljana, Slovenia
37.	2016	Toulouse, France (w/ ESA)
38.	2017	Zvenigorod, Russia (w/ IMBP)
39.	2018	Noordwijk, Netherlands (w/ ESA)
40.	2019	Nagoya, Japan

ISGP 2018 meeting report

The 39th annual meeting do the International Society for Gravitational Physiology (ISGP) took place at the technology center, ESTEC, of the European Space Agency (ESA) in Noordwijk the Netherlands from 18 till 22 June 2018. The meeting was co-organized by ISGP and ESA.

Some 180 participants from Europe, Unites States, Russia, Ukraine, Canada, Japan, Israel, Quatar and even Australia presented their latest results and current concepts in gravity and space related human, animal, and plant related cell and physiology research and countermeasures applications.

Current Concept symposia and other morning symposia on 'Gravity and Spaceflight Effects on Fluid Shifts and Neuro-Ocular Impairment', 'Skeletal Muscle Remodeling during Gravitational Unloading and other Disuse Models' and 'Effects on Radiation' was addressed by 20 invited speakers.

The afternoons were contributed to numerous parallel sessions on e.g. Neuroscience and Psychology, Bone & Muscle, Cell and Animal Biology, Space Medicine and International Projects, Cardiovascular Research, or Plant / Technology / Commercial projects presented in 110 papers. Mondays afternoon was also dedicated to a specific session addressing Trends in Commercializing Space Research. The two poster sessions of 49 registered posters covered similar topics.

The ISGP meeting is characterized by its strong science content of gathering. However, the social events of the program are an integrate part of the meeting and serves both a nice gathering but also fosters discussion and possible future, international, collaborations between participants. This years' social program consisted mainly of the conference dinner at a nice beach club in Noordwijk, a canal boat trip in Amsterdam and the closure day with a visit the historical center of the city of Haarlem. One of the oldest towns in Holland.

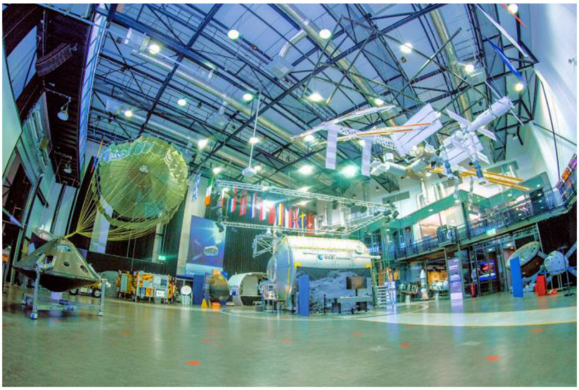
For this meeting we would like to thank the ESA-Human Spaceflight and Robotic Exploration Program, especially Mr. Bernhard Hufenbach and colleagues, for their support regarding the venue and daily audio and video support.

Thanks the VU-University Amsterdam Dental Faculty (ACTA) and VU-Medical Center (VUmc), especially the Dept. Craniofacial Surgery for their support. We want to thank the people of the ESA conference office, in particular Mrs. Irene Saalmink for their assistance in preparing the meeting. Also we want to thank Mr. Astrid Fredriksz for her support during the social events on Thursday evening and Friday.

Finally we need to thank the ESA-ESTEC TEC-MMG section for officially organizing the meeting at ESTEC.

This meeting was also our first opportunity to have our proceeding published with Frontiers journal (Frontiers in physiology, section Environmental, Aviation and Space physiology) who also supported our meeting.

Jack J.W.A. van Loon, local organizers and ISGP president 2017-2018.



Picture of the ESA Erasmus building High Bay in Noordwijk The Netherlands, venue of the 39th ISGP meeting 2018.

Richard Hughson
2018 Nello Pace Award

The Nello Pace Award was established in 1997 by the Galileo Foundation and the International Society for Gravitational Physiology to recognize individuals for performance of outstanding research in, and services to, the field of gravitational physiology. In seeking nominations for this award, many outstanding scientists who have demonstrated these characteristics were identified. The recipient of the 2018 Nello Pace Award, Professor Richard Hughson, is the Schlegel Research Chair in Vascular Aging and Brain Health at the University of Waterloo, and a Fellow of the Canadian Academy of Health Sciences.

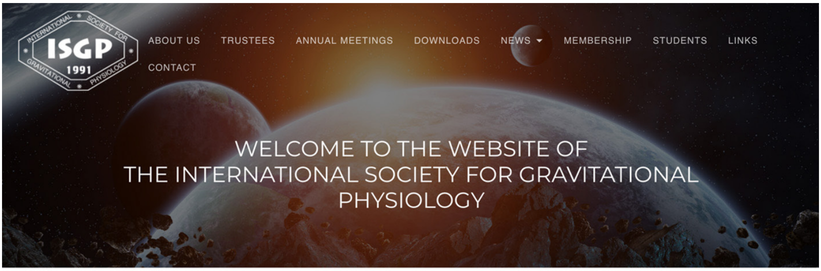
Dr. Hughson received his undergraduate degree from the University of Western Ontario in physiology in 1972, his MSc in physiology from University of British Columbia in 1973, and a PhD in medical science from McMaster University in 1977. He has been a faculty member at the University of Waterloo in Applied Health Sciences since 1977 where he has trained over 80 graduate students and post-doctoral fellows. Dr. Hughson began his career as an exercise cardiovascular physiologist studying the cardiorespiratory responses during exercise transitions. His research was among the first to introduce ultrasound technology to measure exercising muscle blood flow; ultrasound was to become an important research tool in his future work on bed rest and spaceflight. When asked how he became interested in studying astronauts, Dr. Hughson has responded by saying that the great unloading of the body by life in weightlessness is the antithesis of the healthy lifestyle we advocate on Earth with regular physical activity throughout the day. Spaceflight provided an opportunity to study a very healthy person during cardiovascular deconditioning.

The first spaceflight-related experiments conducted by Dr. Hughson and his students involved a simple 4-hour head down bed rest, and studies with lower body negative pressure. In the early days of the research program, graduate students received an honorary diploma in carpentry for construction of devices required for their studies. These simple devices yielded interesting results that started a career in space physiology spanning more than 30 years. At the end of the 4-hours head-down bed rest, 5 of 8 healthy young men were unable to complete a 10-minute tilt test revealing very rapid vascular and neural deconditioning.

Dr. Hughson attended his first ISGP meeting in Lyon, France in 1990, then returned to France later that year to spend his sabbatical period with Professor Claude Gharib at Université Claude Bernard. During his sabbatical year, he conducted at 10-hour study in Lyon with a focus on the initial hormonal and autonomic responses to head-down bed rest, and participated in the 30-day head-down bed rest organized by Centre National d'Etudes Spatiales in Toulouse. In total, Dr. Hughson and Professor Gharib have co-authored 19 publications. The sabbatical in Lyon also introduced Dr. Hughson to Dr. Philippe Arbeille leading to collaborations on multiple bed rest and spaceflight studies including the 60-day bed rest by women in the 2005 WISE project and the 5-day study with artificial gravity in 2010, both conducted at MEDES in Toulouse.

In 2014, Dr. Hughson organized the ISGP meeting in Waterloo, Canada. This meeting included a special symposium on Aging in Space for Life on Earth. He has continued to participate in the ISGP, both scientifically and administratively as an active member of the ISGP Council of Trustees. From his own research as the principal investigator on five spaceflight studies: CCISS, Vascular, BP Reg, Vascular Echo and Vascular Aging, Dr. Hughson noted that astronauts exhibit aging-like changes such as increased carotid artery stiffness and elevated insulin resistance. In 2017, NASA recognized Dr. Hughson and his colleagues with the Compelling Results Award, and in 2018, Dr. Hughson received the NASA Exceptional Scientific Achievement Medal. We are pleased to add to these prestigious awards the 2018 Nello Pace Award in recognition of Richard's outstanding contributions to the field of Gravitational Physiology.

You are invited to visit our ISGP web-site!



About ISGP

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

www.isgp-space.org

In our web site you can:

- Find information about our society
- Get news such as books, events, ….
- Register for free membership
- Find a page dedicated for students
- Download *Journal of Gravitational Physiology* archives
- And a lot more!

Do not hesitate to register for free membership and to suggest contents to be added as downloadable, as new event, or as post-doc and trainee positions open for students!

Final Programme

Sunday 17 June 2018

19:00 **Welcome reception & pre-registration at the Golden Tulip**

21:00 **Noordwijk Beach**

Monday 18 June 2018

08:00 **Registration desk open**

Current Concepts Symposium

Chairs: Charles Fuller, Jack van Loon

Room: High Bay

09:00 **Introduction**

09:10 **Welcome from ESA directorate of technology, engineering and quality**

Torben Hendriks, ESA-TEC-M

09:20 **Welcome from ESA directorate of human and robotic exploration programmes**

Jennifer Ngo-Anh, ESA-HRE-RS

09:30 **Venturing beyond LEO: Research plans for gateway**

William Paloski, NASA JSC, Houston, USA

10:00 **Space food system challenges and integrative solutions for exploration missions**

Grace Douglas, Food lab, NASA JSC, Houston, USA

10:30 Coffee break

11:00 Human milk oligosaccharides: Improving health by prebiotic effects

Stefan Jennewein, Jennewein Biotechnologie GmbH, Bad Honnef, Germany

11:30 Sodium storage and diseases of the aging organism

JensTitze, Duke Univ. Singapore & Univ. Erlangen, Germany

12:00 The human hypergravity habitat, H3: A space flight spin-off for research on obesity and healthy ageing on ground

Jack van Loon, VU-Medical Center Amsterdam & ESA-ESTEC-TEC-MMG, The Netherlands

12:30 Lunch break

Session 1: Analogues & countermeasure research-1

Chairs: Satoshi Iwase / Edwin Mulder

Room: High Bay

14:00 Simulate microgravity on the ground to prepare manned spaceflight

Bareille M[1], *Gauquelin-Koch G*[2], *Bernat M*[1], *Hazane P*[1]

[1]*Medes-imps*, [2]*CNES*

14:15 NASA's use of ground and flight analogs in reducing human risks for exploration

Corbin B[1], *Vega L*[1]

[1]*NASA*

14:30 Preventing lumbar spine injuries in astronauts: An exercise approach for activating the transversus abdominis muscle

Belavy D[1], *Owen P*[1], *Rantalainen T*[1,2], *Scheuring R*[3]

[1]*Deakin University*, [2]*University of Jyväskylä*, [3]*NASA Johnson Space Center*

14:45 Falls and fall-prevention in older persons: Spaceflight meets geriatrics!

Prof Nandu Goswami[1]

[1]Director, "Gravitational Physiology, Aging and Medicine Research Unit", and Chair, Physiology Unit, Otto Loewi Research Center of Vascular Biology, Immunity and Inflammation, Medical University of Graz

15:00 A systematic review on the efficacy of resistive exercise countermeasures in microgravity studies and its ground-based analogues

Miss Leonie Fiebig[1], *Dr. Andrew Winnard, Dr. Mona Nasser, Dr. Björn Braunstein, Dr. David Green, Dr. Jonathan Scott, Dr. Tobias Weber*

[1]European Astronaut Center

15:15 Contribution of the ground reaction forces in the preservation of bone tissue during the locomotor training in long term space missions

Dr. Elena Fomina[1,2,3], *Novikov Valery*[1], *Alexandra Savinkina*[1,4], *Nataliya Lysova*[1], *Svetlana Rezvanova*[1,4], *Tatyana Kukoba1*[2,3]

[1]State Research Center of Russian Federation - Institute of Biomedical Problems of The Russian Academy o Sciences, [2]Moscow State Pedagogical University, [3]RUDN University, [4]Russian State University of Physical Education, Sport, Youth and Tourism (SCOLIPE)

Session 2: Trends in commercialise space research

Chair: Veronica La Regina

Room: Auditorium

14:00 Current scenario: Commercialize space research

VeronicaLa Regina, RHEA for ESA

14:20 ESA – Call for ideas (TIA and HRE)

Jason Hatton, ESA

14:40 Commercial ISS facilities: ICE-cubes

Hilde Stenuit, Space Applications Services

15:00 Commercial ISS facilities: Bartolomeo

Katherine Pegg, Airbus

Session 3: Biology / cell – Animal models-1

Chairs: Jason Hatton / John Love

Room: Multimedia Libray

14:00

Counteracting effects of space flight and hypergravity on human capillary endothelial cells: A comprehensive molecular and morphological study

*Ph.D. Ivana Barravecchia[1], Dr. Chiara De Cesari[1], Ph.D. Olga V. Pyankova[1], Ph.D. Francesca Scebba[1], Ph.D. Mattia Forcato[2], Prof. M. Enrico Pè[1], Dr. Helen A. Foster[3], Prof. Silvio Bicciato[2], Prof. Joanna M. Bridger[3], **Ph.D. Debora Angeloni[1]***

[1]Scuola Superiore Sant'Anna, Institute of Life Sciences, [2]University of Modena and Reggio Emilia, Center for Genome Research, [3]Brunel University, Genome Engineering and Maintenance Network, Institute for Environmental, Health and Society

14:15

Blood vessels from space – results of the SPHEROIDS project

***Dr. Marcus Krüger[1]**, Dr. Markus Wehland[1], MSc Sascha Kopp[1], Dr. Johann Bauer[2], Prof. Sarah Baatout[3], Dr. Marjan Moreels[3], Prof. Marcel Egli[4], Dr. Stefan Riwaldt[1], Prof. Manfred Infanger[1], Prof. Daniela Grimm[1]*

[1]AG Gravitational Biology and Translational Regenerative Medicine, Otto-von-Guericke-University Magdeburg, [2]Max-Planck-Institute of Biochemistry, [3]Expert Group for Molecular and Cellular Biology, Belgian Nuclear Research Centre, 4Bioscience and Medical Engineering, Lucerne University of Applied Sciences and Arts

14:30

Paracrine response of gravisensitive cells to simulated microgravity

***Prof. Ludmila Buravkova[1]**, Mr Andrew Ratusnyy[1], Dr. Marya Ezdakova[1], Dr. Elena Andreeva[1]*

[1]Institute of Biomedical Problems RAS

14:45

Parabolic flight-induced acute hypergravity and microgravity modulates the differentiation potential of embryonic stem cells

***Mr Aviseka Acharya[1]**, Dr. Sonja Brungs[2], Ms Margit Henry[1], Ms Tamara Rotsheyn[1], Ms Nirmala singh Yaduvanshi[1], Ms Lucia Wegener[2], Mr. Simon Jentzsch[2], Professor Jürgen Hescheler[1], Dr. Ruth Hemmersbach[2],*

Professor Helene Boeuf[3], Professor Agapios Sachinidis[1]
[1]University of Köln, [2]German Aerospace Center, Institute of Aerospace Medicine, Gravitational Biology, [3]INSERM-U1026 BioTis

15:00 **Validation of microgravity simulators (random positioning machine and clinostat) using cellular bioassays**

***Dr. Sonja Brungs**[1], Dr. Jens Hauslage[1], Volkan Cevik[1], Kai Wasser[1], PD Dr. Ruth Hemmersbach[1]*
[1]German Aerospace Center, Institute of Aerospace Medicine, Gravitational Biology

15:15 **Mitochondria in endothelial cells in simulated microgravity**

***Dr. Laura Locatelli**[1], Dr. Valentina Romeo[1], Dr. Clara De Palma[1], Dr Sara Castiglioni[1], Dr. Alessandra Cazzaniga[1]*
[1]University of Milan

15:30 **Coffee break & poster session 1**

Session 4: Countermeasure research-2

Chair: Barbara Corbin / Pierre-François Migeotte
Room: High Bay

16:30 **Human performance in altered-gravity environments**

***Prof. Ana Diaz Artiles**[1]*
[1]Texas A&M University

16:45 **Influence of acceleration levels on jump performance during centrifugation**

***Prof. Markus Gruber**[1], Dr. Andreas Kramer[1], Dr. Jakob Kümmel[1]*
[1]University Konstanz

17:00 **Effect of artificial gravity with exercise on spaceflight deconditioning in humans, and project for assessment of artificial gravity in H-II transfer vehicle in international space station**

***Prof. Satoshi Iwase**[1]*
[1]Aichi Medical University

17:15 **Biomechanics during flywheel resistance exercise – effects of the gravity vector**

MSc Maria Jönsson[1,2], *MD PhD Hans E Berg*[3,4], *PhD Lena Norrbrand*[1,2], *PhD Lanie Gutierrez-Farewik*[5], *MD PhD Patrik Sundblad*[6,7], *PhD Michael S Andersen*[8], *MD PhD Ola Eiken*[1,2]

[1]Dept. of Environmental Physiology, CBH, KTH Royal Institute of Technology, [2]Swedish Aerospace Physiology Centre, SAPC, [3]Dept. of Orthopaedics, Karolinska University Hospital, [4]CLINTEC, Karolinska Institutet, [5]KTH Mechanics, Royal Institute of Technology, [6]Dept. of Clinical Physiology, Karolinska University Hospital, [7]Inst. for Laboratory Medicine, Karolinska Institutet, [8]Dept. of Materials and Production, Aalborg University

17:30 **Virtual reality technology and exercise in artificial gravity and bed rest settings as a countermeasure for spaceflight deconditioning**

Dr. Gabriel G. De La Torre[1], *Dr. Ana Diaz-Artiles*[2], *Jesper Jorgensen*[4], *Dr. Andreas Vogler*[3]

[1]University of Cadiz, [2]Texas AM University, [3]Andreas Vogler Studio, [4]University of Roskilde

17:45 **Six weeks of exercise training with the Functional Re-adaptive Exercise Device (FRED) increases lumbar multifidus cross-sectional area and improves patient reported function in people with chronic, non-specific low back pain**

Ms Kirsty Lindsay[1], *Prof. Nick Caplan*[1], *Prof Paul Hodges*[5], *Prof Julie Hides*[3], *Dr Tobias Weber*[4], *Dr Sauro Salomoni*[5], *Dr Jonathon Scott*[4], *Dr Enrico De Martino*[6], *Dr Andrew Winnard*[1], *Ms Elizabeth Young*[1], *Ms Gunda Lambrecht*[4], *Prof Dorothee Debuse*[2]

[1]Northumbria University, [2]LUNEX University, [3]Griffith University, [4]Space Medicine Office, EAC ESA, [5]University of Queensland, [6]Aalborg University

Session 5: Interactive session: Getting prepared to use the new ISS commercial facilities

Chairs: Veronica La Regina

Room: Auditorium

16:30 **Identification of research and development areas**

18:00 **Brainstorming of scientific objectives**

Brainstorming / preliminary sketching of space experiments

Session 6: Biology / cell – Animal models-2

Chairs: Monica Monici / Hisashi Kato

Room: Multimedia Library

16:30

Wound healing in weightlessness: An in vivo study on the medicinal leech (hirudo medicinalis)

Dr. Francesca Cialdai[1], *Dr. Desirée Pantalone*[2], *Prof. Daniele Bani*[3], *Prof. Paolo Romagnoli*[3], *Prof. Angela Maria Rizzo*[4], *Prof. Fabio Celotti*[4], *Dr. Alessandra Colciago*[4], *Dr. Elettra Sereni*[5], *Dr. Francesco Ranaldi*[5], *Dr. Monica Monici*[1]

[1]ASAcampus Joint Lab., ASA Res. Div., Dept. of Experimental and Clinical Biomedical Sciences, University of Florence, [2]Dept. of Surgery and Translational Medicine, University of Florence, [3]Dept. of Experimental and Clinical Medicine, University of Florence, [4]Dept. of Pharmacological and Biomolecular Sciences, University of Milan, [5]Dept. of Experimental and Clinical Biomedical Sciences, University of Florence

16:45

Hypergravity impact on cell traction forces

Julia Eckert[1,2,3], *Jack J.W.A. van Loon*[3,4], *Lukas M. Eng*[2], *Thomas Schmidt*[1]

[1]Physics of Life Processes, Leiden Institute of Physics, Leiden University, [2]School of Science, Department of Physics, Technische Universität Dresden, [3]Life & Physical Science, Instrumentation and Life Support Laboratory (TEC-MMG), ESA/ESTEC, [4]DESC (Dutch Experiment Support Center), Dept. Oral and Maxillofacial Surgery / Oral Pathology, VU University Medical Center & Academic Centre for Dentistry Amsterdam (ACTA)

17:00

Growing tissues in space

Prof. Daniela Grimm[1], *Dr. Marcus Krüger*[2], *MSc Sascha Kopp*[2], *Dr. Markus Wehland*[2], *Professor Manfred Infanger*[2], *Dr. Johann Bauer*[3]

[1]Department of Biomedicine, Aarhus University, [2]AG Gravitational Biology and Translational Regenerative Medicine, Otto-von-Guericke-University-Magdeburg, [3]Max-Planck-Institute of Biochemistry

17:15

Tissue engineering research on the international space station

Mr John Love[1]

[1]NASA

17:30 **A ground-based facility for artificial gravity allowing translational research from cellular to human physiology**

Mr Timo Frett[1], Michael Arz[1], Guido Petrat[1], Dr Christian Liemersdorf[1], PD Dr Ruth Hemmersbach[1] , [1]German Aerospace Center (DLR e.V.)

17:45 **Mechanoregulation of proliferation, differentiation, senescence and survival of bone marrow primary osteoprecursor cells**

Dr. Cassandra Juran[1], Dr. Elizabeth Blaber[1], Dr. Eduardo Almeida[2]
[1]Universities Space Research Association (USRA) at NASA Ames Research Center, [2]NASA

Student initiative

Room: Auditorium

18:00 **Students corner: Presentation of SELGRA**

Jeremy Rabineau, Université libre de Bruxelles, Belgium

18:30 **End of Day 1**

Tuesday 19 June 2018

Plenary session: Skeletal muscle remodeling during gravitational unloading and other disuse models

chairs: Carlo Reggiani / Boris Shenkman

Room: High Bay

09:00 **Support afferentation is a master of postural muscle activity, cytoskeleton stability and proteostasis**

Boris Shenkman, SSC RF Institute of Biomedical Problems, RAS, Moscow, Russia

09:30 **Novel transitional approaches how to modulate titin filament based myofibrillar load sensing**

Siegried Labeit, Universitätsmedizin Mannheim, University of Heidelberg, Mannheim, Germany

10:00 **Disuse skeletal muscle atrophy on earth and in space: Cellular, proteomic and molecular adaptations**

Roberto Bottinelle, University of Pavia, Pavia, Italy

10:30 **Coffee break**

11:00 **The impact of disuse on skeletal muscles is exacerbated by aging Carlo Reggiani, Univ. Padova, via Italy**

11:30 **Contraction signalling in skeletal muscle: How is specificity achieved?**

Martin Flück, Balgrist Campus, Univ. of Zurich, Zurich, Switzerland

12:00 **General discussion**

12:30 **Lunch break**

Session 7: Neuroscience-1

Chair: Stefan Schneider / Alexander Stahn

Room: High Bay

14:00 **Regional cerebral blood flow during head-down tilt bed rest. Combined with 0.5% CO_2 and neuro-ocular impairment – results from the VaPER study**

Dr. Donna Roberts[1]

[1]Medical University of South Carolina

14:15 **Alterations of the cortical control of locomotions in the long-term spaceflights revealed by fMRI**

***Inesa Kozlovskaya**[1], Ekaterina Pechenkova[2], Inna Nosikova[1], Angelique Ombergen van[3], Liudmila Litvinova[2], Ilya Rukavishnikov[1], Floris Wyuts[3], Ben Jeurissen[4], Valentin Sinitsyn[2], Alena Rumshiskaya[2], Elena Tomilovskaya[1]*

[1]Institute of Biomedical Problems (ras), [2]Radiology Department, Federal Center of Treatment and Rehabilitation, [3]Antwerp Universtiy Research Center for Equilibrium and Aerospace (AUREA), University of Antwerp, [4]Imec/Vision Lab, University of Antwerp

14:30 **Hyper-gravity promotes motor learning in goldfish and humans**

Shohei Miura[1], Yuki Takagi[1], Takafumi Kashima[1], Kohei Urase[1], Shuntaro Miki[1], ***Prof. Yutaka Hirata[1]***

[1]Chubu University College of Engineering

14:45 **Exercise as a countermeasure for impaired brain function? - Evidence from the RSL bed rest study**

Mrs Anika Werner[1,3]*, Mrs Katharina Brauns[1], Prof. Hanns-Christian Gunga[1], Prof. Dr. Simone Kühn[4], Dr. Alexander Christoph Stahn[1,2]*

[1]Charite Universitatsmedizin Berlin, Center for Space Medicine and Extreme Environments Berlin, [2]University of Pennsylvania, Perelman School of Medicine, [3]Normandie Université, UMR INSERM U 1075 COMETE, [4]University Medical Center Hamburg-Eppendorf, Dept. of Psychiatry and Psychotherapy

15:00 **Development and functional validation of a ground-based analog for post-spaceflight sensorimotor/neurovestibular impairment: The wheelchair head immobilization paradigm**

Mr Jordan Dixon[1]*, Dr Torin Clark[1]*

[1]University of Colorado (Boulder)

15:15 **Motor and cognitive functions in Parkinson's disease patients across the program of "dry immersion"**

Professor Alexander Meigal[1]*, Professor Liudmila Gerasimova-Meigal[1], Olesya Tretjakova[1], Kirill Prokhorov[1], Professor Natalia Subbotina[1], Nina Popadeikina[1], Docent Irina Sayenko[2]*

[1]Petrozavodsk State University, [2]State Scientific Center "Institute of Biomedical Problems" (RAS)

Session 8: Bone & muscle

Chairs: Ruth Globus / Yoshinobu Ohira

Room: Multimedia Library

14:00 **ESA bedrest cocktail: Effects of an anti-oxidant and an anti-inflammatory cocktail on the prevention of skeletal muscle deconditioning during a 2-month head down tilt bedrest**

Coralie Arc-Chagnaud[1,2]*, Théo Fovet[1], Thomas Brioche[1], Rémi Roumanille[1], Guillaume Py[1], Angèle Chopard[1]*

[1]University of Montpellier; INRA, UMR 866 Dynamique Musculaire et Métabolisme, [2]Freshage Research Group-Dept. Physiology-University of Valencia, CIBERFES, INCLIVA

14:15 Estimation of gait characteristics during walking in lower gravity environment using a wearable device

Mr. Léo Lamassoure[1], *Mr. Keisuke Araki[1], Dr. Akihito Ito[1,3], Dr. Kiyotaka Kamibayashi[2,3], Dr. Yoshinobu Ohira[2,3], Dr. Nobutaka Tsujiuchi[1,3]*

[1]Graduate School of Engineering, Doshisha University, [2]Graduate School of Health and Sports Science, Doshisha University, [3]Research Center for Space and Medical Sciences, Doshisha University

14:30 Investigation of additional low-level axial body load on neuromuscular responses during running in simulated lunar gravity

Miss Julia Attias[1], *Mr Alexander Suss[3], Dr Katya Mileva[2], Professor Thais Russomano[1], Dr David Green[1,3]*

[1]King's College London, [2]London South Bank University, [3]KBR Wyle, Space Medicine Office, European Astronaut Centre

14:45 Analysis of the locomations strategy during walking under ground and reduced gravitational loads on musculoskeletal system

Dr. Alexey Shpakov[1], *Dr. Anton Artamonov[1]*

[1]Research Institute For Space Medicine Federal Research Clinical Center of Federal Biomedical Agency of Russia

15:00 Modulation of MLC2 regulatory protein and AKT signaling factor by phosphorylation/glycosylation states in human skeletal muscle after short-term dry immersion and longer bed rest studies

Prof. Laurence Stevens[1], *Marie Pourrier[1], Laetitia Cochon[1], Valerie Montel[1], Prof. Bruno Bastide[1]*

[1]Universite de Lille - Urepsss

15:15 Protein synthesis alterations in isolated soleus muscle after ex vivo eccentric exercise following gravitational unloading

Mr Sergey Tyganov[1], *Mr Timur Mirzoev[1], Mr Sergey Rozhkov[1], Mr Boris Shenkman[1]*

[1]SSC RF Institute of Biomedical Problems, RAS

Session 9: Plant / technology / commercial

Chairs: Iliya Bulavin / Yair Glick

Room: Auditorium

14:00

GRAVI-2 space experiment: The effects of statolith location on early stages of gravity sensing pathways in lentil roots

Dr François Bizet[1], Dr Veronica Pereda-Loth[2], Dr Nicole Brunel[1], Claire Szczpaniak[3], Irène Hummel[4], David Cohen[4], Philippe Label[1], Eric Badel[1], ***Valerie Legué[1]***

[1]Université Clermont Auvergne, INRA, PIAF, F-63000 Clermont-Ferrand, [2]GSBMS, University of Toulouse, [3]Université Clermont Auvergne,CICS, F-63000 Clermont-Ferrand, [4]INRA, Université de Lorraine, UMR EEF, 54280 Champenoux

14:15

"We fly your research in microgravity": The airbus commercial service products for microgravity research

Mr Christian Bruderrek[1]*, Mrs Maria Birlem[1], Mr Philipp Schulien[1], Mrs Noemie Bernede[1]*

[1]Airbus Defence and Space

14:30

Enhanced concept of a multipurpose bioreactor for pilot processing in space environments

Mrs Ann Delahaye[1], ***Mr Dries Demey[1]***

[1]Qinetiq Space

14:45

Observation of larger fluctuation of mass values of peptides, as compared to the predicted values due to the tidal forces

Serhiy Souchelnytskyi

College of Medicine, Proteomics Facility, Qatar University, Doha, Qatar

15:00

Fabrication of patterned colloidal photonic crystals on stretchable PDMS films

Miss Vanja Miskovic[1]*, Dr. Christophe Minetti[1], Dr. Hatim Machrafi[1], Prof. Frank Dubois[1], Dr. Carlo Saverio Iorio[1], Mr. Patrick Queeckers[1]*

[1]Universite libre de Bruxelles

15:15 **10 years of the Large Diameter Centrifuge (LDC): Overview hyper-g life sciences research @ ESTEC**

Jack Van Loon[1,2], *Alan Dowson*[2], *Jutta Krause*[3], *Pedro Raposo*[4], *Francois Gaubert*[2], *Robert Lindner*[2], *José Gavira Izquierdo*[6], *Torben K. Henriksen*[7]

[1]VUmc Amsterdam, [2]ESA-ESTEC-TEC-MMG, [3]ESA-ESTEC-HRE-PPD, [4]Zeugma, [5]ESA-TEC-MMG, [6]ESA-TEC-MM, [7]ESA-TEC-M

15:00 **Coffee break & poster session 2**

Session 10: Neuroscience-2

Chairs: Daniela Santucci / William Paloski

Room: High Bay

16:30 **From Antarctica to Alzheimers – Exercise helps to prevent cognitive decline**

Prof. Stefan Schneider[1]

[1]German Sport University Cologne

16:45 **Postural stability of cosmonauts after long-term space flights**

Dr. Nikita Shishkin[1], *Mr. Vladimir Kitov*[1], *Mrs. Tatiana Shigueva*[1], *PhD Elena Tomilovskaya*[1], *Prof. Inesa Kozlovskaya*[1]

[1]RF SSC-Institute of Biomedical Problems RAS

17:00 **BDNF – A key biomarker for assessing acute stress responses and brain plasticity during spaceflight?**

Dr. Alexander Stahn[1,2], *Katharina Brauns*[1], *Anika Werner*[1], *Dr David Dinges*[2], *Dr Mathias Basner*[2], *Dr Simone Kuehn*[3], *Dr Hanns-Christian Gunga*[1]

[1]Charité Universitätsmedizin Berlin, [2]University of Pennsylvania, Perelman School of Medicine, [3]University Medical Center Hamburg-Eppendorf, Dept. of Psychiatry and Psychotherapy

17:15 **Changes in intrinsic functional brain connectivity after first-time exposure to parabolic flight**

Dr. Angelique Van Ombergen[1], *Prof. Floris Wuyts*[1], *Dr. Ben Jeurissen*[1], *Prof. Jan Sijbers*[1], *Floris Vanhevel*[2], *Steven Jillings*[1], *Prof. Paul M. Parizel*[1,2], *Prof. Stefan Sunaert*[3], *Prof. Paul H. Van de Heyning*[2], *Prof. Vincent Dousset*[4], *Prof. Steven Laureys*[5], *Dr. Athena Demertzi*[5]

[1]University of Antwerp, [2]Antwerp University Hospital, [3]KU Leuven - University of Leuven, [4]University of Bordeaux, [5]University of Liège

17:30 Changes in neuronal activity and episodic memory after 30 days of isolation and confinement

Mrs Anika Werner[1,3], *Mrs Katharina Brauns*[1], *Prof. Dr. Hanns-Christian Gunga*[1], *Prof. Dr. Simone Kühn*[4], *Dr. Alexander Christoph Stahn*[1,2]

[1]*Charité Universitätsmedizin Berlin, Center for Space Medicine and Extreme Environments Berlin,* [2]*University of Pennsylvania, Perelman School of Medicine,* [3]*Normandie Université, UMR INSERM U 1075 COMETE,* [4]*University Medical Center Hamburg-Eppendorf, Dept. of Psychiatry and Psychotherapy*

17:45 Morphofunctional peculiarities of ischemic and hemorrhagic injuries of the brain in rats under microgravity effects modelling

Dr. Mikhail Baranov[1], *Prof. Aleksander Paltzin*[2,3], *Prof. Galina Romanova*[2], *Dr. Fatima Shakova*[2]

[1]*Federal Scientific Clinical Center of Fmba of Russia,* [2]*Institute of General Pathology and Pathophysiology,* [3]*Russian Medical Academy of Postgraduate Education*

18:00 End of Day 2

Session 11: Biology / cel – Animal models-3

Chairs: Laurence Stevens / Ruth Hemmersbach

Room: Auditorium

16:30 Microvaculature on a chip

Mr Mehdi Inglebert[1], *Mrs Daria Tsvirkun*[3], *Mr Alexei Grichine*[4], *Mr Alain Duperray*[4], *Mr Chaouqi Misbah*[2], *Mr Lionel Bureau*[2]

[1]*Univ. Grenoble Alpes, LIPHY,* [2]*CNRS, LIPHY,* [3]*Research Center for Obstetrics, Gynecology and Perinatology,* [4]*INSERM, IAB*

16:45 Tissue repair and regenaration in space and on earth

Dr. Monica Monici[1], *Dr Francesca Cialdai*[1], *Dr Michele Balsamo*[2], *Dr Liyana Popova*[2], *Eng Alessandro Donati*[2], *Prof Daniele Bani*[3], *Prof Paolo Romagnoli*[3], *Eng Jack J.W.A. van Loon*[4], *MDr Desirée Pantalone*[5]

[1]*ASAcampus Joint Lab., ASA Res. Div., ASA srl & Dept. of Experimental and Clinical Biomedical Sciences, University of Florence,* [2]*Kayser Italia Srl,* [3]*Dept. of Experimental and Clinical Medicine, University of Florence,*

[4]Dept. Oral and Maxillofacial Surgery, ACTA/VU Medical Center, Vrije University, [5]Dept. of Surgery and Translational Medicine, University of Florence

17:00

Regulation of gene expression in the testes, heart and lungs of mice under long-term modelling microgravity

*Mr. Sergey Loktev[1], **Dr. Irina Ogneva[1,2]***

[1]Institute for biomedical problems RAS, [2]I.M. Sechenov First Moscow State Medical University

17:15

C. elegans as a model for understanding spaceflight induced muscle decline

***Dr. Amelia Pollard[1]**, Dr Christopher Gaffney[4], Dr Colleen Deane[2], Mr Michael Cooke[2], Miss Jennifer Hewitt[3], Dr Bethan Phillips[1], Professor Nathaniel Szewczyk[1], Dr Siva Vanapalli[3], Dr Timothy Etheridge[2]*

[1]University of Nottingham, [2]University of Exeter, [3]Texas Tech University, [4]Lancaster University

17:30

The SERiSM project: Modulation of osteogenic markers in human Blood-derived stem cells aboard the ISS during the VITA Mission of the Italian space agency

***Prof. Mauro Maccarrone[1]**, Dr. Giulia Merlini[2], Dr. Cristina Ruggiero[2], Dr. Sara Piccirillo[3], Dr. Giovanni Valentini[3], Dr. Gabriele Mascetti[3], Mr. Michele Balsamo[4], Dr. Natalia Battista[5], Dr. Monica Bari[2], Dr. Alessandra Gambacurta[2]*

[1]Department of Medicine, Campus Bio-Medico University of Rome, [2]Department of Experimental Medicine and Surgery, Tor Vergata University of Rome, [3]Italian Space Agency, [4]Kayser Italia S.r.l., [5]Faculty of Biosciences and Technology for Food, Agriculture and Environment, University of Teramo

17:45

Maintaining muscle health in C. elegans: New protective compounds and methods

***Dr. Amelia Pollard[1]**, Christopher Gaffney[4], Jennifer Hewitt[3], Siva Vanapalli[3], Roberta Torregrossa[2], Matthew Whiteman[2], Nathaniel Szewczyk[1], Timothy Etheridge[2]*

[1]University of Nottingham, [2]University of Exeter, [3]Texas Tech University, [4]Lancaster University

18:00

End of Day 2

Wednesday 20 June 2018

Plenary session: Radiation

Chairs: Christine Hellweg / Peter Norsk

Room: High Bay

09:00 **The journey to mars and intracellular signaling pathways: Effects of space radiation**

Christine Hellweg, DLR Institute of Aerospace Medicine, Cologne, Germany

09:45 **Gravity, radiation and age-related tissue degeneration: Experimental models to identify shared mechanisms**

Ruth Globus, NASA Ames, Moffett Field, USA

10:30 **Coffee break**

11:00 **Heart in space: Effect of the extraterrestrial environment on the cardiovascular system**

Richard Hughson, Schlegel-University of Waterloo Research Institute for Aging, Waterloo, Canada

11:45 **Will space radiation stop human space exploration? health effects, astronaut radioresistance & countermeasures**

Sarah Baatout, Belgian Nuclear Research Center, SCK-CEN, Mol, Belgium

12:30 **Lunch break**

Session 12: Metabolism / nutrition

Chairs: Jochum Zange / Lichar Dillon

Room: High Bay

14:00 **Effects of antioxidants on bone turnover markers in 6° head-down tilt bed rest**

***Miss Katharina Austermann**[1], Dr. Natalie Baecker[1], Dr. Rolf Fimmers[2], Dr. Peter Stehle[1], Dr. Scott Smith[3], Dr. Martina Heer[1]*

[1]Department of Nutrition and Food Sciences, Nutritional Physiology, University of Bonn, [2]Department of Medical Biometry, Informatics and

Epidemiology, University of Bonn, [3]Human Health and Performance Directorate, NASA Lyndon B. Johnson Space Center

14:15

The effects of 60 days bed rest on the physical and metabolic characteristics of young, healthy men

Miss Kiera Ward[1], Dr Diane Cooper[1], Dr Donal O'Gorman[2]

[1]Athlone Institute of Technology, [2]Dublin City University

14:30

Role of skeletal muscle atrophy and inflammation in microgravity-induced iron misdistribution. Potential perspectives to fight spaceflight anemia

Dr. Frédéric Derbré[1], *Mr. Kévin Nay[1], Dr. Nicolas Pierre[2], Dr. / M.D Thibault Cavey[3,4], Dr. Luz Lefeuvre-Orfila[1], Mrs. Dany Saligaut[1], M.D Martine Ropert[3,4], Dr. / M.D Olivier Loréal[3]*

[1]Laboratory "Movement, Sport and health Sciences " (M2S) - University of Rennes / ENS Rennes, [2]Liège University - GIGA Institute, [3]INSERM, University of Rennes, INRA, Institut NUMECAN (Nutrition Metabolisms and Cancer) UMR A1341, UMR S1241, [4]Department of Biochemistry, CHU Rennes

14:45

Metabolic response of rats to chronic centrifugation at a small Radius

Charles A. Fuller[1], *Amy L. McElroy[1], Tana M. Hoban-Higgins[1]*

[1]University of California, Davis

15:00

Resistive vibration exercise and nutritional supplementation during 21 days of head-down tilt bed rest: Effects on cartilage health in relation to morphological changes of thigh muscles

Dr. Anna-Maria Liphardt[1,2], *Prof. Dr. Felix Eckstein[3,4], Vera Bolte[1], Dr. Torben Dannhauer[3,4], Dr. Eva-Maria Steidle-Kloc[3], Prof. Dr. Gert-Peter Brüggemann[1], PD Dr. Anja Niehoff[1,5]*

[1]German Sport University Cologne (DSHS Köln), Biomechanik & Orthopädie, [2]Friedrich-Alexander-University Erlangen-Nuremberg, Internal Medicine 3 - Rheumatology & Immunology, Universitätsklinikum, [3]Paracelsus Medical University Salzburg & Nuremberg, Instiute of Anatomy, [4]Chondrometrics GmbH, [5]University of Cologne, Medical Faculty, Cologne Center for Musculoskeletal Biomechanics (CCMB)

15:15 Prevention of spaceflight-induced bone loss: A promising dietary countermeasure

Dr. Ann-Sofie Schreurs[1], *Dr. Candice Tahimic*[3], *Mrs. Sonette Steczina*[4], *Mrs. Moniece Lowe*[4], *Dr. Josh Alwood*[2], *Dr. Ruth Globus*[2]

[1]NASA, USRA, [2]NASA, [3]KBR/Wyle Laboratories, [4]Blue Marble Space Institute of Science

Session 13: Immune system / respiratory / radiation

Chairs: Debora Angeloni / Francesco Pampaloni

Room: Auditorium

14:00 Dysregulation of cellular-mediated immune response in an in vitro model due to exposure to simulated microgravity and simulated psychological stress

Mr Richard Thomas Deyhle Jr[1,2], *Doctor Bjorn Baslet*[1], *Professor Sarah Baatout*[1,2], *Doctor Marjan Moreels*[1]

[1]SCK•CEN, Belgian Nuclear Research Centre, [2]Ghent University, Department of Molecular Biotechnology

14:15 GRAIN V2.0 (influence of altered gravity on immune responses demonstrated with neutrophil migration performance) migration and activation of immune cells in altered gravity

Dr. Dominique Moser[1], *Dr. Shujin Sun*[2], *Dr. Ning Li*[2], *Katharina Biere*[1], *Marion Hörl*[1], *Sandra Matzel*[1], *Dr. Cora Thiel*[3], *Dr. Yuxin Gao*[2], *Prof. Oliver Ullrich*[3], *Prof. Mian Long*[2], *Prof. Alexander Choukèr*[1]

[1]Hospital of The Ludwig Maximilians University, [2]Chinese Academy of Sciences, [3]University of Zurich

14:30 Gravitational stress during parabolic flights induced changes in human leukocyte subsets

Dr. Ulrik Stervbo[1], *Dr. Toralf Roch*[2], *Dr. Tina Kornprobst*[2], *Dr. Gerald Grütz*[2], *Prof. Birgit Sawitzky*[2], *PhD Andreas Wilhelm*[2], *PhD Francis Lacombe*[3], *Kaoutar Allou*[3], *Dr. Markus Kaymer*[4], *Prof. Timm Westhoff*[1], **Dr. Felix S. Seibert**[1], *Prof. Nina Babel*[1]

[1]Ruhr University Bochum; University Hospital Marien Hospital Herne, [2]Berlin-Brandenburg Center for Regenerative Therapies; Charité-Universitätsmedizin Berlin, [3]Laboratoire d'hématologie, CHU de Bordeaux, Hôpital Haut-Lévêque, [4]Beckman Coulter GmbH

14:45 The Coenzyme Q10 (CoQ10) as countermeasure for retinal damage onboard the international space station: The CORM project

Dr. Matteo Lulli[1], *Dr. Francesca Cialdai*[2], *Dr. Leonardo Vignali*[2], *Dr. Monica Monici*[2], *Dr. Alessandro Cicconi*[3], *Dr. Stefano Cacchione*[3], *Dr. Alberto Magi*[1], *Dr. Michele Balsamo*[4], *Dr. Marco Vukich*[4], *Dr. Gianluca Neri*[4], *Dr. Alessandro Donati*[4], *Prof. Sergio Capaccioli*[1]

[1]University of Florence, [2]ASAcampus Joint Laboratory, [3]Sapienza University of Rome, [4]Kayser Italia srl

15:00 Gradual reduction of exhaled nitric oxide during the preflight preparation and inflight periods in ISS astronauts

Dr. Lars L Karlsson[1], *Dr. Alain Van Muylem*[2], *Prof. Dag Linnarsson*[1]

[1]Karolinska Institutet, [2]Erasme University Hospital and Université Libre de Bruxelles

15:15 IMMUNO3D: Effects of simulated microgravity and hypergravity on a three-dimensional model of human bone marrow

Dr. Francesco Pampaloni[1], *Dr. Sonja Brungs*[2], *Mrs. Berit Reinhardt*[1], *Dr. PD Ruth Hemmersbach*[2], *Prof. Dr. Ernst H.K. Stelzer*[1]

[1]Goethe University Frankfurt, Buchmann Institute for Molecular Life Sciences (BMLS), [2]DLR – German Aerospace Center, Institute of Aerospace Medicine, Gravitational Biology Department

15:30 Coffee break & poster session 3

Session 14: Space medicine & international cooperation

Chairs: Martina Heer / Victor Demaria-Pesce

Room: High Bay

16:30 Welcome – opening remarks

Martina Heer, Univ. of Bonn, Germany

16:35 Landmarks of history of international cooperation in human space exploration

Victor Demaria-Pesce, ESA-EAC, Cologne Germany

16:40

The European astronaut centre: A hub for human spaceflight education

David Green, KBRwyle / European Astronaut Centre

16:50

Cardiospace French-Chinese cooperation in space physiology: Lessons learnt and perspectives

Mr Jean-christophe Lloret[1], Dr Ming Yuan[2], Mr Laurent Arnaud[1], Dr Xuemin Yin[2], Dr Guillemette Gauquelin[1], Dr Yinhui Li[2]

[1]French Space Agency, [2]Astronaut Center of China

17:00

We'll go nowher but together

Dr. Laurence Vico-pouget[1]

[1]Inserm U1059 University of Lyon, University Jean Monnet

17:10

Animal studies in bion-m missions: Benefits of scientific cooperation

Dr. Olga Vinogradova[1], *Dr. Boris Shenkman[1], Dr. Vladimir Sychev[1]*

[1]SRC RF Institute of Biomedical Problems RAS, Russia

17:20

NASA space medicine research for exploration

Dr. Erik Antonsen[1]

[1]Nasa

17:30

International scientific and medical cooperation: A must for Human Space Exploration

Dr. Guillaume Weerts[1], *Dr. Victor Demaria-Pesce[1]*

[1]Esa

17:40

Questions

17:50

Conclusions

Victor Demaria-Pesce

18:00

End of Day 3

19:30

Symposium dinner

Tulum Tulum

Zeereep 104

2202 NW Noordwijk

Session 15: Psychology / neuroscience

Chairs: Rainer Herpers / Fabio Ferlazzo

Room: Auditorium

16:30

Evaluation of anxiety in situation of short-term microgravity (EVA-0G): Sensitivity of cognitive parameters

Miss Cécile Guillot[1], *Doctor Jean-Philippe Hainaut*[1], *Doctor Cécile Langlet*[1], *Professor Benoît Bolmont*[1]

[1]*University of Lorraine*

16:45

Effects of long-term immobilization on affective picture processing – an ERP study

Mrs Katharina Brauns[1], *Mrs Anika Werner*[1], *Prof. Hanns-Christian Gunga*[1], *Dr. Alexander Christoph Stahn*[1,2]

[1]*Charité Universitätsmedizin Berlin, Center for Space Medicine and Extreme Environments Berlin,* [2]*University of Pennsylvania, Perelman School of Medicine*

17:00

Locomotion on the Earth after long-duration space flights as step to locomotion on other celestial bodies

Dr. Nataliya Lysova[1], *Mr. Vladimir Kitov*[1], *Dr. Elena Fomina*[1]

[1]*RF SRC – Institute of Biomedical Problems, Russian Academy of Sciences*

17:15

Changes in the characteristics of voluntary movements after long term space flights

Dr. Nikolay Osetskiy[1], *Mr. Vladimir Kitov*[1], *Dr. Inna Sosnina*[1], *Dr. Natalia Lysova*[1], *Mrs. Lyubov Amirova*[1], *Dr. Marissa Rosenberg*[2], *PhD Igor Koffman*[2], *Prof. Millard Reschke*[2], *Dr. Ilya Rukavishnikov*[1], *PhD Elena Tomilovskaya*[1], *Pfor. Inesa Kozlovskaya*[1]

[1]*RF SSC Institute of Biomedical Problems RAS,* [2]*NASA Johnson Space Center*

17:30

Acute weightlessness impairs spatial updating performance

Dr. Alexander Stahn[1,2], *Anika Werner*[1], *Katharina Brauns*[1], *Dr Stephane Besnard*[3], *Dr Pierre Denise*[3], *Dorothee Grevers*[1], *Dr Thomas Wolbers*[4], *Dr Martin Riemer*[4], *Dr Simone Kuehn*[5], *Dr Hanns-Christian Gunga*[1]

[1]*Charité Universitätsmedizin Berlin,* [2]*University of Pennsylvania, Perelman School of Medicine,* [3]*Normandie Université, UMR INSERM U 1075*

COMETE, [4]German Center for Neurodegenerative Diseases - Site Magdeburg, [5]University Medical Center Hamburg-Eppendorf, Dept. of Psychiatry and Psychotherapy

17:45 Sympathetic activity during acute simulated microgravity

Mr Marc Kermorgant[1], Dr Marc Labrunée[1,2], Dr Thomas Geeraerts[3,4], Dr Eric Schmidt[5], Dr Nathalie Nasr[1,6], Dr Alexandra Weckel[7], Dr Alexander Choukèr[8], Dr Jean-Michel Senard[1,9], ***Dr Anne Pavy-Le Traon[1,6]***

[1]UMR INSERM 1048, Institute of Cardiovascular and Metabolic Diseases (I2MC), [2]Department of Rehabilitation, University Hospital of Toulouse, [3]Department of Anesthesiology and Intensive Care, University Hospital of Toulouse, [4]Toulouse NeuroImaging Center – ToNIC, UMR 1214, Inserm / Université Toulouse III – Paul Sabatier, [5]Department of Neurosurgery and Institute for Neurosciences, University Hospital of Toulouse, [6]Department of Neurology and Institute for Neurosciences, University Hospital of Toulouse, [7]Department of Otorhinolaryngology and Otoneurology, University Hospital of Toulouse, [8]Department of Anaesthesiology, "Stress and Immunity" Laboratory, University Hospital of Munich, [9]Department of Clinical Pharmacology, University Hospital of Toulouse

18:00 End of Day 3

19:30 Symposium dinner

Tulum Tulum
Zeereep 104
2202 NW Noordwijk

Thursday 21 June 2018

Plenary session: Effect of gravity and spaceflight on fluid shifts and neuro-ocular impairment

Chairs Inessa Kozlovskaya / Alan Hargens
Room: High Bay

09:00 Introduction to spaceflight and fluid shifts

Inessa Kozlovskaya, IMBP, Moscow, Russia

09:15 Spaceflight associated neuro-ocular syndrome during exploration missions

Brandon Macias, Medical Univ. South Carolina, Charleston, SC USA

09:45 **Intracranial adaptation to spaceflight: Results from the retrospective review of brain MRI scans of ISS and shuttle astronauts**

Donna Roberts, KBRwyle, Houston, USA

10:15 **Mechanisms of endothelium effects on murine arteries during spaceflight**

Olga Vinogradova, IMBP, Moscow, Russia

10:45 **Coffee break**

11:15 **Intracranial hemodynamics in space and on earth**

Mark Wilson, Imperial College London, UK

11:45 **Artificial gravity to reverse headward fluid shifts**

Lonnie Petersen, Univ. California, San Diego, USA

12:15 **Summary of spaceflight and fluid shifts**

Alan Hargens, Univ. California, San Diego, USA

12:30 **Lunch**

Session 16: Cardiovascular-1

Chairs: Liudmila Gerasimova-Meigal / Richard Hughson

Room: High Bay

14:00 **Preliminary results for jugular vein volume and middle cerebral vein velocity increase during 6 month ISS spaceflight**

Prof. Philippe Arbeille[1], *Dr Kathryn Zuj*[1], *Dr Brandon Macias*[2], *Dr Doug Ebert*[2], *Dr Steven Laurie*[2], *Pr Scott Dulchavsky*[3], *Dr Mike Stenger*[4], *Dr Alan Hargens*[5]

[1]*UMPS-CERCOM University Hospital Tours,* [2]*KBRwyle,* [3]*Henry Ford nnovation Institut and Hospital ,* [4]*Cardiovascular & Vision Lab,NASA Johnson Space Center,* [5]*Dept of Orthopaedic Surgery, UCSD Medical Center, La Jolla*

14:15 **The mechanisms of endothelium influences in murine arteries differently affected in spaceflight**

Dr. Olga Vinogradova[1,2], *Dr. Dina Gaynullina*[1,2], *Ms Oksana Kiryukhina*[1], *Dr Olga Tarasova*[1,2]

[1]SRC RF Institute of Biomedical Problems RAS, [2]M.V. Lomonosov Moscow State University

14:30

A comparison of squatting exercise on a centrifuge and with terrestrial attraction

Dr. Jochen Zange[1], *Timothy Piotrowski*[1], *Prof. Dr. Jörn Rittweger*[1]

[1]DLR, Deutsches Zentrum für Luft- und Raumfahrt

14:45

High-intensity training as cardiovascular countermeasure for 60-day bed rest

Dr. Martina Anna Maggioni[1,2], *Dr Paolo Castiglioni*[3], *Prof. Giampiero Merati*[2], *Mr Stefan Mendt*[1], *Ms Katharina Brauns*[1], *Ms Anika Werner*[1], *Prof Hanns-Christian Gunga*[1], *Dr Alexander Stahn*[1,4]

[1]Charité Universitätsmedzin Berlin, Center for Space Medicine and Extreme Environments Berlin, [2]Università degli Studi di Milano, Department of Biomedical Sciences for Health, [3]IRCCS Don Gnocchi Foundation, [4]University of Pennsylvania, Perelman School of Medicine

15:00

Evaluation of combined effects of lunar gravity simulation and the altered magnetic field on cardiovascular system of healthy volunteers

Dr. Yury Gurfinkel[1], *Dr. Mikhail Baranov*[2], *Dr. Roman Pishchalnikov*[3]

[1]Lomonosov Moscow State University, Laboratory of blood microcirculation head; [2]Research Institute for Space Medicine, Federal Biomedical Agency of Russia, [3]Prokhorov General Physics Institute of the Russian Academy of Sciences (GPI RAS)

15:15

Differences between left and right ventricular cardiac output during (simulated) hyper-to micro-gravity transitions

Mr Lutz Thieschäfer[1], *Dr. Jessica Koschate*[1], *Dr. Uwe Drescher*[1], *Dr. Andreas Werner*[2,3], *Dr. Daniel Dumitrescu*[4], *Dr. Uwe Hoffmann*[1]

[1]Institute of Physiology and Anatomy, German Sport University Cologne, [2]German Air Force - Center of Aerospace Medicine, Aviation Physiology Training Center, Aviation Physiology Diagnostics and Research, [3]Center for Space Medicine and Extreme Environments, Institute of Physiology, [4]Cologne Heart Center, Division of Cardiology

Session 17: Sleep / neuroscience

Chairs: Donna Roberts / Charles Fuller

Room: Auditorium

14:00

How sleep restriction and fragmentation affect the autonomic nervous system – An intervention study

Miss Naima Laharnar[1], *Miss Maria Zemann*[1], *Miss Joanna Fatek*[1], *Dr. Alexander Suvorov*[2], *Mr. Mark Belakovskiy*[2], *Prof. Oleg Orlov*[2], *Dr. Martin Glos*[1], *Prof. Thomas Penzel*[1], *Prof. Ingo Fietze*[1]

[1]*Charité-Universitätsmedizin Berlin,* [2]*Russian Academy of Science - Institute of Biomedical Problems*

14:15

Isolation, sleep, cognition and neurophysiological responses – An investigation in the Human Exploration Research Analog (HERA)

Mr Timo Klein[1,2], *Ms Andrea Rossiter*[3], *Mr Jan Weber*[1], *Dr Tina Foitschik*[1], *Dr Brian Crucian*[4], *Dr Stefan Schneider*[1,2], *Dr Vera Abeln*[1]

[1]*German Sport University Cologne,* [2]*University of the Sunshine Coast,* [3]*King´s College London,* [4]*NASA Johnson Space Center*

14:30

Alterations in resting state electrocortical activity after 60 days of bed rest

Mrs Katharina Brauns[1], *Mrs Anika Werner*[1], *Prof. Hanns-Christian Gunga*[1], *Dr. Alexander Christoph Stahn*[1,2]

[1]*Charité Universitätsmedizin Berlin, Center for Space Medicine and Extreme Environments Berlin,* [2]*University of Pennsylvania, Perelman School of Medicine*

14:45

What space tells us about sleep

Dr. Alain Gonfalone[1]

[1]*European Space Agency*

15:00

Modelling brain injuries under altered gravity conditions: Understanding brain plasticity

Dr. Ilaria Cinelli[1]

[1]*NUIG*

15:15

Biological rhythms and decision-making performance of high arctic residents during summer and winter

Dott. Pierpaolo Zivi[1], *Prof. Vittorio Pasquali*[1], *Prof. Stefano Sdoia*[1], *Prof. Fabio Ferlazzo*[1]

[1]Department of Psychology - Sapienza University of Rome

15:30 Coffee break & poster session 4

Session 18: Cardiovascular-2

Chairs: Marc-Antoine Custaud / Nandu Goswami

Room: High Bay

16:30 Influence of otolithic afferents on the cardiovascular system during a 3-days dry immersion

Phd Steven De Abreu[1], *Dr Shigehiko Ogoh*[3], *Pr Pierre Denise*[1,2], *Pr Hervé Normand*[1,2]

[1]University of CAEN, [2]CHU de CAEN, [3]University of TOYO

16:45 Blood pressure and heart rate variability in Parkinson's disease patients under "dry immersion"

Prof. Liudmila Gerasimova-Meigal[1], *Prof. Alexander Meigal*[1]

[1]Petrozavodsk State University

17:00 Central blood pressure and pulse wave velocity before and after six months in space

Mr Fabian Hoffmann[1], ***Mr Stefan Möstl***[1], *Dr Elena Luchitskaya*[2], *Mrs Irinia Funtova*[2], *Dr Roman Baevsky*[2], *Prof Jens Tank*[1]

[1]DLR (German Aerospace Center), [2]Institute for Biomedical Problems

17:15 24-hr ambulatory bp and cerebral hemodynamics in crewmembers: Arterial stiffness and cerebrovascular pulsatility

Prof. Richard Hughson[1], *Dr. Katelyn Wood*[1], *Ms. Danielle Greaves*[1], *Prof. Philippe Arbeille*[2]

[1]Schlegel-UWaterloo Research Institute For Aging, [2]University of Tours

17:30 Altered venous function during long-duration spaceflights

Dr Jeanne Hersant[1], *Dr Kathryn Zuj*[2], *Dr Ana De Holanda*[1], *Dr Guillemette Gauquelin Koch*[3], *Pr Claude Gharib*[4], *Dr. Jacques-olivier Fortrat*[1]

[1]Faculté De Médecine CNRS 6214 Inserm 1083, [2]University of Waterloo, [3]Centre National d'Etudes Spatiales, [4]Faculté de Médecine Lyon Est

17:45 **Remote echography onboard the ISS fully controlled from the ground CNES Space center – application in isolated medical centre on earth (200 patients)**

Prof. Philippe Arbeille[1], *PHD Didier Chaput[2], PHD Arielle Depriester[3], PHD Olivier Belbis[2], PHD Alain Maillet[3], PHD Patrice Benarroche[2], PHD Sebastien Barde[2]*

[1]UMPS-CERCOM University Hospital Tours, [2]CADMOS - CNES , [3]MEDES

18:00 **End of Day 4**

Session 19: Space analogues / microgravity model / medication

Chairs: Erik Antonsen / Arnaud Runge

Room: Auditorium

16:30 **Cardiac autonomic modulation during 14-month Overwintering at the Antarctic station neumayer III**

Dr. Martina Anna Maggioni[1,2], *Dr. Paolo Castiglioni[3], Prof. Giampiero Merati[2,3], Mr Stefan Mendt[1], Prof. Hanns-Christian Gunga[1], Ms. Katharina Brauns[1], Ms. Anika Werner[1], Dr. Alexander Stahn[1,4]*

[1]Charité Universitätsmedzin Berlin, Center for Space Medicine and Extreme Environments, [2]Università degli Studi di Milano, Department of Biomedical Sciences for Health, [3]IRCCS Don Gnocchi Foundation, [4]University of Pennsylvania, Perelman School of Medicine

16:45 **Astronaut training in weightlessness using virtual reality**

Dr. Amaury Solignac[1], *Dr. Vincent Rieuf[1], Mr. Jean-Francois Clervoy[2], Mr. Thierry Gharib[3]*

[1]I.C.E.B.E.R.G., [2]ESA, [3]Novespace

17:00 **Sex-specific brain adaptations during short-term isolation and confinement: Results from the NASA HERA C3 mission**

Dr. Alexander Stahn[1,2], *Anika Werner[1], Katharina Brauns[1], Dr David Dinges[2], Dr Mathias Basner[2], Dr Hanns-Christian Gunga[1], Dr Simone Kuehn[3]*

[1]Charité Universitätsmedizin Berlin, Center for Space Medicine and Extreme Environments Berlin, [2]University of Pennsylvania, Perelman School of Medicine, [3]University Medical Center Hamburg-Eppendorf, Dept. of Psychiatry and Psychotherapy

17:15

Role of axial and support unloading in development of hypogravitational motor syndrome

Dr. Elena Tomilovskaya[1], *Dr. Ilya Rukavishnikov*[1], *Dr. Tatiana Kukoba*[1], *Mrs. Tatiana Shigueva*[1], *Ms. Inna Sosnina*[1], *Mrs. Lyubov Amirova*[1], *Prof. Inessa Kozlovskaya*[1]

[1]RF SSC - Institute of Biomedical Problems RAS

17:30

Proteomic investigation of human skeletal muscle before and after 70 days of head down bed rest with or without exercise and testosterone countermeasures

Dr. Lichar Dillon[1], *Dr. Kizhake Soman*[1], *Dr. John Wiktorowicz*[1], *Ms. Ria Sur*[1], *Dr. Daniel Jupiter*[1], *Mr. Christopher Danesi*[1], *Mrs. Kathleen Randolph*[1], *Mr. Charles Gilkison*[1], *Dr. Larry Denner*[1], *Dr. William Durham*[1], *Dr. Randall Urban*[1], *Dr. Melinda Sheffield-Moore*[1]

[1]University of Texas Medical Branch

17:45

Dose tracker: An iOS app for collection of medication use data from volunteer crewmembers on the international space station

Dr. Virginia Wotring[1]

[1]Baylor College of Medicine

18:00

The Effects of long-duration space flight on seletal muscle: Electrically-evoked and voluntary properties of a slow muscle

Prof. Yuri Koryak[1], *Inessa Kozlovskaya, Steven Siconolfi, John Gilbert*

[1]State Scientific Center of the Russian Federation Institute of Biomedical Problems of the Russian Academy of Sciences, [2]2Space Biomedical Research Institute NASA-JSC, [3]RUG Life Sciencen

18:15

End of Day 4

Friday 22 June 2018

09:00 **Tour in haarlem**

16:30

Poster session - Monday 18 / Tuesday 19 June

1

Is artificial gravity able to protect the musculoskeletal system in a murine model of knee osteoarthritis?

Dr. Benoit Dechaumet[1], Dr. Damien Cleret[1], Mr Norbert Laroche[1], Mr Arnaud Vanden-Bossche[1], Pr. Marie- Hélène Lafage-Proust[1], ***Dr. Laurence Vico-pouget[1]***

[1]Inserm, U1059, University of Lyon, University Jean Monnet

2

Nuclear–cytoplasmic traffic of class iia histone deacethylases in rat soleus muscle at the early stage of gravitational unloading

Mrs Natalia Vilchinskaya[1]*, Mr Boris Shenkman[1]*

[1]SSC RF-Institute of Biomedical Problems, RAS

3

Differentiation of mesenchymal stem cells into osteoblasts under simulated microgravity

Dr. Gabriela Chiritoiu[1], Stefana Iosub[2], Alexandru Nistorescu[3], Adrian Dinculescu[3], Dr. Florin Jipa[2], ***Dr. Cristian Vizitiu[3]****, Dr. Felix Sima[2], Dr. Stefana Petrescu[1]*

[1]Institute of Biochemistry, Romanian Academy, Splaiul Independentei 296, Bucharest, Romania, [2]Center for Advanced Laser Technologies (CETAL), National Institute for Laser, Plasma and Radiation Physics (INFLPR), Atomistilor 409, 0077125 Magurele, Romania, [3]Institute of Space Science (ISS), 409 Atomistilor Street, Magurele, Romania

4

Bone remodelling study using strontium enriched hydroxyapatite nanoparticles

Prof. Angela Maria Rizzo[1]*, Dr. Getano Campi[2], Dr. Francesco Cristofaro[3], Dr. Giuseppe Pani[1], Dr. PaolaAntonia Corsetto[1], Dr. Barbara Pascucci[2], Prof. Livia Visai[3]*

[1]Department of Pharmacological and Biomolecular Sciences, Università Degli Studi di Milano, [2]Institute of Crystallography, CNR, [3]Department of Molecular Medicine, University of Pavia

5

Nuclear accumulation of HSP70 protein in mouse skeletal muscles in response to reloading following unloading

Prof. Katsumasa Goto[1], *Mr. Antonios Apostolopoulus*[2], *Mrs. Ayane Nakamura*[1], *Prof. Yoshinobu Ohira*[3]

[1]*Graduate School of Health Sciences, Toyohashi SOZO University,*
[2]*Graduate School of Space Physiology and Health, King's College London,*
[3]*Research Center for Space and Medical Sceinces, Doshisha University*

6

Analysis and characterization of bone tissue using modeled microgravity analogues as tissue engineering models

Dr. Vivek Mann[1], *Dr. Alamelu Sundaresan*[1], *Dr. Daniela Grimm*[2], *Dr. Thomas Corydon*[2], *Dr. Stefan Riwaldt*[2], *Dr. Sascha Kopp*[3], *Mr. Elvis Okoro*[1], *Dr. Janne Reseland*[4]

[1]*Texas Southern University,* [2]*Aarhus University,* [3]*Otto-Von - Guericke Universit,* [4]*University of Oslo*

7

Effect of clomipramine on lipid raft disorders in soleus muscle of rats exposed to short-term hindlimb unloading

Prof. Irina Bryndina[1], *Prof. Alexey Petrov*[2], *Prof. Andrey Zefirov*[2], *Dr Maria Shalagina*[1], *Mr Alexey Sekunov*[1], *Mr Vladimir Protopopov*[1]

[1]*Izhevsk State Medical Academy,* [2]*Kazan State Medial University*

8

The effect of 30 day hindlimb unloading and overload on murine bone marrow stromal progenitors

Mrs Elena Markina[1], *Mrs Irina Andrianova*[1], *Mr Andrey Shtemberg*[1], *Mrs Ludmila Buravkova*[1]

[1]*State Scientific Center of The Russian Federation - Institute of Biomedical Problems (ibmp)*

9

The definition of the cellular and molecular mechanisms of plants gravisensitive

Dr. Olga Artemenko[1]

[1]*Institute of Botany Nasu*

10 **Clinorotation impacts the plasmalemma lipid bilayr and its functional domains–rafts in plant cells**

*Prof. Elizabeth Kordyum[1], **PhD Iliya Bulavin[1]**, Dr. Olena Nedukha[1], Tamara Vorob'eva[1]*

[1]Institute of Botany

11 **Investigation of plant tolerance to radioactive environment of space flights**

Dr. Galina Shevchenko[1]

[1]Institute of Botany, NAS Ukraine

12 **Response of calcium ions to hypergravity**

Dr. Olena Nedukha[1]

[1]Institute of Botany

13 **HSP90s and HSP70s stabilize root gravitropic response in Arabidopsis**

Dr. Liudmyla Kozeko[1]

[1]Institute of Botany, NAS of Ukraine

14 **Clinorotation impact on respiration and photosynthesis of pea plants under the low light conditions**

Dr. Vasyl Brykov[1]

[1]M.G. Kholodny Institute of Botany, NAS of Ukraine

15 **"Rhizogenesis in vitro" from leaf explants as a model for studying root cell differentiation under real and simulated microgravity**

Dr. Iliya Bulavin[1]

[1]Institute of Botany

16 **Adaptive responses in mammals to alterad gravitational environments**

*Dr. Arianna Racca[1], Dr. Nadia Francia[1], Dr. Sara Tavella[2], **Dr. Daniela Santucci[1]***

[1]Center for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, [2]Department of Oncology, Biology and Genetics, University of Genova

17

HypoCampus – effects of long-duration spaceflight on spatial cognition and its neural basis

Dr. Alexander Stahn[1,2], *Katharina Brauns*[1], *Anika Werner*[1], *Dr Stephane Besnard*[3], *Dr Pierre Denise*[3], *Dr Tom Hartley*[4], *Dr Bernhard E. Riecke*[5], *Dr Thomas Wolbers*[6], *Dr Mathias Basner*[2], *Dr David Dinges*[2], *Dr Hanns-Christian Gunga*[1], *Dr Simone Kuehn*[7]

[1]Charité Universitätsmedizin Berlin, Center for Space Medicine and Extreme Environments Berlin, [2]University of Pennsylvania, Perelman School of Medicine, [3]Normandie Université, UMR INSERM U 1075 COMETE, [4]University of York, Dept. of Psychology, [5]Simon Fraser University, School of Interactive Arts and Technology, [6]German Center for Neurodegenerative Diseases - Site Magdeburg, [7]University Medical Center Hamburg-Eppendorf, Dept of Psychiatry and Psychotherapy

18

Plantar mechanical stimulation prevents neurochemical alterations in the hippocampus induced by stimulated microgravity

Ms. Anna Berezovskaya[1], *Mr. Sergey Tyganov*[2], *Dr. Boris Shenkman*[2], *Dr. Margarita Glazova*[1]

[1]Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, [2]Institute of Biomedical Problem Russian Academy of Sciences

19

Effect of endurance exercise training on neurogenesis of adipose-derived stem cells, isolated from fat-depot in Wistar rats

Dr. Hisashi Kato[1,2], *Dr. Yoshinobu Ohira*[1,2], *Dr. Tetsuya Izawa*[1,2]

[1]Graduate School of Health and Sports Science, Doshisha University, [2]Research Center for Space and Medical Sciences, Doshisha University

20

Cardiovascular response to different doses of applied external pressure

Miss Elizabeth Bird[1], *Mr Alan Hargens*[1], *Mrs Lonnie Petersen*[1]

[1]University of California, San Diego

21

Dynamic foot stimulation prevents calcineurin/NFATc1 inactivation and slow-to-fast shift in rat soleus muscle under unloading

Mrs Christina Sharlo[1], *Mr Sergei Tyganov*[1], *Ms Inna Paramonova*[1], *Ms Olga Turtikova*[1], *Mr Boris Shenkman*[1]

[1]The Russian Federation State Research Center – Institute of Biomedical Problems of The Russian Academy of Sciences

22 **The influence of body size and exercise countermeasures on resources required for human exploration missions**

Dr Jonathan Scott[1,2], *Dr David Green*[1,2,3], *Dr Guillaume Weerts*[2]

[1]Wylelabs GmbH, [2]Space Medicine Office, European Astronaut Centre, European Space Agency, [3]Centre of Human and Aerospace Physiological Sciences, King's College London

23 **Zinc oxide and graphene as sensing platforms towards enabling skin wound healing assessment in space**

Dr. Gemma Rius[1], *Dr Elisabet Prats-Alfonso*[2], *Dr Marta Duch*[1], *Dr Jaume Esteve*[1], *Prof. Philippe Godignon*[1]

[1]Institut de Microelectronica de Barcelona IMB-CNM-CSIC, [2]Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)

24 **Influence of the transformed environment on the yield of soybean plants and resistance to soybean mosaic virus**

Prof. Lidiya Mishchenko[1], *Associate Prof. Ivan Mishchenko*[2], *Scientific Researcher Alina Dunich*[1]

[1]Taras Shevchenko National University of Kyiv, [2]National University of Life and Environmental Sciences of Ukraine

25 **Eight days of Earth reambulation worsen bone loss induced by 1-month spaceflight in the major weight-bearing ankle bones of mature mice**

Dr. Laurence Vico-pouget[1]

[1]Inserm U1059 University of Lyon, University Jean Monnet

Poster Session - Wednesday 20 / Thursday 21 June

26 **EEG response of volunteers of different sexes who are in a rotating short-range centrifuge**

Miss Daria Schastlivtseva[1], PhD Tatiana Kotrovskaya[1], PhD Milena Koloteva[1], Prof. Yuriy Bubeev[1]

[1]SSC RF - Institute of Biomedical Problems of RAS

27

VaPER-Study: Strict adherence of 6°-Head Down Tilt Bed Rest – An Improvement to the Ground-Based Microgravity Analogue?

Dr. Melanie von der Wiesche[1], **Alexandra Noppe**[1], Freia Paulke[1], Dr. Edwin Mulder[1]

[1]DLR - German Aerospace Center, Institute of Aerospace Medicine

28

Individualized dose of exercise for counteracting astronaut post-flight orthostatic intolerance

Prof Ferdinando Iellamo[1,2], **Dr Giuseppe Caminiti**[1], Dr Vincenzo Manzi[1], Miss Serena Selli[1], Dr Maurizio Volterrani[1]

[1]S.raffaele Irccs, [2]University of Tor Vergata

29

Proteomics of microparticles derived from Endothelial cells after modelled Microgravity conditions

Mrs Daria Kashirina[1], Mr Andrey Ratushny[1], Mrs Olga Zhidkova[1], Dr Alexey Kononikhin[1,2], Prof Irina Larina[1], Prof Lyudmila Buravkova[1]

[1]Institute For Biomedical Problems – Russian Federation State Scientific Research Center Ras, [2]Institute of Energetic Problems of Chemical Physics RAS

30

The dynamic adaptive response of endothelial cells to simulated microgravity

Dr. Alessandra Cazzaniga[1], Laura Laura Locatelli[1], Dr Roberta Scrimieri[1], Dr Sara Castiglioni[1]

[1]University of Milan

31

Vascular echo: Faster central and peripheral pulse wave velocity in astronauts while on international space station

Mrs Danielle Greaves[1], Dr. Richard Hughson[1], Dr. Philippe Arbeille[2]

[1]University of Waterloo, [2]University of Tours Francois Rabelais

32

Changes observed on the inotropic state of the heart between first and second month in microgravity, assessed by CARDIOVECTOR-1

Mr Jeremy Rabineau[1]*, Dr Irina Funtova*[2]*, Dr Elena Luchitskaya*[2]*, Pr Philippe van de Borne*[1]*, Pr Jens Tank*[3]*, Dr Pierre-François Migeotte*[1]

[1]*Université Libre De Bruxelles,* [2]*Institute of Biomedical Problems,* [3]*Deutsches Zentrum für Luft- und Raumfahrt*

33 The effect of microgravity on central aortic blood pressure

Dr. Felix S. Seibert[1]*, Fabian Bernhard*[1]*, Dr. Ulrik Stervbo*[1]*, Sinthuya Vairavanathan*[1]*, Dr. Frederic Bauer*[1]*, Dr. Benjamin Rohn*[1]*, Dr. Nikolaos Pagonas*[1]*, Prof. Nina Babel*[1]*, Prof. Joachim Jankowski*[2]*, Prof. Timm H. Westhoff*[1]

[1]*Ruhr University Bochum; University Hospital Marien Hospital Herne,* [2]*Institute for Molecular Cardiovascular Research; RWTH Aachen University*

34 Cardiac mechanical function measurement by kino-cardiography: Effect of acute postural changes vs RSL 60 days head-down tilt test

PhD Applicant Amin Hossein[1]*, MSci Farhana Pinky*[1]*, PhD Applicant Damien Gorlier*[1]*, PhD Applicant Jérémy Rabineau*[1]*, Prof Philippe Van De Borne*[3]*, Prof Antoine Nonclercq*[2]*, PhD Pierre-François Migeotte*[1]

[1]*Université Libre de Bruxelles, LPHYS,* [2]*Université Libre de Bruxelles, BEAMS,* [3]*Université Libre de Bruxelles, Cardiology Dept.*

35 Cardiac deconditioning after the 60-days ESA-RSL head-down bed-rest: wearable monitoring of heart kinetic energy and machine learning

Mr Damien Gorlier[1]*, Ms Federica Landreani*[2]*, Prof Philippe van de Borne*[1]*, Mrs Irina Funtova*[3]*, Prof Jens Tank*[4]*, Dr Enrico Caiani*[2]*, Dr Pierre-François Migeotte*[1]

[1]*Université Libre de Bruxelles,* [2]*Politecnico di Milano,* [3]*Institute for Biomedical Problems,* [4]*DLR Institute of Aerospace Medicine*

36 Gravitational stress during parabolic flights induced changes in human leukocyte subsets

Dr. Ulrik Stervbo[1]*, Dr. Toralf Roch*[2]*, Dr. Tina Kornprobst*[2]*, Dr. Gerald Grütz*[2]*, Prof. Birgit Sawitzky*[2]*, PhD Andreas Wilhelm*[2]*, PhD Francis Lacombe*[3]*, Kaoutar Allou*[3]*, Dr. Markus Kaymer*[4]*, Prof. Timm Westhoff*[1]*,* ***Dr. Felix S. Seibert***[1]*, Prof. Nina Babel*[1]

[1]*Ruhr University Bochum; University Hospital Marien Hospital Herne,*

[2]Berlin-Brandenburg Center for Regenerative Therapies; Charité-Universitätsmedizin Berlin, [3]Laboratoire d'hématologie, CHU de Bordeaux, Hôpital Haut-Lévêque, [4]Beckman Coulter GmbH

37

Influence of short-term suborbital flight factors on human lymphocytes functional activity

***Dr. Irina Alchinova**[1], Mrs. Margarita Polyakova[1], Prof. Mikhail Karganov[1], Dr. Mikhail Baranov[1], Mr. Nikolay Mullin[2], Mr. Sergey Kalinkin[3], Mr. Kirill Morozov[3], Mr. Nikolay Balugin[4],*
Dr. Vladimir Yushkov[4]
[1]Research Institute For Space Medicine, Federal Biomedical Agency of Russia, [2]Skolkovo Institute of Science and Technology , [3]Moscow Polytechnic University, [4]Central aerological observatory

38

Altered anandamide metabolism in microgravity: The "RESLEM" experiment

***Prof. Mauro Maccarrone**[1], Dr. Monia Di Tommaso[2], Mr. Gianluca Neri[3], Mr. Alessandro Donati[3], Dr. Natalia Battista[2], Dr. Monica Bari[4]*
[1]Department of Medicine, Campus Bio-Medico University of Rome, [2]Faculty of Biosciences and Technology for Food, Agriculture and Environment, University of Teramo, [3]Kayser Italia S.r.l., [4]Department of Experimental Medicine and Surgery, Tor Vergata University of Rome

39

Radiation-induced DNA damage in plasmid DNA model

***Dr. Katerina Pachnerova Brabcova**[1], Prof. Lembit Sihver[2], Dr. Egor Ukraintsev[3], Dr. Marie Davidkova[1], Dr. Christian J. Schwarz[4]*
[1]Nuclear Physics Institute, Czech Academy of Sciences, [2]Atominstitut, Technische Universität Wien, [3]Institute of Physics, Czech Academy of Sciences, [4]ESTEC ESA

40

Effect of microgravity on characteristics of the accuracy control of movements

***Tatiana Shigueva**[1], Vladimir Kitov[1], Nicolay Osetskiy[1], Liubov Amirova[1], Dr Elena Tomilovskaya[1], Prof. Inesa Kozlovskaya[1]*
[1]State Scientific Center of the Russian Federation – Institute of Biomedical Problems of the Russian Academy of Sciences

41

The european active dosemeter on ISS

*Matthias Dieckmann, **Dr. Ulrich Straube**[2], Dr. Thomas Berger[3],*
Dr. Matthias Dieckmann[1]
[1]ESA, [2]EAC, [3]DLR

42 **Effect of imitated microgravity on plasma membrane epicotyls and roots in plant**

***Dr. Olena Nedukha**[1], Prof Elizabeth Kordyum, assist Tamara Vorob'eva,*
Dr Vladimir Grakhov
[1]Institute of Botany

43 **The somatogravic illusion during centrifugation: Sex differences**

***Prof. Rainer Herpers**[1,2,3], Prof. Laurence R. Harris[2], Ms Meaghan McManus[2],*
Mr. Thomas Hofhammer[1], Ms Alexandra Noppe[4], Mr Timo Frett[4],
Prof. Michael Jenkin[2]
[1]Bonn-Rhein-Sieg University of Applied Sciences, [2]York University,
[3]University of New Brunswick, [4]German Aerospace Centre (DLR), Institute of Aerospace Medicine

44 **The probable neural mechanisms of thick-toed geckos' attachment to the surfaces in weightlessness**

*Dr. Valeriy Barabanov[1], **Dr. Victoria Gulimova**[1], Dr. Alexandra Proschina[1],*
Dr. Anastasia Kharlamova[1], Dr. Rustam Berdiev[2], Prof. Sergey Saveliev[1]
[1]Research Institute of Human Morphology, [2]Research and educational center for wild animal rehabilitation, Faculty of Biology, M.V. Lomonosov Moscow State University

45 **Unraveling non-cancer effects of spaceflight: How are the brain and skin affected in astronauts?**

***Miss Greta Lamers**[1,2], Dr. Marjan Moreels[1], Dr. Mieke Verslegers[1],*
Prof. Dr. Sarah Baatout[1,2]
[1]Radiobiology Unit, Interdisciplinary Biosciences Expert Group, SCK•CEN / Belgian Nuclear Research Centre, [2]UGent, Faculty of Bioscience Engineering

46 **Age Peculiarities in the disease structure of cosmonauts after finishing their flight activity**

***Mr Serguei Zakharov**[1], Mrs. Ekaterina Rudenko[1], Mrs. Oksana Novikova[1]*
[1]The Research Institute For Space Medicine Federal Research Clinical Center of Federal Biomedical Agency of Russia

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[1]*Kayser Italia Srl*

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[1]*Universitätsklinikum Magdeburg*, [2]*Aarhus University*

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[1]*Universitätsklinikum Magdeburg*, [2]*Max-Planck-Institut für Biochemistry*, [3]*Department of Biomedicine, Aarhus University*

Extended Abstracts of This Proceedings

Personalized exercise training program as a countermeasure to orthostatic intolerance after space flights

Ferdinando Iellamo[1,2]*, Maurizio Casasco[3], Chiara Fossati[4], Giuseppe Caminiti[2], Maurizio Volterrani[2]

[1]Dipartimento di Scienze Cliniche e Medicina Traslazionale, Università degli Studi di Roma Tor Vergata, Roma, Italy
[2]Dipartimento di Scienze Motorie e Internistiche, IRCCS San Raffaele Pisana, Roma, Italy
[3]Federazione Medico Sportiva Italiana (FMSI), Rome, Italy
[4]Dipartimento di Scienze della Salute, Foro Italico University of Rome, Rome, Italy
**iellamo@uniroma2.it*

INTRODUCTION

Approximately 83% of astronauts experience pre-syncope and even fainting during upright position on landing days after long-term space flights. The inability to adequately elevate peripheral vascular resistance (i.e. vasoconstriction) along with the hypovolemia occurring during flight are considered the main factors of post-flight orthostatic intolerance 2, 3, 10.

Among the countermeasures tested against orthostatic intolerance, in-flight physical exercise has been the most obvious, because of its predictable capability to positively affect exercise capacity, autonomic nervous system regulation, muscle strength, and power, all of which are impaired as a consequence of weightlessness. Accordingly, 2–3 hours per day are devoted by astronauts to physical activity during flights. Yet, despite widespread in-flight utilization, physical exercise has proven only partially effective in counteracting orthostatic intolerance after space flights.

It is arguable that most of the inconsistencies about the effectiveness of exercise reflect the poor knowledges that exist on the optimal dose of exercise (i.e. intensity and volume) to be performed to achieve a given physiological benefit, in this case orthostatic tolerance. To date, physical activity of astronauts during space flights has been mainly self-selected using conventional levels of dynamic exercise (according to general recommendations) in addition to some forms of resistance exercises.

Recently, a new training method, referred to as the "individualized TRaining IMPulses" (TRIMPi)8, which is an individually determined, integrated measure of responses to physical load, that permits to account, in a single term, for both intensity and volume effects of endurance exercise training, has been developed. By this method, the dose of exercise has been repeatedly reported to affect neural cardiovascular regulation in on-ground studies.5

We report the case of an astronaut who performed a TRIMPi-based training program on board of the ISS during the expeditions 52/53 of the NASA/ASI-sponsored "Missione Vita" in order to investigate the feasibility and effectiveness of this structured, individually tailored, exercise training program in preventing/improving space flight-induced orthostatic intolerance and its underlying neural mechanisms.

A 60 years old astronaut who took part to the NASA/ASI-sponsored Missione Vita for 139 days performed a TRIMPi-based training program during the last two months of permanence on the ISS (according to the time-schedule allotted by NASA to this experiment).

Spectral analysis of heart rate (HR), blood pressure (BP) variability and baroreflex sensitivity (BRS, by mean of the sequences technique) were used as consolidated methodologies to assess the neural control of the cardiovascular system 9 during orthostatic stress before and after flight.

The astronaut performed an active orthostatic test (10 min supine rest followed by 20 min of unaided standing-up with continuous one-lead ECG and continuous non-invasive BP recording (by Finometer device) before flight and 4 days after landing (time-constraints by NASA). The astronaut underwent a pre-flight progressive exercise test on a treadmill with continuous ECG monitoring and intermittent capillary sampling for blood lactate determination in order to establish the personalized exercise program by TRIMPi.8

The TRIMPi-based exercise training program was performed on alternate days. Each 30-min exercise session consisting in treadmill running, at pre-flight determined training loads as calculated by the TRIMPi, was performed during astronaut's usual scheduled physical activity time so as to not disturb his daily routines. The astronaut's HR was monitored by a cardiotachograph during the whole exercise training sessions and downloaded periodically to the Earth.

N adverse effects were reported during exercise training.

In comparison to pre-flight, the orthostatic tolerance test (OT) performed after flight showed a greater decrease in BP on going from supine to standing position (SAP: -30 vs – 10 mmHg; DAP – 10 vs -5 mmHg). This was accompanied by a greater increase in HR (+17 vs +8 b/min). No change in BRS was observed between pre-flight and post-flight OT.

After-flight, OT showed a greater increase in the Low-Frequency (LF) component of HRV (indicator of mainly sympathetic modulation) and a greater decrease in the High-Frequency (HF) component of HRV (indicator of vagal modulation) (Fig. 1) with a shift in the sympatho-vagal balance toward a greater cardiac sympathetic activation (as expressed by the LF/HF ratio, Fig.2, left panel).9 The LF component of systolic BP variability (reflecting the

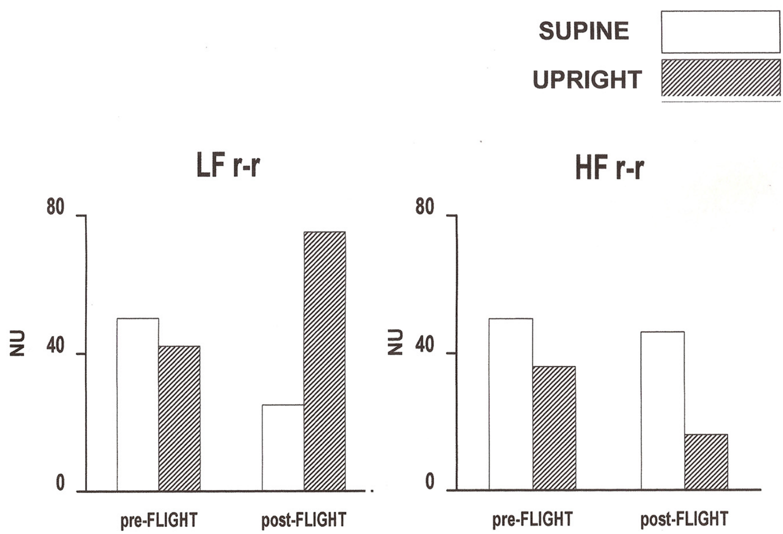

FIGURE 1: Low-frequency and High Frequency components of Heart Rate Variability recorded during pre-flight and post-flight orthostatic tolerance test. LF r-r=Low-frequency, HF r-r=High-Frequency, nu=normalized units.

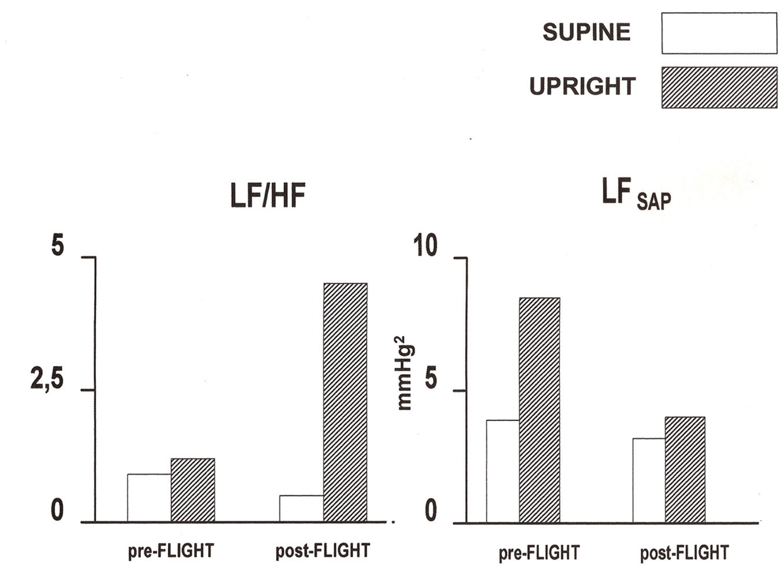

FIGURE 2: Cardiac LF/HF ratio and low-frequency component of systolic blood pressure variability recorded during pre-flight and post-flight orthostatic tolerance test. LF-SAP= Low-frequency component of systolic blood pressure variability.

sympathetic activation at peripheral vascular level) in response to changing posture from supine to upright was less after-flight than pre-flight (Fig. 2, right panel), paralleling the greater decrease in BP.

These findings suggest that prolonged exposure to actual microgravity induces an impairment of neural sympathetic mechanisms controlling peripheral vasoconstriction resulting in a greater decrease in blood pressure on the assumption of the upright posture. This greater post-flight decrease in blood pressure is opposed by an increase in sympathetic activation at cardiac level that induces a greater HR response, possibly preventing an excessive decrease in BP, and (likely) the appearance of symptoms of orthostatic intolerance. Exercise training by TRIMPi might be involved in inducing these autonomic responses.

DISCUSSION

This report is in agreement with several previous studies indicating the central role of sympathetic nervous system in affecting orthostatic tolerance. Blaber et al.1 used HRV to investigate the differences in autonomic regulation of the heart in a group of 29 astronauts who did (non-finishers) or did not (finishers) experience post-flight orthostatic intolerance. Finishers and non-finishers had an increase in sympathetic activity with stand on pre- flight, yet only finishers retained this response on landing. Non-finishers also had lower sympatho-vagal balance and higher pre-flight supine parasympathetic activity than finishers. These results suggest that post-flight impairment in autonomic control of the heart and vasculature may contribute to orthostatic intolerance. Moreover, it has been reported that during Lower Body Negative Pressure (LBNP), Muscle Sympathetic Nerve Activity (MSNA) was lower before symptoms of pre-syncope in orthostatic intolerant subjects, whereas the activation of MSNA was preserved in tolerant subjects after short-term bed rest.7 These results support the hypothesis of reduced peripheral sympathetic activity in subjects with orthostatic intolerance, as indirectly confirmed by our experiment.

The TRIMPi-based exercise training employed in this case during a long-lasting space flight might have acted as a physiological stimulus for increasing sympathetic cardiac activity on standing up after flight. Indeed, Iellamo et al.5 have reported that very intensive endurance training shifted the cardiovascular autonomic modulation from a parasympathetic toward a sympathetic predominance in elite athletes. The same group6 reported a curvilinear dose-response relationship between individualized training load (by TRIMPi) and autonomic nervous system functioning parameters with an increase in the LF component of HR variability, at peak exercise training load in patients suffering from chronic heart failure, who shares several pathophysiological changes with humans exposed to prolonged weightlessness.

In keeping with this concept, a study performed by Iellamo et al.4 during the tragically ended STS 107 spacelab mission, suggested that dynamic exercise in microgravity environment might potentiate some sympathetic activity-enhancing mechanisms, such as the muscle metaboreflex.

Overall, the findings of the present report indicate the feasibility of an on ground-determined, individually tailored, training protocol as an exercise-based countermeasure to be employed during prolonged space manned missions to counteract post-flight orthostatic intolerance. Further researches on a larger number of individuals would be mandatory for a better understanding of the role of TRIMPi methodology in reducing orthostatic intolerance after space flights

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Keywords: microgravity, orthostatic intolerance, TRIMP method, autonomic control, heart rate variability, orthostatic intolerance test, TRIMPi method training, autonomic dysfunction, long-lasting flight

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Effectiveness of resistive exercise countermeasures in bed rest to maintain muscle strength and power – A Systematic Review

Leonie Fiebig[1]*, Andrew James Winnard[2], Mona Nasser[3], Bjoern Braunstein[4], Jonathan Scott[1], David Green[1], Tobias Weber[1]

[1]*European Astronaut Centre (EAC), Cologne, Germany*
[2]*Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom*
[3]*Peninsula Dental School, Plymouth University, Plymouth, United Kingdom*
[4]*Institut für Biomechanik und Orthopädie, Deutsche Sporthochschule Köln, Cologne, Germany*
**leonie.fiebig@hotmail.de*

BACKGROUND

The evolution of exercise hardware and prescriptions have allowed astronauts to be exposed for long durations to microgravity (μG) and to return to Earth's gravitational field in relatively good physical condition [1]. However, not all astronauts appear to benefit equally from the prescribed exercises with the majority still experiencing multi-systems space physiological deconditioning [1]. Furthermore, future deep space explorations beyond low earth orbit will impose additional constraints upon the ability to perform exercise counter-measures. For instance, the size of exploration vehicles and cis-lunar stations [2; 3] will prohibit the use of relatively heavy and large hardware currently used on the International Space Station (ISS) [1; 4].

Therefore, exercise effectiveness to counteract space deconditioning needs to be further improved to (a) minimize the anticipated risks that future missions beyond low earth orbit will constitute [5]; and to (b) assure that after prolonged exposure times to μG astronauts will be able to operate autonomously and safely in extra-terrestrial (hypo-) gravity environments [6].

Data from exercise countermeasure studies that have been conducted in space, or in terrestrial analogues constitute a valuable knowledge base that should be taken into account for the development of novel exercise regimen and exercise hardware. To this date, however, there has not been a systematic approach to synthesize the findings of these studies and the flow of information between physiological research and operational implementation is frequently sub-optimal. It is therefore the aim of this project to synthesize data from both space- and terrestrial analogue-studies to help inform the development of novel exercise concepts and hardware for exploration missions.

The broad scope of a series of systematic literature reviews seeks to evaluate the effect of active exercise countermeasures, passive countermeasures (e.g. centrifugation) and nutritional countermeasures on musculoskeletal, cardiovascular, and cardiopulmonary parameters in microgravity and its ground-based analogues. The data presented at this conference include initial results of this project, focusing on the effectiveness of various resistive exercise protocols to maintain muscle strength and power in bed rest.

MATERIAL AND METHODS

Guidelines of the Cochrane Collaboration [7] and tools provided by the Aerospace Medicine Systematic Review Group [8] were used to conduct this review.

Search strategy

PubMed, Web of Science, Embase, NASA's and ESA's life sciences archives (Life Science Data Archive, Technical Reports Server and Erasmus Experiment Archive), as well as the database of the German Aerospace Centre (elib) and the Institute of Electrical and Electronics Engineers (IEEE) were searched.

Criteria for included studies

Initially found studies were screened using the Cochrane PICO-model: Participants (Patients: P) had to be human adults (men or women). The intervention (I) had to be either a space mission, a bed rest study, or a dry immersion study that used an active, passive, or nutritional countermeasure. Control groups (Comparison: C) had to refrain from countermeasure use. Outcomes (O) had to be either a cardiopulmonary, a cardiovascular, or a musculoskeletal parameter. Only studies with a minimum duration of five days conducted in μG, bed rest, or dry immersion were included. Full texts were obtained of all studies that met the above criteria.

Calculation of effect sizes

Calculation of effect sizes was performed to compare mean effects between control and intervention groups. The effect sizes were bias corrected using weighted (accounting for n = sample size) pooled standard deviations as per Hedge's g method [9]. Thresholds of <-0.2, -0.2, 0.2, 0.6, 1.2, and >1.2 were defined as negative, trivial, small, moderate, large, and very large effects between control and intervention groups [10]. To compare pre- and post-differences for each group, mean differences (post mean value – pre mean value) were calculated.

RESULTS

The initial search resulted in 4031 studies, with 10 included in the present analysis (Figure 1). The final 10 included studies were either randomized controlled,

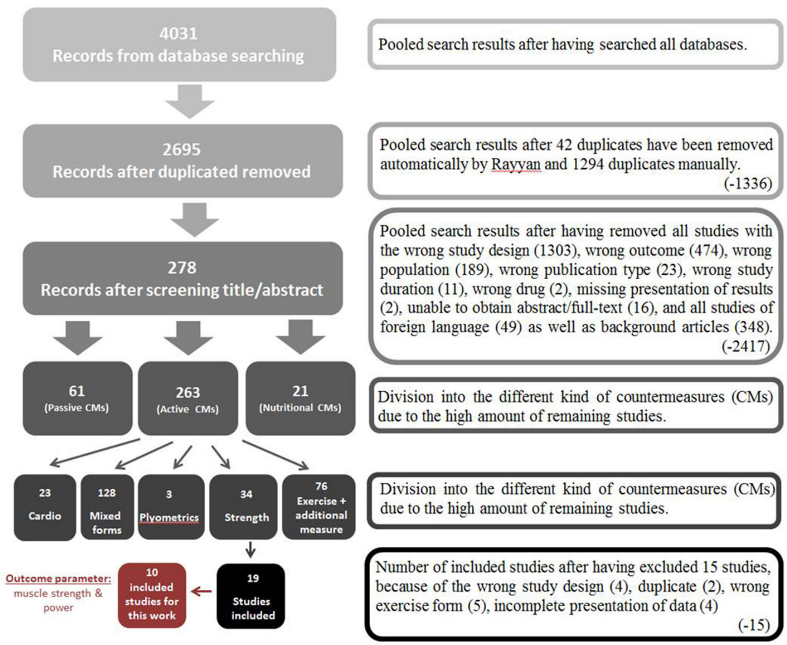

FIGURE 1: Literature search and screening procedure. Literature screening was performed using the Rayyan web application [11]. CM =countermeasure.

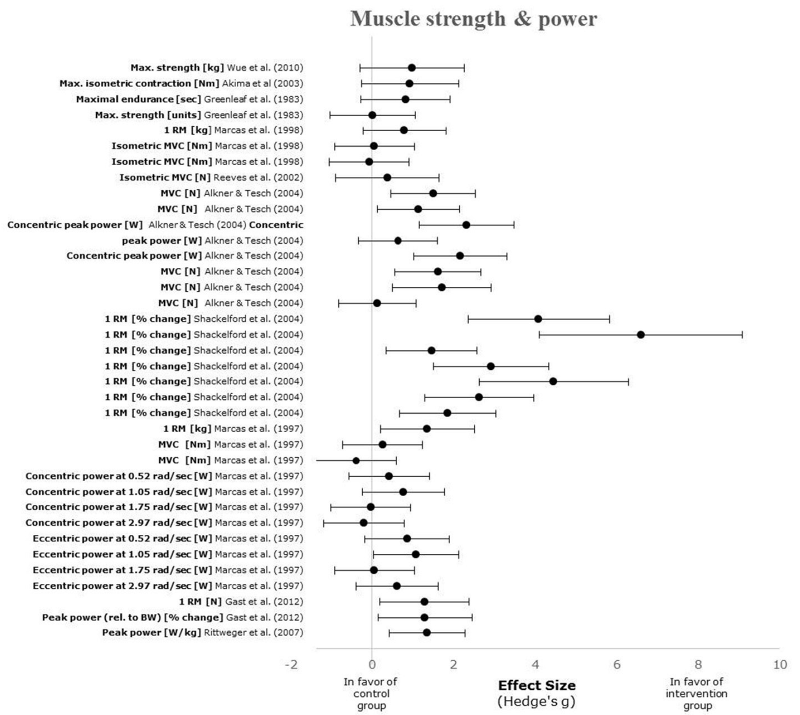

FIGURE 2: Effect sizes with confidence intervals (set at 95%) of parameters for muscle strength and power.

or controlled studies. The effect sizes are presented in Figure 2. Seven of the parameters analyzed parameters show a very large effect, nine a large effect, 10 a moderate effect, three a small effect, seven a trivial effect, and one a negative effect. A summary of the main study characteristics is shown in Figure 3. In the intervention groups, 19 of the 37 outcome parameters decreased after bed rest, whereas in the control group all outcome parameters decreased.

DISCUSSION

The main findings of the present study were (1) that effect sizes show a wide range and that (2) approximately half of the intervention groups and (3)

Author + Year	BR days	Training program	Outcome parameter	Measured muscle / movement	Mean pre-post difference IG	Mean pre-post difference CG
Wu et al. 2010	30	5x5-10 squats, 5x10-15 calf press, (60-80% of MVC), 5days/week	Max. strength [kg]	Knee flex/ex OR plantar flex/ex	20 (±30)	-12 (±29)
Akima et al. 2003	20	Leg press & plantar flexion, 5x10reps/day (70% of MIF) on a total of 16 days	Max. isometric contraction [Nm]	Plantar flexors	1 (±15)	-12 (±10)
Greenleaf et al. 1983	14	Leg extension, 30 sets of 1 min duration/day at 21% of max. force	Maximal endurance [sec]	Handgrip	-11 (±18)	-29 (±23)
			Max. strength [units]	Handgrip	4 (±159)	0 (±139)
Marcas et al. 1998	14	Leg press, 5x8 reps (80-85% of 1RM), every other day	1 RM [kg]	Leg press	1 (±30)	-21 (±21)
			Isometric MVC [Nm]	Knee extension	33 (±48)	-36 (±24)
			Isometric MVC [Nm]	Knee flexion	-15 (±35)	-13 (±17)
Reeves et al. 2002	90	4x14 calf press, 2-3x/week (intensity not specified)	Isometric MVC [N]	Plantar flexor	-1316 (±487)	-1602 (±785)
Alkner & Tesch 2004	90	4x7 supine squat, 4x14 calf press, every 3rd day (intensity in % of max. force not specified)	MVC [N]	Supine squat 90°	-257 (±219)	-705 (±329)
			MVC [N]	Supine squat 120°	-631 (±488)	-1395 (±741)
			Concentric peak power [W]	Supine squat ?°	18 (±129)	-328 (±151)
			MVC [N]	Calf press 90°	-818 (±577)	-1216 (±603)
			Concentric peak power [W]	Calf press 90°	-28 (±95)	-264 (±111)
			MVC [N]	Knee extension 120°	-79 (±29)	-131 (±32)
			MVC [N]	Knee extension 90°	-61 (±28)	-107 (±23)
			MVC [N]	Plantar flexion 90°	-45 (±26)	-48 (±19)
Shackelford et al. (2004)	119	*Upper body workout* (biceps curls, triceps press-downs, upright rowing, bench press, prone rowing, single-leg heel raises, bilateral heel raises) , 2-6 sets, 5-11reps (max.-effort set) depending on week, 3 days/week *Lower body workout* (back extensions, hip abduction, wide-stance horizontal leg press, narrow-stance leg press, lying knee-ups, single-leg heel raises, bilateral heel raises), 2-6 sets, 5-11reps (max.-effort set) depending on week, 3 days/week *Heel raises*, 5x20reps, 6 days/week	1 RM [% change]	Leg press	74 (±31)	-29 (±7)
			1 RM [% change]	Heel raises	50 (±14)	-37 (±9)
			1 RM [% change]	Knee ups	86 (±79)	-6(±6)
			1 RM [% change]	Left abduction	110 (±56)	-26 (±19)
			1 RM [% change]	Right abduction	102 (±32)	-26 (±19)
			1 RM [% change]	Bench press	60 (±32)	-12 (±13)
			1 RM [% change]	Back extension	26(±25)	-16 (±13)
Marcas et al. 1997	14	Plantar flexion, 5x6reps to failure, every other day	1 RM [kg]	Plantar strength	23 (±34)	-20 (±23)
			MVC [Nm]	Plantar flexion	-8 (±40)	-16 (±10)
			MVC [Nm]	Dorsiflexion	-4 (±7)	-1 (±7)
			Concentric power at 0.52 rad/sec [W]	Dorsi/plantar flexion	-1 (±18)	-8 (±11)
			Concentric power at 1.05 rad/sec [W]	Dorsi/plantarflexion	-5 (±31)	-27 (±22)
			Concentric power at 1.75 rad/sec [W]	Dorsi/plantarflexion	-14 (±69)	-12 (±34)
			Concentric power at 2.97 rad/sec [W]	Dorsi/plantarflexion	-29 (±116)	-9 (±71)
			Eccentric power at 0.52 rad/sec [W]	Dorsi/plantarflexion	4 (±18)	-11 (±14)
			Eccentric power at 1.05 rad/sec [W]	Dorsi/plantarflexion	11 (±23)	-15 (±23)
			Eccentric power at 1.75 rad/sec [W]	Dorsi/plantarflexion	-2 (±50)	-5 (±43)
			Eccentric power at 2.97 rad/sec [W]	Dorsi/plantarflexion	23 (±110)	-48 (±108)
Gast et al. 2012	60	Bilateral leg press (75-80% of MVC), single leg heel raises (1.3x BW), double leg heel raises (1.8x BW), back & forefoot raise (1.5x BW), 1 set until failure (max. 12 reps), 3x/week	1 RM [N]	Leg press	39 (±240)	-206 (±101)
			Peak power (rel. to BW) [% change]	CMJ	-11.3 (±10)	-27 (±13)
Rittweger et al. (2007)	60	4x7 supine squats, 4x14 calf press , every 3 days	Peak power [W/kg]	CMJ	-4 (±5)	-13 (±7)

FIGURE 3: Study characteristics presenting mean differences (±SD). Decreases are marked in red, increases are marked in green. IG = intervention group; CG = control group; lRM = one repetition maximum; max= maximum/maximal; Oex = flexion; ex= extension; reps= repetitions; BW = body weight.

all control groups had negative mean differences between pre- and post-values. This shows that treatment effects of resistive exercise regimen vary significantly across interventions, and that all control groups and half of the intervention groups lost muscle strength and power.

The majority of effect sizes are in favor of resistive exercise intervention groups compared to control groups. Consequently, and unsurprisingly, resistive exercise during bed rest appears superior to no countermeasure in preserving muscle strength and power. However, effect sizes vary significantly across outcome parameters. Of the 39 parameters that were included, most effect sizes show a small to very large effect [10] in favor of the intervention groups. Differences among study characteristics such as training protocols, study durations and quality, subject groups and their baseline values, or differences between test protocols employed to measure muscle strength and power might account for this variation and should be addressed in follow-up studies.

To conclude, resistive exercise appears not always to be sufficient to maintain muscle strength and power during bed rest. Differences among study characteristics need to be further analyzed to explain different results.

Other forms of exercise (e.g. concurrent training, high intensity interval training, plyometric training) should also be investigated to optimize exercise countermeasure prescriptions for human space flight to preserve muscle strength and power of astronauts.

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Keywords: bed rest, muscle strength, space flight, exercise countermeasures, muscle power

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Hyper-gravity promotes motor learning in goldfish and humans

Yutaka Hirata*, Shohei Miura, Yuki Takagi, Takafumi Kashima, Kohei Urase, Shuntaro Miki

Department of Robotic Science and Technology, Chubu University, Kasugai, Japan
**yutaka@isc.chubu.ac.jp*

INTRODUCTION

Microgravity imposes various effects on the human body such as malfunctioning of the cardiovascular system, weakening of muscle strength, reducing calcium from bones, and inducing space motion sickness (Moore, 1996). Further, under micro-gravity, physical movements of astronauts and cosmonauts are slow and somewhat awkward. Motor control systems of our body are continuously calibrated by interacting with gravity on the earth, thus require re-adjustments in the brain motor areas when the gravitational environment is changed. This is not only due to the direct effects of gravity on the mass of the body, but to the effects on sensory systems such as vestibular and proprioceptive systems as well. Although some astronauts have made subjective reports informally, scientific evidence on motor learning under different gravitational environments is missing. In the present study, we address this issue by evaluating learning curves of oculomotor neural integrator adaptation in goldfish and prism adaptation of hand-reaching task in humans under different gravity conditions.

METHOD AND RESULT IN GOLDFISH

We first performed oculomotor neural integrator (NI) adaptation experiments (instability adaptation; Major et al., PNAS, 2004) in goldfish under hyper-gravity (1.5G) and normal gravity conditions. Seven fish (12 - 15cm) were used for the hyper-gravity experiment and the same fish underwent the normal gravity experiment as well. Four fish experienced the hyper-gravity experiment first, while the other 3 fish underwent the normal gravity experiment first. The hyper-gravity environment was created by centrifugation with the apparatus we developed for this experiment. In each condition, the experiment lasted for 2 hours for NI adaptation. Between the two experiments, each fish was given at least 1 week interval in its home water tank. Averaged learning curves of NI time constant (Major et al., PNAS, 2004) over the all fish used under hyper-gravity and normal gravity conditions were compared. As the result, faster learning rate and greater learning amount were found in the mean learning curve of hyper-gravity condition than those in the learning curve of normal gravity condition. Thus, motor learning in goldfish, at least NI adaptation, was promoted under hyper-gravity environment.

METHOD AND RESULT IN HUMANS

Then we conducted motor learning experiments in humans under hyper-gravity (2G) and normal gravity conditions. The motor learning task we employed is prism adaptation of hand-reaching (Martin et al., 1996) which has been a popular motor learning task, and the cerebellum has been identified to crucially be involved (Hanajima, et al., 2015). As in the goldfish experiment, the hyper-gravity environment was created by centrifugation with the GyroLab system. Six healthy male subjects (age 21-42, average 24.7 years old) underwent both hyper-gravity and normal gravity experiments. Each experiment consisted of 20 hand-reaching maneuvers to a visual target on a touch screen at 45 cm in front of the subject without wearing prism goggles, followed by 60 hand-reaching maneuvers to the same target with the prism goggles on. The timing and interval of reaching maneuvers were controlled by the audio instruction recorded before the experiments. The subjects were instructed to close their eyes before they started each reaching maneuver. After each reaching, touched location was immediately marked on the screen, and they were asked to open their eyes to visually confirm where they touched relative to the visual target. Each subject underwent 1G and 2G experiment multiple times (> 4 times for each condition) in a randomized order. Between experiments, subjects were asked to make hand-reaching as many times as possible under normal gravity without wearing prism googles so that they completely readapted to normal visual environment under normal gravity. Averaged learning curves of prism adaptation under hyper-gravity and normal gravity were calculated for each subject, and were compared in each subject. As the result, faster learning rate was found in the mean learning curve of hyper-gravity condition than that in the learning curve of normal gravity condition in all the subjects participated. Therefore, motor learning in humans, at least prism adaptation of hand reaching was promoted under hyper-gravity.

DISCUSSION AND HYPOTHESIS

Why is motor learning promoted under hyper-gravity in both goldfish and humans? The cerebellum has been implicated to play a crucial role in goldfish NI adaptation and in human hand-reaching prism adaptation. An augmented gravitation input would significantly increase vestibular input to both the goldfish and human cerebellum, in particular, the vestibulocerebellum (VCB). Thus, a working hypothesis for accelerated motor learning under hyper-gravity was envisioned to be an up-regulated Purkinje cell activity enabling more efficient modification of synaptic efficacy. If so then it should be possible to accelerate motor learning by using another stimulus that up-regulates Purkinje cell activity such as that observed in the primate VCB (flocculus) in which Purkinje cells exhibit higher dc firing rates in light than in dark (Hirata and Highstein, 2001). We have also confirmed that similar up-regulation of Purkinje cells in VCB occurs in goldfish (Miki and Hirata, unpublished observation).

EXPERIMENT TO TEST THE HYPOTHESIS

We then tested this experimental paradigm in the goldfish VCB responsible for NI adaptation by inducing motor learning utilizing two types of visual stimuli. Either white spots were displayed as a stimulus on a black background (Darker) or black spots on white background (Brighter). Image contrast of the Darker stimulus was adjusted to evoke an equivalent optokinetic behavior as that induced by the Brighter stimulus so that behavioral error during training would be comparable under the two conditions. The same eight goldfish were used for both Brighter and Darker stimulus experiments. Instability adaptation paradigm was employed. Each NI training experiment lasted for 2 hours as in the hyper-gravity experiment. As a result, a significantly faster learning rate was found with the Brighter than the Darker visual stimulus. This finding supports the hypothesis that Purkinje cell up-regulation alone may produce more efficient changes in synaptic efficacy contributing to the neural basis of motor learning.

CONCLUSION

Motor learning under hyper-gravity is promoted in humans and goldfish, at least in prism adaptation of hand reaching (human) and oculomotor neural integrator adaptation (goldfish) that we employed in the current study. Similarly, Brighter stimulation promoted motor learning of oculomotor neural integrator adaptation in goldfish. Taken together, it is suggested that cerebellar Purkinje cell up-regulation caused by constant increases of sensory input may accelerate cerebellar synaptic plasticity, and result in promoted motor learning.

Keywords: cerebellum, oculomotor, eye movement, prism adapation, synaptic plasiticty

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The somatogravic illusion during centrifugation: Sex differences

Rainer Herpers[1,2,3]*, Laurence Roy Harris[3], Meaghan McManus[3], Thomas Hofhammer[2], Alexandra Noppe[4], Timo Frett[4], Michael Jenkin[3], David Scherfgen[2]

[1]University of New Brunswick Fredericton, Fredericton, Canada
[2]Hochschule Bonn-Rhein-Sieg (H-BRS), Sankt Augustin, Germany
[3]Centre for Vision Research, York University, Ontario, Canada
[4]Institut für Luft- und Raumfahrtmedizin, Deutsches Zentrum für Luft- und Raumfahrt (DLR), Cologne, Germany
**rainer.herpers@h-brs.de*

INTRODUCTION

Maintaining orientation in an environment with non-Earth gravity (1 g) is critical for an astronaut's operational performance. Such environments present a number of complexities for balance and motion. For example, when an astronaut tilts due to ascending or descending an inclined plane on the moon, the gravity vector will be tilted correctly, but the magnitude will be different from on earth. If this results in a mis-perceived tilt, then that may lead to postural and perceptual errors, such as mis-perceiving the orientation of oneself or the ground plane and corresponding errors in task judgment.

Rotation on a centrifuge offers a unique opportunity to vary the direction of the gravity vector without physical tilt, that is, without co-activation of the semicircular canals during the simulated tilt. The tilt angle simulated is the tilt of the simple vector sum of gravity and the acceleration added by the centrifuge. Perceiving acceleration as tilt is the well-known somatogravic effect [Mach1875, Clark1951]. Sustained linear acceleration together with gravity creates a single gravito-inertial force (GIF). Under normal gravity conditions, sustained linear acceleration in the transverse plane can create an illusion of tilt - the somatogravic illusion - in which the entire GIF is interpreted as corresponding to gravity. However, the magnitude of the effect, i. e. the fraction of the GIF that is interpreted as gravity, has not been well quantified in the sagittal plane. We, therefore, varied the added acceleration to induce a somatogravic illusion and measured the perceptual effects using a haptic rod to indicate the perceived direction of gravity.

Previous experiments measuring the somatogravity illusion have used a swinging gondola, hypergravity and a visual assessment method [Tribukait1999, Tribukait2006]. Our experiments used a haptic rod outside of the participant's view, ruling out effects of eye torsion, and a fixed chair mounted so that the participant sat upright and nose-out to provide a controlled and constant direction of otolith stimulation over a physiologically valid range of acceleration forces.

METHODS AND MATERIAL

The somatogravic illusion was measured during prolonged backwards centripetal acceleration created by centrifugation simulating different pitched-forward tilts. The perception of tilt during centrifugation was compared to the perception of physical tilt generated using a motion platform (Moog).

For both setups (Moog and centrifuge), 5 male and 5 female participants, all healthy and between 20 and 45 years old, sat upright in a chair in the dark, with their head lightly restrained, and judged the orientation of a rod relative to gravity (see Figure 1). Different participant groups were used for each setup.

Each participant in the centrifugation section was selected by a medical screening done at the German Aerospace Center's Aeromedical Center. The medical examination consisted of a clinical-chemical analysis (glucose, creatinine, urea, uric acid, SGOT, SGPT, µGT, total cholesterol, HDL and LDL), hematology (blood count), urine analysis (glucose, protein, urobilinogen), resting ECG, exercise test to verify endurance capacity, standing test for orthostatic tolerance assessment and a medical history. At the time of medical examination, participants were familiarized with the conduction of the study, including the procedure of centrifugation and the associated OTL protocols. All volunteers underwent a comprehensive clinical assessment and gave a written informed consent prior to the study.

For each centrifugation, participants underwent four experiment sessions plus two control sessions on two days. Between the two experiment sessions on a single day, a 30 min break was planned.

Participants sat in a fixed-position upright chair looking outwards on a short arm centrifuge with their head lightly restrained. The head position was used to define the radius for estimation of centripetal acceleration.

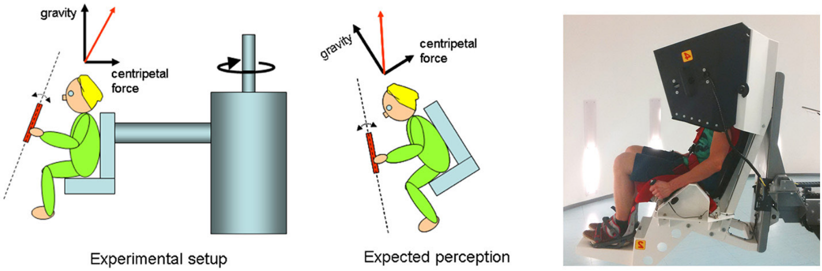

FIGURE 1: Experimental setup. Participants were sitting on a centrifuge facing outwards. Their field of view was blocked by a metal hood (right). Participants manipulated a haptic rod to indicate the perceived direction of gravity.

A haptic rod about 20 cm in length mounted on a potentiometer was mounted so that the participant could touch it with one hand. The haptic rod was mounted on a servo-controlled motor that rotated in the parasagittal plane. The participant's hand rested on the rod throughout the experiment and so there was no issue of centrifugal forces. The rod's orientation was varied using an adaptive psychophysical staircase that honed in on the perceived direction of gravity. The participant was instructed to press a button (operated by the other hand) to indicate when the setting is satisfactory. The computer recorded the orientation and time stamp of the setting along with the rotation condition.

The GIF was swung between 0°, 22.5° or 45° (6 combinations of change in GIF) by the addition of a centripetal acceleration of between 0 g and 1 g. Participants had to make a rod setting continuously throughout the entire time they were on the centrifuge including during transitions to different rotational speeds. Once each target speed had been reached, it was maintained for 180 s.

RESULTS AND DISCUSSION

For centrifugation, all males showed a substantial somatogravic illusion with a gain (perceived tilt over simulated tilt) of 0.46, whereas 4 of 5 females did not experience it at all and continued to identify the direction of gravity correctly despite the tilt of the GIF (see Figure 2 A, B).

For physical tilt using the Moog motion platform, a similar sex asymmetry was found (gain for males: 1.2, gain for females: 0.49, see Figure 2 C, D).

The low gain observed for females suggests a restraining influence of a strong idiothetic vector acting as a prior indicating that gravity is continuously aligned with the body. Results indicate that sex should be taken into account when assessing balance or perceived orientation in situations where the direction of gravity is not aligned with the body. Examples include changes of body orientation during normal changes of posture, responding to imposed tilt

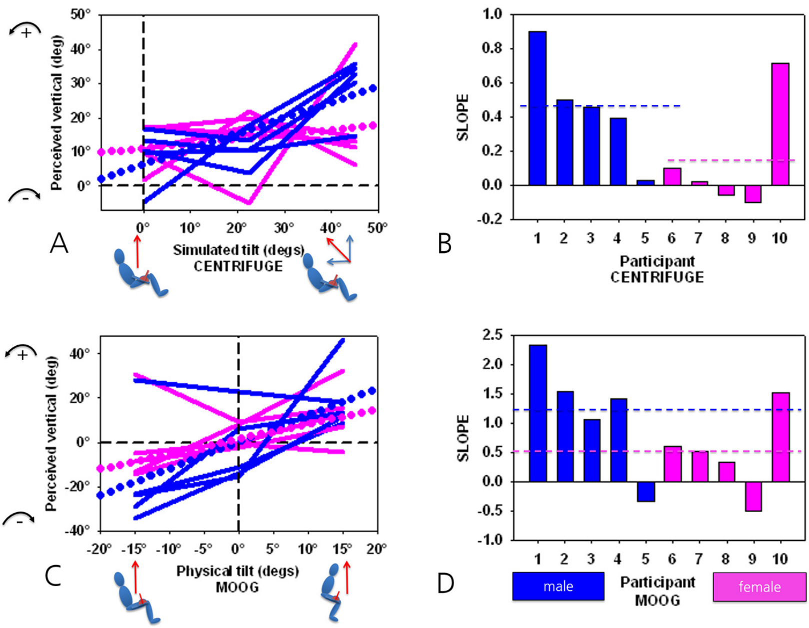

FIGURE 2: Participants' perception of tilt compared to simulated tilt (by centrifugation, **A**) and physical tilt (using the Moog motion platform, **C**) and the corresponding linear regression slopes (**B, D**). It can be observed that female participants are more capable of correctly identifying the direction of gravity despite the tilt of the GIF.

(such as when bumped into while walking) or during conditions of prolonged acceleration such as when driving or piloting an aircraft.

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Keywords: centrifugation, vestibular system, perception of upright, somatogravic Illusion, gravito-inertial force

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Virtual reality as a countermeasure for physical training in bed rest and artificial gravity conditions

Gabriel G. De La Torre[1]*, Andreas Vogler[2], Robert John Stone[3], Ana Diaz Artiles[4], Jesper Jorgensen[5], Julie Bienertova-Vasku[6]

[1]*University of Cádiz, Cádiz, Spain*
[2]*Vogler Studio, Munich, Germany*
[3]*University of Birmingham, Birmingham, United Kingdom*
[4]*Department of Aerospace Engineering, Texas A&M University, College Station, United States*
[5]*Roskilde University, Roskilde, Denmark*
[6]*Masaryk University, Brno, Czechia*
**gabriel.delatorre@uca.es*

Physics-based simulations are helping mission planners on the Journey to Mars providing virtual environments to test vehicle and system performance in simulated deep space environments. NASA has relied heavily on mission simulators to prepare astronauts for every conceivable contingency. Mercury, Gemini, and Apollo crews spent one third or more of their total training time in simulators. Lunar landing crews used simulators for more than half of their training time. Currently, Sidekick, the project to put the Microsoft HoloLens (a virtual/augmented reality system) onboard the ISS, is designed to empower the crew with assistance when and where they need it. The goal of the Sidekick project is to increase ISS utilization and scientific return by accelerating crew activities.

VR is usually described as "an advanced form of human-computer interface that allows the user to interact with and become immersed in a simulated, computer-generated environment in a naturalistic fashion" (Schultheis & Rizzo, 2001, p. 82). Specifically, what distinguishes VR from other media or communication systems is the sense of presence (Riva, 2007, 2008): VR can be considered the leading edge of a general evolution of interfaces such as television, computer and telephone whose ultimate goal is the full immersion of the human sensorimotor channels into a vivid and interactive experience. The higher sense of presence induced by VR may be used to elicit optimal experiences that will support the process of change and adaptation under space conditions. Use of virtual reality (VR) technology in health is increasing with multiple applications, for example to improve walking in people post-stroke and brain injury patients and other physical rehabilitation situations. The hardware and software used to create these systems has varied but has predominantly been constituted by projected environments with users walking on treadmills and more recently cycling. Transfer of training from the virtual environment to real-world exercise has positive research support.

In Space research, exercise is currently one of the most important countermeasures to mitigate the negative physiologic adaptation the body experiences in microgravity. Regular exercise routines improve not only the overall physiological state, but also cognitive function and psychological well-being. There has been development on several appropriate exercise protocols such as the Integrated Resistance and Aerobic Training Study (I-RAT; SPRINT), which combines high intensity and continuous aerobic exercise as well as resistive exercise. This exercise protocol has been successfully tested and validated in a 14-day bed rest study (Ploutz-Snyder et al., 2014) and more recently in a 70-day bed rest study (Ploutz-Snyder et al., 2018). The SPRINT exercise research program is currently in evaluation on the ISS (Hackney et al., 2015; Ploutz-Snyder et al., 2018).

The combination of this exercise protocols with VR may both improve the physical condition and psychological experience associated with it (Plante et al., 2003). In our research we propose to compare traditional supine configuration using cycling ergometers (see figure 1) with newly tested by MIT sideways position (Artiles et al., 2015), both with virtual reality technology. The sideways configuration offers two advantages over a left-side down, or more traditional supine position. First, it faces “into the wind”, given the centrifuge´s clockwise direction of rotation. Left-side down lateral decubitus position would be equivalent if the direction of rotation of the centrifuge were counterclockwise. Second, it minimizes the effects of Coriolis acceleration during knee flexion/extension in a rotating environment. Previous studies have shown that Coriolis forces can induce mediolateral knee deflections during centrifuge supine squats. The proposed lateral orientation of subjects minimizes (or eliminates) these deflections.

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Keywords: bed rest, psychology, physical exercise, artificial gravity, centrigugal testing

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The regulation of the DNA methylation in the ovaries of mice under 23-days antiorthostatic suspension

Maria A. Usik[1,2], Irina V. Ogneva[1,2]*

[1]*I.M. Sechenov First Moscow State Medical University, Moscow, Russia*
[2] *Cell biophysics laboratory SSC RF, Institute for biomedical problems RAS, Moscow, Russia*
**iogneva@yandex.ru*

INTRODUCTION

Weightlessness has a negative impact on various systems of the human body, including on the cardiovascular, muscle and skeletal systems, which can impede the deep space exploration (Watenpaugh D.E., Hargens A.R., 1996; Riley D.A. et al., 2000; Vico L., Hargens A., 2018). Despite the long history of research, the mechanisms of perception and transduction of mechanical stresses at the cellular level have not been adequately studied. In particular, the effect of microgravity is changes in the expression of various genes (Gershovich P.M. et al., 2008; Pan Z. et al., 2008), however, the reasons for this are still unclear. In mammals, one way to regulate expression is to change the DNA methylation level. Therefore, the aim of this work was to estimate the total DNA methylation level, the content of the intermediate product 5-hydroxymethylcytosine (5hmC) and the enzymes controlling these processes (DNA methylases DNMT1, DNMT3a, TET demethylases and HDAC1 deacetylase) in the ovaries of mice after long-term antiorthostatic suspension.

MATERIALS AND METHODS

Microgravity effects were simulated using the Ilyin-Novikov standard model of antiorthostatic suspension modified by Morey-Holton (Morey-Holton et al., 2005). During the experiment, the animals were kept in standard vivarium conditions with food and water ad libitum. The animals were randomly divided into two groups: C (n=7), control group; HS (n=7), the 23-day suspension group. At the end of the suspension, after euthanizing the animals, the ovaries were isolated, weighed, and immediately frozen for subsequent isolation of nucleic acids and proteins. All the procedures conducted with animals were approved by the Commission on Biomedical Ethics of the Institute of Biomedical Problems.

Total DNA was isolated from the frozen tissues using a DNA isolation kit (Synthol, Russia) based on a phenol/chloroform method. The total methylation level was estimated through restriction analysis (MspI/HpaII) by EpiJET DNA Methylation Kit (Thermo Scientific, USA), the content of 5hmC – by dot-blotting with specific antibodies and proteins content – by Western blot with specific antibodies (all primary antibodies – Abcam, UK).

RESULTS

The total methylation level in the ovaries was reduced after 23 days of hindlimb suspension by 20% ($p < 0.05$) for the internal cytosine and by 10% ($p < 0.05$) for the external cytosine in the 5′-CCGG-3′ loci (Figure 1, A). The content of 5hmC in the ovaries did not change in the HS group compared to the group C (Figure 1, B).

The content of S-phase DNA methylase DNMT1 and de novo methylase DNMT3a did not change after antiorthostatic suspension (Figure 2, A). But the relative content of the active demethylase TET2 increased after suspension by 16% ($p < 0.05$), although there were no changes in TET3 content (Figure 2, B).

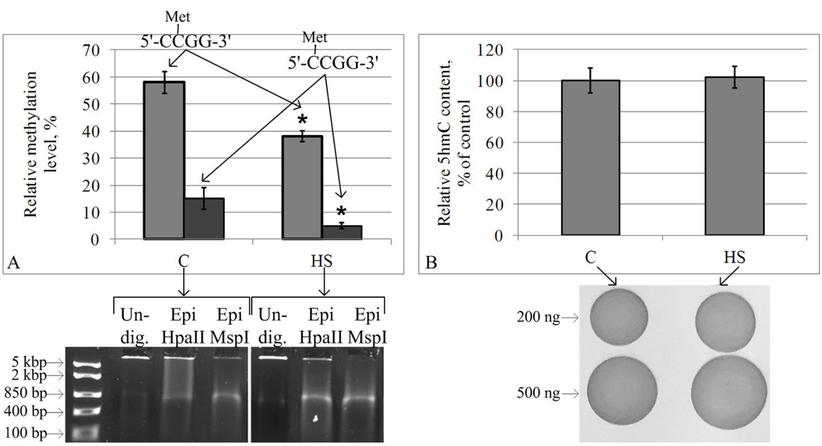

FIGURE 1: Total methylation (**A**) and 5hmC (**B**) levels with typical pictures. * – $p < 0.05$ in comparison with group C. Undig. – undigested genomic DNA; Epi HpaII – genomic DNA digested with Epi HpaII; Epi MspI – genomic DNA digested with Epi MspI.

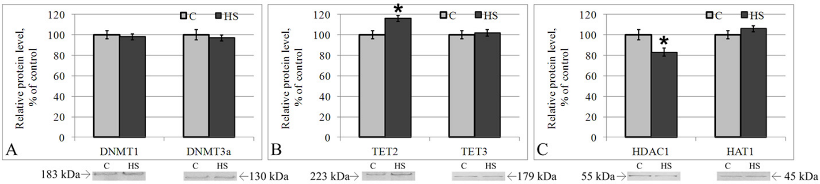

FIGURE 2: Relative methylation/demethylation fermentas and HDAC1 protein levels with typical western-blots. * – p < 0.05 in comparison with group C. (**A**) – methylases DNMT1 and DNMT3a; (**B**) – demethylases of the TET-family; (**C**) – deacetylase HDAC1.

Content of histone acetylase HAT1 did not change, but the histone deacetylase HDAC1 content decreased after disuse by 17% ($p < 0.05$) in comparison with control level (Figure 2, C).

DISCUSSION

The obtained results indicate that in the ovarian cells of mice, after a 23-day antiorthostatic suspension, a hypomethylated state was established, in the absence of changes in the content of 5hmC. However, Western blot data indicate that the content of DNMT1 and DNMT3a methylases did not change, while the TET2 demethylase content increased. In this case, it can be assumed that the establishment of a hypomethylated state is due to active complete demethylation of the target sites, without accumulation of an intermediate 5hmC product. At the same time, the activity of TET2 demethylase may be related to the observed decrease of the deacetylase HDAC1 content, which, in turn, is capable of deacetylating TET proteins, inhibiting their activity in complex with DNMT1 (Zhang Y.W. et al., 2017).

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Keywords: DNA methylation, ovary, epigenetic regulation, histone deacetylase (HDAC) 1, microgravity (μg)

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Pilot study on the effects of early exposure to hypergravity on the behavioural and cerebellar development of CD-1 mice

Silvia Schwartz[1], Nadia Francia[2], Sara Tavella[3], Maria Egle De Stefano[4], Daniela Santucci[2]*

[1]*Center for Life NanoScience@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy*
[2]*Center for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy*
[3]*DIMES, University of Genoa & IRCCS Polyclinic Hospital San Martino, Genova, Italy*
[4]*Department of Biology and Biotechnology "Charles Darwin", Sapienza University, Rome, Italy.*
daniela.santucci@iss.it

The nervous system is known to be highly sensitive to environmental alterations. Nevertheless, changes induced by exposure to altered gravity have not been fully understood. In this study we investigated the effects of hypergravity on motor coordination and cerebellum structural development of CD-1 mice exposed to centrifugation-induced 2g hypergravity (HG) from post-natal day (PND) 1 to PND21. Not rotated mice (stationary control, SC), and mice exposed to 1g (rotational control, RC) were used as controls. Behavioural endpoints were assessed at PND7, 14 and 21, while cerebellar structure was analysed at PND7. Results showed that PND7 HG mice exhibited a delay in the Righting response, while Pole grasping and Auditory startle responses were negatively affected only in RC mice. On PND21, an impaired Dowel test performance was observed in the HG group. Histological analysis showed alterations in cerebellar granule cell migration of rotated mice, HG animals being more affected than RC ones. These data suggest that altered gravity delays cerebellar development, which results in impaired motor coordination performances. Further understanding how cerebellum responds to altered gravity could provide insights into its implications in several neurological disorders, and contribute to the development of therapeutic tools and strategies.

INTRODUCTION

The possibility that exposure to an altered gravitational environment may interfere with the development and maturation of the nervous system is relevant for vertebrate physiology.

A number of animal studies have shown that changes in sensorimotor behaviour are associated with brain structural changes (Aizikov and Markin, 1981). The cerebellum, renowned for its role in motor coordination, is affected by exposure to hypergravity (Lalonde 2003). While it is difficult to perform extensive developmental studies in space, hypergravity paradigms using centrifuges provide a fruitful way to study the effects of gravity changes on biological organisms (Serova et al., 1993). However, a few studies have directly correlated changes in motor behaviour with cerebellar structural alterations.

In this pilot study, CD-1 mice were exposed from PND1 to PND21 to centrifuge-induced gravity (see Francia et al., 2006 for methodological details and behavioural tests). Three experimental groups were designed: stationary controls (SC), rotational control (RC, 1g) and hypergravity (HG, 2g). Both sensorimotor reflexes and motor coordination were assessed at PND7, 14 and 21, while histological analyses were conducted at PND7, peak day of granule cell migration, and when several genes important for cell cycle progression and differentiation are either up- or down-regulated transiently (Kagami and Furuichi, 2001).

RESULTS

At PND7, a significant delay in the emergence of the Righting reflex (returning to the feet when placed on the back) was observed in HG mice (Fig. 1A). By contrast, impairment in Pole grasping (grasping a toothpick with forepaws) and Startle responses (whole body startle response to a laud hand clap) were observed exclusively in RC animals (Fig. 1B, C).

To assess balance skills, the Dowel test (walking on a pencil) was performed at PND14 and 21. While no major differences were observed between SC, RC and HG mice at PND14, a significant impairment was seen at PND 21 in HG mice (Fig. 1D, E), confirming a specific delay in motor coordination development in mice exposed to hypergravity.

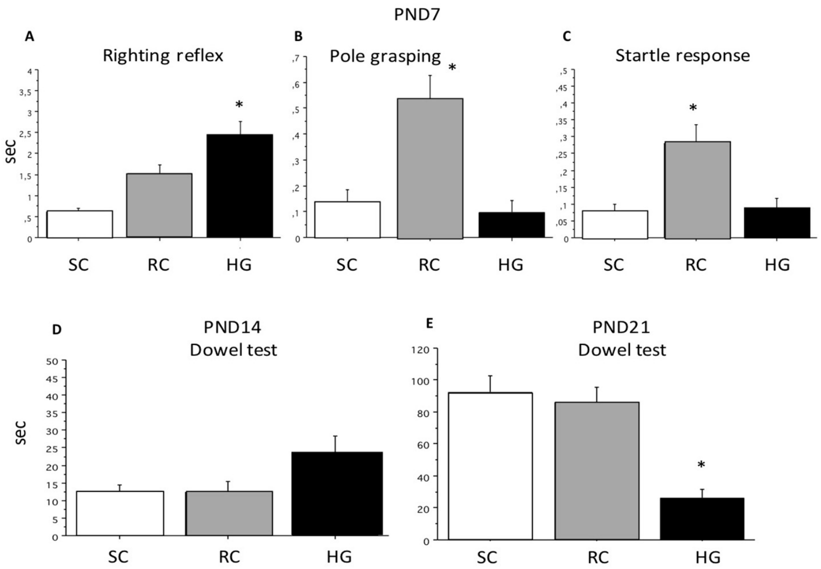

FIGURE 1: Battery of behavioral tests analyzing motor coordination of mice exposed to altered gravitational acceleration. Righting reflex, Pole grasping and Auditory startle tests were used at PND 7 (upper panel) and Dowel test at PND 14 and 21 (lower panel). (**A**) Righting reflex. Hypergravity (HG) mice show a significant delay in the emergence of reflex (F (2.68) = 7.68, p <0.001 Bonferroni post-hoc correction) compared to stationary control (SC) and rotational control (RC) groups. (**B**) In Pole grasping test, only mice of the RC group show a significant delay in the emergence of this response compared to the other two (F (2.68) = 7.01; p <0.01). (**C**) As observed in the Pole grasping test, the RC group manifests a significant delay in the emergence of the Auditory startle response (F (2, 66) = 3.026, p <0.05). (**D, E**) Dowel test results from PND 14 and PND 21 mice, respectively. Exposition to 2g negatively affects the ability to maintain equilibrium in PND 21 mice (F (2.48) = 10.545; p <0.02), whereas a slight, but non-significant, improvement is observed in PND 14 mice.

Histological analyses were performed on paraffin-embedded cerebellum slices. In SC mice (Fig. 2A-C), maturing granule cells are still mainly localized in the external germinal zone (EGZ), where they form a compact cellular area. Differentiating granule cells migrate to final destination, either isolated or in chains, across the Purkinje cell (PC) layer, which are organizing in the characteristic monolayer. (Fig. 2B,C). In HG mice (Fig. 2D-F), the EGZ appear to be thicker and more cell-packed compared to SC mice, with the inner layer

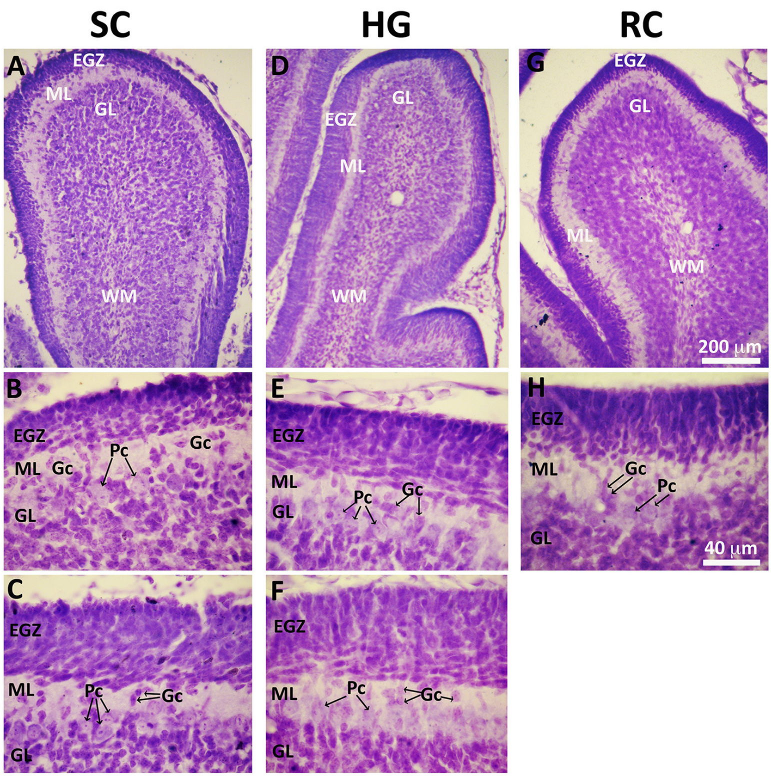

FIGURE 2: Histological analysis on PND7 mouse cerebellum in control and hypergravity conditions. **(A-C)** SC mouse group. A: At PND 7, cerebellar folia show the expected subdivision in external granular zone (EGZ) -where immature granule cells are located-, molecular layer (ML) – through which granule cell migrate-, the granule cell layer (GL) and the central white matter (WM). B,C: Higher magnification of the cortical portion of the cerebellum. Migrating granular cells (Gc) are seen crossing the ML and reach their definitive layer (GL), crossing the Purkinje cell (Pc) layer. Pc are disposing in a monolayer, a distinctive feature of maturation. **(D-F)** HG mouse group. D: Gross organization of cerebellar folia is similar to the SC group. E,F. At higher magnification. The EGZ appear larger, and the immature Gc more packed, than in the SG mice, as a consequence, the ML is narrower compared to control groups and Pc appear distributed on multiple layers. **(G-H)** RC mouse group. F: Gross organization of cerebellar folia is similar to the SC group. G-H. At higher magnification, the organization of the cortical layers appears to be somehow in between the SC and the HG conditions. Scale bar: low magnifications, 200 µm; high magnifications, 40 µm.

of granule cells distributing with a different orientation compared to outer ones. This implies that the PC layer get closer to EGZ, suggesting a sort of hold up of granule cell migration. PC mono-layer organization also appears delayed. In RC mice (Fig. 2G,H), these features are intermediate between the two groups, although closer to the HG mice.

CONCLUSIONS

Results on mice exposed to hypergravity during the first three weeks of postnatal life indicates that this stimulus influences the behavioural profile, with a delay in the mode and time of reflex occurrences. In particular, the investigated behavioural endpoints are directly related to the ontogeny of neuromotor functions and represent sensitive indicators of the level of maturation of nervous system and cerebellum in particular.

On PND7 the Auditory startle response and the Pole grasping were affected in RC mice, while only the Righting reflex was impaired in HG, confirming a specific response to hypergravity exposure. Of a note, Righting reflex requires an appropriate integration between vestibular and somatosensory inputs in order to make postural adjustments when the body is displaced from its normal vertical position. Moreover, significant changes in the ability to remain in balance in the Dowel test, emerged only in PND 21 HG mice The non-linear trend of behavioural responses, in which some effects were more marked at PND7 and PND 21, is in agreement with data previously reported (Sajdel-Sulkowska et al., 2001) and confirms the existence of specific windows of vulnerability during critical phases of development.

In agreement with previous data, in which a reduction in the cerebellar mass was observed in rats chronically exposed to gravitational acceleration during the perinatal period (Sajdel-Sulkowska et al., 2005), this pilot study on CD-1 mice suggests that early exposure to hypergravity causes an increase in the EGZ thickness and a reduction in the space between this and the PC layer. This suggests a slower migration rate of granule cells towards their final destination compared to control, with possible alteration in the wiring of cerebellar circuits responsible for the vestibular system proper activity. Indeed, in the first 20 PND, the cerebellum is in its most plastic period, characterized by granule cell maturation and migration from the EGZ, in which they are tightly clustered, through the PC layer, down to their final destination. It is particularly interesting how these anatomical and morphological changes parallel the behavioral ones, confirming that perinatal exposure to hypergravity interferes with correct CNS wiring. This could end up in short-, medium- or long-term repercussions, which may be more or less pronounced, depending on the targeted system, e.g. sensorimotor, neuromotor, cognitive, emotional. Our preliminary data indicate both morphological and behavioral changes in early postnatal mice exposed to hypergravitational stimuli, which can be perceived as a "delay" in our "gravitational system", but that can be also interpreted as a plastic reorganization of the developing nervous system in a "challenging" environment. Further studies will be devoted to analyse later stages of cerebellar development, when its different neuronal types have fully matured and reached their final destination.

ACKNOWLEDGEMENT

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Keywords: cerebellum, hypergravity, development, animal model, animal behaviour

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Effects of unloading condition on wound healing process: Experiments with hirudo medicinalis

Francesca Cialdai[1]*, Desire' Pantalone[2], Daniele Bani[3], Paolo Romagnoli[3], Angela Maria Rizzo[4], Fabio Celotti[4], Alessandra Colciago[4], Elettra Sereni[1], Francesco Ranaldi[1], Monica Monici[1]

[1]Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università degli Studi di Firenze, Florence, Italy
[2]Department of Surgery and Translational Medicine, Careggi University Hospital, Florence, Italy
[3]Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italy
[4]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy
**francesca.cialdai@unifi.it*

Wound healing is a fundamental process for survival. It makes an organism able to repair a damage and restore physiological condition. The whole process involves the coordinated interaction of many different cell populations and biochemical molecules, such as proteins, proteases, growth factors, and extracellular matrix (ECM) components. Wound healing consist of four precisely programmed phases that follow one another and partly overlap. The events of each phase must happen in a definite and regulated manner. Hemostasis is the first phase and begins immediately after wounding. It is characterized by vascular constriction and fibrin clot formation. The clot and surrounding wound tissue release pro-inflammatory cytokines and growth factors, such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Once bleeding is controlled, immune cells migrate into the wound (chemotaxis) and promote the inflammatory phase, which is characterized by the sequential infiltration of neutrophils, macrophages, and lymphocytes. During this phase bacteria and cell debris are removed from the wound and soluble factors are released. They induce cell migration and also stimulate cell division in the next phase.

The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction.

In the last phase, the remodeling phase, collagen is reorganized and realigned along tension lines while apoptosis and phagocytosis of apoptotic bodies lead to the removal of unnecessary cells. In this phase, regression of many of the newly formed capillaries occurs, so that vascular density in the wound progressively decreases.

Interruptions, aberrancies, or lags in the process can lead to delayed wound healing or non-healing chronic wounds. Among the many factors which regulate the repair mechanisms, mechanical factors play a critical role during the healing process [Agha et al., 2011]. Also gravitational alterations can affect tissue repair: most of the studies on wound healing in μg conditions show delay and impairment in wound and fracture healing, mostly due to changes in the inflammatory and in remodeling phase [Delp, 2008; Radek et al., 2008].

Therefore, the possibility to carry out research in unloading conditions represents an unique tool to increase our knowledge on wound healing biomechanics and the role of gravity in the process.

Moreover, in the perspective of longer-term space missions, with a growing number of astronauts involved, wound healing must be regarded as a serious problem, because the risk of injury due to traumatic events or unexpected emergency surgery will increase while medical evacuation times to Earth will become incompatible with the urgency of implementing assistance procedures. Therefore, it is crucial to understand the impact of microgravity (μg) on the mechanisms underlying wound healing.

In the frame of these studies, we developed an in vivo model of wound healing based on leeches [Hirudo Medicinalis]. The model is obtained by performing a wound and a suture on the back surface of the animal. This invertebrate represents an interesting model, since the sequence of events occurring during the healing is similar as in vertebrates. Moreover, the leech is very resistant and, once nourished, can survive for some months without eating. For this reasons, it can be considered one the most suitable living beings to be used for experiments both in modeled and real μg, even during long-term space missions.

For the experiments in modeled μg, animals were anesthetized with a 10% ethanol solution. Surgical wounds (10 mm length, 2 mm depth) were performed on the dorsal surface of the animals (at about the 15th metamere) and sutured (DAFLON 4/0 wire). For the exposure to modeled μg, each leech was housed in a T25 flask filled with leech culture medium. μg was modeled by a Random Positioning Machine (RPM), rotation speed 60°/s, about 10–3 × g. After exposure to modeled μg, animals were anesthetized again and fixed in formaldehyde 4%, then dissected. Differences in wound healing between animals exposed to unloading conditions and controls (1xg) were analyzed by morphological imaging of the wound (Fig. 1), histology, autofluorescence and immuno-fluorescence microscopy. The results demonstrated that modeled microgravity induces a significant delay in wound closure and repair processes as proved by altered epithelium formation and matrix organization.

In order to counteract the microgravity-induced impairment in wound healing, we assayed in the leech model the effect of biochemical (Platelet Rich Plasma) and physical (NIR laser radiation) factors known to promote tissue repair. PRP is a concentrate of growth factors (normally released by platelets upon activation) which is obtained by blood centrifugation. The use of red and infrared radiation to favor wound healing is documented in literature applied in clinics. Our previous studies have shown that NIR laser radiation improves the cell energy metabolism, favors the formation of endothelial cell monolayers, improves the production and assembly of ECM components as fibronectin, proteoglycans and collagen I [Monici et al., 2011].

The results of preliminary experiments demonstrated that in the leech model both PRP and NIR laser application speed up and foster the healing process.

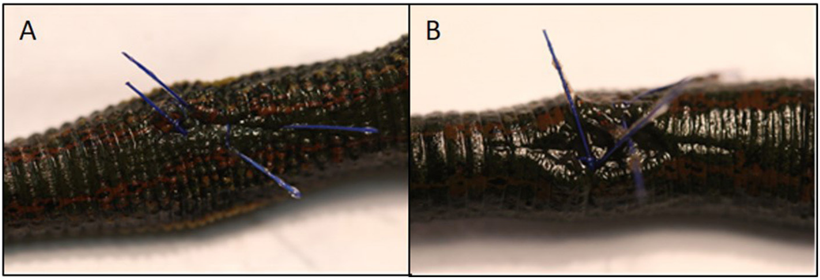

FIGURE 1: Effect of modeled microgravity (RPM) on in vivo model of wound healing. A Control; B Exposed to microgravity.

Keywords: wound healing, in vivo model, platelet rich plasma (PRP), hirudo medicinalis (Annelida), microgravity (μg), NIR laser radiation

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Morphofunctional peculiarities of ischemic and hemorrhagic injuries of the brain in rats at the modeling of the effects of microgravitation

Mikhail Baranov[1]*, Alexander Paltsyn[2], Galina Romanova[2], Fatima Shakova[2]

[1]*Federal Research and Clinical Center, Federal Medical-Biological Agency, Moscow, Russia*
[2]*Research Institute of General Pathology and Pathophysiology, The Russian Academy of Medical Sciences, Moscow, Russia*
**baranovm2000@mail.ru*

Information on the effects of microgravity at the central nervous system (CNS) is still fragmentary. At the same time, cognitive functions: memory, ability to learn and analyze situations, make decisions - can also change under microgravity. Besides, cerebro-vascular changes can impact the onset and course of both cerebral ischemic and hemorrhagic stroke if it occurs during the space flight.

PURPOSE

To assess microgravity effects at the formation of specific features in the development of brain injuries of various etiology.

The rat suspension model was used for modeling microgravity.

Several experiment series were made in which suit suspension (SS) of various duration preceded to or was made after experimental brain injury.

For the experimental model of ischemic stroke, photochemical thrombosis of blood vessels in the prefrontal cortex was used.

Cognitive functions of rats' brain were studied by developing a conditioned passive avoidance reflex (CPAR) [1]. CPAR stability was defined with latent period (LP).

Model of hemorrhagic stroke. A transplant consisting of crushed hemostatic collagen sponge and blood plasma of rabbit was injected into the cortex motor zone in the left hemisphere into the area which is responsible for movements of animal's right front paw.

There were several groups of rats in this experiment:

Group 1. Typical hemorrhagic stroke. Transplant contains acellular blood plasma.

Group 2. Typical hemorrhagic stroke. Transplant contains blood plasma rich with platelets containing angiogenesis stimulation factors (PRP).

Group 3. Typical stroke. Transplant (PRP). 7 days of SS after stroke.

Group 4. 7 days of SS. Typical stroke. Transplant (PRP).

Group 5. 2 days of SS. Typical stroke. Transplant (PRP).

Group 6. Vivarium control.

Animals' functional state was assessed with their motor activity. Motor activity was examined before surgery and on day 7 after it using test "walking on bar".

Morphological findings were used for defining: vasculature development, blood vessels caliber, neurons content, gliocytes, infiltrate cells; location density of binuclear neurons.

RESULTS

Microgravity negative impact at rats' cognitive functions was revealed even without additional pathological damage.

After exposure to microgravity in EG, average values of motor activity and CPAR indices were not significantly different from the baseline values. It was noted decrease in LP CPAR. In CG, motor activity was reliably reduced in comparison to initial levels after 14 days of staying in normal vivarium conditions, while CPAR latent period remained unchanged. Decreased motor activity in the control group and a conditioned passive avoidance reflex demonstrate that cognitive functions were preserved, since animals show less "interest" to repeated presentations to familiar "open field" device environment and keep in their memory unpleasant sensations of electric shock (CPAR). On the other hand, findings in experimental animals can be regarded as an adverse SS effect at cognitive functions. Less transition latent period indicates weakened conditioned passive avoidance reflex; animals have motor activity on the "open field" similar to the pre-experimental level what shows weak fixation of the information obtained during the first testing.

Than we studied an impact of ischemic stroke at cognitive functions and at the structure of brain prefrontal cortex. Animals were divided into control and experimental groups. For 14 days, controls were in vivarium, while experimental animals were in SS. On day 7, ischemic stroke was induced with the described procedure in both groups.

We can state that stroke in the prefrontal cortex causes significant memory impairments in rats of both groups, but there are no differences between the groups.

At the next stage, hemorrhagic stroke was chosen as a model of pathological process in brain. Number of binuclear neurons per unit area in rat's cerebral cortex was taken as an indicator of regenerative processes.

The highest mortality rate was in the group in which SS started after stroke modeling; and the lowest mortality rate was in Group 5 in which stroke was preceded by a short-term simulated microgravity.

The best restoration of motor function was in animals from Groups 4 and 5 in which hemorrhagic stroke was preceded by exposure to SS. The worst results were in Group 3.

Morphological examination of brain preparations from animals of Group 1 showed extravasates in the infiltrate, neoangiogenesis. However, vessels grow slowly; they are mostly of small diameter.

Group 2 - much more blood vessels. Vessels were of larger caliber and they were arterioles and venules. Significantly more macrophages; they were most often located in the vessel wall.

Morphological changes in Group 3 demonstrated signs of acute circulatory disorders. Serious circulation impairments - extravasates- which were not seen in Group 2, are often met in Group 3. Many longitudinally cut capillaries which are invisible under normal circulation, now become clearly seen and are indicative of disorders in blood outflow and blood stasis.

Group 4 has a larger number of newly formed vessels than Groups 1 and 3. These vessels are capillaries and more matured vessels - venules and arterioles. All animals of this group had extravasates at the border of infiltrate and penumbra which mainly consisted of loosely lying erythrocytes and rarely met dense clusters. There were no other circulatory disorders, such as blood stasis.

Group 5 has the largest number of newly formed vessels, both in the infiltrate and penumbra. There are no circulatory disorders, such as blood stasis or extravasates.

Groups 3 and 5 significantly differ from the controls with density of binuclear neurons locations. In Group 5, density of fusion locations in the stroke area significantly exceeds similar density at the same part of intact hemisphere. Injury triggers regeneration, but simultaneously it inhibits this regeneration with pathogenic changes in the stroke focus. That is why, regeneration intensity is always a ratio of stimulating and inhibiting factors. In Group 5, this ratio promoted regeneration what was manifested both morphologically and functionally.

The overall good result in Group 5 can be probably explained with effects of short-term microgravity which preceded damage; it increased venous pressure and thereby, contributed to collapse or dilatation of collaterals and anastomoses.

Keywords: rats, morphology, microgravity, hemorragic stroke, ischemic stroke

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Age peculiarities in the disease structure of cosmonauts after finishing their flight activity

Serguei Zakharov

Research Institute for Space Medicine, Federal Research Clinical Center of Federal Biomedical Agency of Russia, Moscow, Russia
docsu@mail.ru

INTRODUCTION

The reliability and safety of manned space programs are provided not only via the perfection of technical and control systems, but also via the perfection of medical health care and improved working capacity of space crews which implies questions of their prolonged professional life. The latter system is aimed to protect and maintain cosmonauts health and working capacity at all stages of their professional activity.

PURPOSE

To find out age-specific features in the morbidity structure of cosmonauts who have finished their flight activity.

MATERIALS AND METHODS

An in-depth survey of 26 cosmonauts aged 50-88 who took part in space flights and by now have finished their flight activity has been performed. The cosmonauts were divided into 3 age groups: Group A - second adult age, up to 60 years (n = 8); Group B - elderly age from 61 to 74 (n = 9); Group C - old age from 75 to 90 (n = 9).

Clinical diagnoses were coded according to the International Classification of Diseases, revision 10. The structure of disease incidence in cosmonauts is presented in percentage of the total number of examined cosmonauts.

RESULTS

In Group A, all the subjects had diseases of their digestive system and diseases of their urogenital system. Then, by prevalence, diseases of the musculoskeletal system, endocrine system, and eye diseases followed. Pathologies in the circulatory and respiratory systems were diagnosed in 75% of the examined.

Diseases of the circulatory system which affect 100% of the examined individuals occupy the first place in the disease prevalence both in Group B and in Group C. Diseases of the digestive system were also revealed in all examined subjects in Group B. 89% of the cosmonauts complained of problems with their urogenital system. Diseases of the musculoskeletal system and eye diseases were found in 78%. Diseases of the respiratory system and ear diseases in this group are twice lower than in group A and yield 44% and 33%, respectively. In addition to the above-mentioned differences, researchers registered neoplastic pathology in Group B.

All cosmonauts in Group C have diseases of their circulatory system, digestive system, and endocrine system. Diseases of the respiratory system and urogenital system were diagnosed in 78% of the examined. All cosmonauts in this group had surgical interventions what explains decreased incidence of eye and urogenital diseases in this age group.

CONCLUSIONS

1. Currently, 100% morbidity of the digestive system in all three investigated groups of cosmonauts is difficult to explain. Probably, it is due to the involvement of many factors and systems of the body into the pathogenesis of this group of diseases.

2. The lower percentage of circulatory system diseases in group A is associated with initially better health state in the cosmonauts. This effect is neutralized in older cosmonauts from Groups B and C.

3. If to follow the trend from younger Group A to older Group C, one can see a regular tendency in increasing the number of nosologies in every cosmonaut including neoplasms and surgical interventions.

Keywords: disease, morbidity, cosmonauts, peculiarities, activity - individual

Citation: *Zakharov S(2019). Age peculiarities in the disease structure of cosmonauts after finishing their flight activity. Front. Physiol. Conference Abstract: 39th ISGP Meeting & ESA Life Sciences Meeting. doi: 10.3389/conf.fphys.2018.26.00023*

Dose Tracker: An iOS app for collection of medication use data from volunteer crewmembers on the International Space Station

Virginia Wotring[1]*, LaRona Smith[2]

[1]Center for Space Medicine, Baylor College of Medicine, Houston, United States
[2]NASA-Johnson Space Center, Space Medicine Division, JES Tech, Houston, United States
**vwotring@gmail.com*

INTRODUCTION

During space flight, the body undergoes a number of physiological changes that would be expected to result in altered interactions with administered medications, but it is not yet known if, or to what extent, clinically-relevant pharmacology changes occur. The potential for alteration in either pharmacokinetics (PK, how the body handles administered medications) or pharmacodynamics (PD, how administered medications act upon the body) has long been a concern. Potential causes of spaceflight associated PK alterations include cephalad fluid shifting, which could alter absorption of orally-administered drugs and/or distribution of drugs to target tissues. There have been reports of a changes in gastrointestinal transit time (1), calcium absorption (2), and in the amounts of liver enzymes involved in drug metabolism (3–5). Regarding pharmacodynamics, tissue remodeling may alter cell phenotypes and expression of cell membrane receptors that serve as drug targets; it is well-demonstrated that bone (6) and muscle (7) exhibit significant remodeling during spaceflights. PK/PD studies are routine on Earth, but are not very feasible in spaceflight, requiring dozens to hundreds of subjects, and multiple blood draws over the course of 8-16 hours. This observational epidemiological study was a noninvasive attempt toward addressing the paucity of PK/PD evidence by regular direct questioning of crewmember volunteers regarding their user experiences with medications used during missions.

METHODS

App development

An examination was made of methods used previously to collect medication usage data. Mission medical records and physician notes only captured

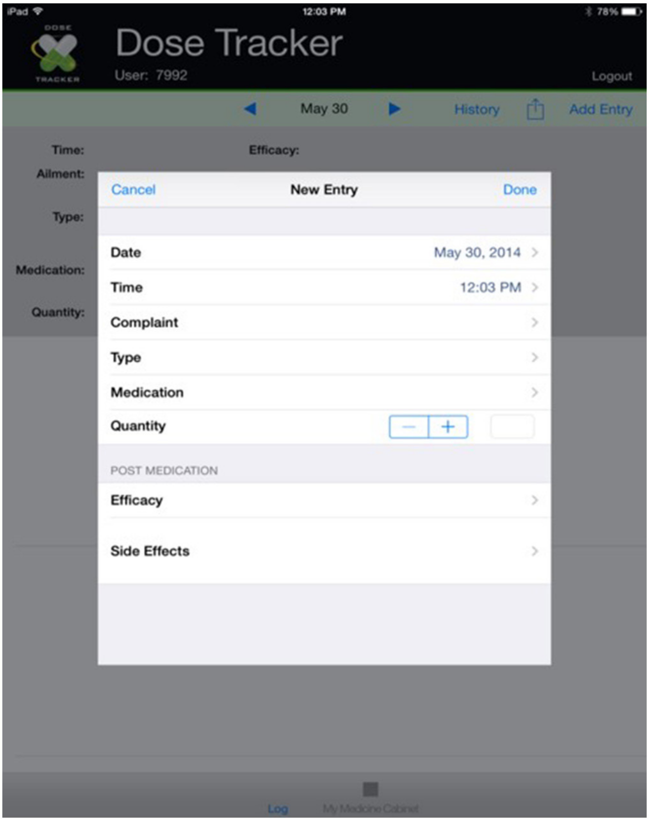

FIGURE 1: Screen shot of Dose Tracker main data entry page. The current date and time were used as defaults, but could be altered by the user to dates and times in the past. Designed to leverage the known contents of the ISS medical kit, the app showed likely choices of medications for each user-entered complaint, and likely side effects for each medication. Users could also freely type in entries.

data deemed relevant by flight surgeons or other clinical personnel, and did not routinely capture data that would be pertinent to potential PK/PD alterations. Post-flight medical debrief questionnaires could be lengthy, had low response rates and/or missing data (8), and did not include perceived side effects or efficacy.

For this study, a tablet-based questionnaire was designed to provide a streamlined individualized data entry system for the user. Specific questions regarding medication use were asked of each participating crewmember, somewhat different from the questions that physicians ask regarding patient health. These included subject's perception of drug efficacy (including pain ratings on the Wong-Baker scale for indications involving pain) and questions regarding the occurrence, severity and timing of any side effects.

The data collection process was streamlined with flexible programming that leveraged the limited medication choices aboard, the doses available, typical dosing frequency, and side effects associated with each medication (Figure 1). For chronically-used medications like a daily vitamin, the app permitted subjects to make a single medication usage entry that covered a specific range of dates. The study team requested input on the app from JSC Pharmacy, flight surgeons and crew during development and testing. The app was tested during 4 HERA missions at JSC, which was a very low-fidelity analog for pharmacological purposes since medication use is not typically permitted in this analog.

IMPLEMENTATION

This study was approved by the Institutional Review Boards of Baylor College of Medicine and NASA Johnson Space Center. Approximately one year prior to a planned ISS mission, astronaut subjects were briefed regarding the study and were consented to participate. Subjects were trained on app usage and were asked to record all medication usage, including prescription, over-the-counter, and nutritional supplements. Pre-flight or post-flight, each participating crewmember recorded their medication usage for a duration equivalent to their on-orbit mission, so that their ground medication usage frequencies, doses, and perceptions could be compared to those recorded during their spaceflight mission.

Coded (de-identified) data were delivered weekly to a secure server for analysis by study investigators. The study was written and approved for a total of 24 subjects, in order to yield at least 6 subjects using common medications. One aim of the study was to acquire sufficient in-flight medication usage data to provide the necessary variance and effect-size information required to properly power future studies when they are needed.

RESULTS

Seven subjects consented to participate during the abbreviated study period. One subject dropped before the beginning of data collection. Five subjects completed both flight and ground data collection; one additional subject completed only data collection during flight.

A total of 5766 records of medication use were collected. There were 20.63 $\pm$ 8.47 entries per subject per flight week; 15.62 $\pm$ 4.58 per ground week (mean $\pm$ SEM).

Notably, the app collected 49 reports of no medication use in a given week of data collection, providing positive confirmation that a crewmember was not using medications. Medical records had no such indication, forcing researchers to instead rely on the (possibly incorrect) assumption that no recorded data means no medication usage.

Subjects also provided feedback and usability of the app, which was mixed. A number of lessons learned have been captured regarding the need for multiple secure login steps, time zone issues on Earth, issues with the Apple iOS operating system, and NASA processes for software updates and data transfers. Procedures to ensure subject privacy were cited as the reason for multiple complaints. One subject requested that data be shared with their flight surgeon. Using the app to provide information for medical care was not planned, and required special approval from the JSC IRB to arrange. Data sharing between the research study and the clinic was successfully managed for the mission duration and indicates a level of clinical interest in the data collected by the study.

DISCUSSION

The study demonstrated a significant increase in the amount of medication usage information being collected. Self-reports of medication use collected by the app were higher than found in medical records, by more than an order of magnitude (9). This is now being used as rationale to update medical operations requirements for the collection of this type of information on the ISS.

The funding agency, NASA Human Research Program, elected to end this study well before its planned 24 subjects could be completed. With only 5 subjects, it is not possible to achieve the aims of collecting data regarding inflight PK or PD changes. It is hoped that the lessons learned will prove useful for future attempts to record astronaut medication use in a timely and complete fashion.

ACKNOWLEDGEMENT

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Keywords: pharmacology, pharmacy, pharmacokinetics, pharmacodynamics, microgravity, spaceflight

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Human performance in altered-gravity environments

Ana Diaz Artiles

Department of Aerospace Engineering, Texas A&M University, College Station, United States
adartiles@tamu.edu

Astronauts undergo important physiological deconditioning in space due to the weightless environment. Some of the most common issues include bone loss, muscle atrophy, cardiovascular deconditioning, spaceflight-associated neuro-ocular syndrome, and neurovestibular alterations. Currently, several countermeasures are in place that have notably reduced the detrimental effects of weightlessness, but they require significant crew time and resources. New approaches, possibly combining novel and current countermeasures are needed, especially for longer missions such as a trip to Mars where astronauts will not have the ground support usually provided when landing on Earth.

Artificial Gravity (AG) has often been proposed as a multi-system countermeasure capable of mitigating most of the physiological deconditioning occurring in reduced gravity levels, particularly if combined with exercise (Clément 2017; Clément, Charles, and Paloski 2016). Artificial gravity has never been tested in space as a countermeasure, but multiple ground studies have shown its benefits in various physiological systems, including cardiovascular, neurovestibular, and musculoskeletal systems. Despite the potential benefits, many questions still remain about its implementation. Aspects such as the appropriate gravity level, centrifuge configuration, radius, angular velocity, exposure time, exercise modality, exercise protocol, or safety concerns are still unanswered, and require further investigation.

Our artificial gravity research program at the Bioastronautics and Human Performance group at Texas A&M University focuses on investigating human performance in altered-gravity environments to inform future AG implementation as a spaceflight countermeasure. We are using both experimental and computational approaches to develop quantitative models to predict partial-gravity dose-response relationships (i.e. the relationship between a physiological response and the magnitude of G-level) and test the new models in experiments with human subjects. Our current and future research platforms include a short-radius centrifuge, a tilting bed-platform, parabolic flights, and a reduced-gravity treadmill.

In our previous research efforts, we conducted a human experiment using a short-radius centrifuge to quantify the effects of gravity level and cycling exercise intensity on multiple aspects of human physiology, including cardiovascular responses, foot forces, and comfort. This was the first study to characterize and quantify the transient cardiovascular response in human subjects to a combination of multiple levels of artificial gravity and exercise intensity in a short-radius centrifuge. All subjects tolerate well the centrifugation protocol, and our quantitative results showed that the addition of artificial gravity to exercise could provide a greater cardiovascular benefit than exercise alone (Diaz Artiles 2015; Diaz, Heldt, and Young 2015; Diaz, Trigg, and Young 2015). Using these experimental data, we developed and validated a unique lumped-parameter model of the cardiovascular system that includes both the effects of gravity gradient and ergometer exercise. Identical centrifugation and exercise profiles were simulated and compared to the experimental data and results show that the model is capable of reproducing the cardiovascular changes due to both centrifugation and exercise, including the dynamic responses during transitions between the different phases of the protocol (Diaz Artiles 2015; Diaz Artiles, Heldt, and Young 2016).

Current and future research efforts include a human experiment using the tilt-platform to study cardiopulmonary responses to ergometer exercise in multiple simulated hypo-gravity conditions (Berg and Diaz Artiles 2017; Perez, Navarro Tichell, and Diaz Artiles 2018). Tilt paradigms are commonly used to simulate hypo-gravity conditions. The principle is to recreate the fluid shift from the lower extremities towards the upper part of body that occurs when the gravitational force is partially or completely removed. A head-down tilt (HDT) of -6 degrees is widely accepted as a microgravity analog. Similarly, we can recreate the partial fluid shift conditions at different gravity levels by using the appropriate tilt angles (Head-up tilt or HUT) and considering the gravitational component in the head to-toe direction (Gz) We are particularly interested in the following environments: microgravity: -6° head down tilt; Moon: +9.5° head up tilt (HUT); Mars: +22.3° HUT; and upright. These four conditions will provide a good quantitative set of data to generate the gravitational dose-response curve of the cardiopulmonary system during exercise in the hypo-gravity domain.

Additionally, we are implementing a comprehensive sensitive analysis of our cardiovascular model using Latin Hypercube Sampling/Partial Rank Correlation Coefficient (LHS/PRCC) techniques to study output changes due to subject variability. Simulations are also being conducted under different gravitational conditions, both constant gravity environments as well as artificial gravity created by a short-radius centrifuge, which generates a strong gravity gradient along the body Gz direction. (Alonso and Diaz Artiles 2018). Results from this study will provide quantitative information about the effect of individual differences on cardiovascular responses to orthostatic stress. Additional modeling efforts include the extension of the current capabilities of this model by introducing pulmonary and metabolic effects, as well as long-term effects, including time-dependencies in model parameters and blood volume regulation mechanisms.

Keywords: exercise, computational modeling, centrifuge, cardiovascular responses, human spaceflight, artificial gravity, human experiments, space countermeasure, orthostatic intolerance, gravitational dose-response curves

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Tissue repair and regeneration in space and on earth

Monica Monici[1*], Francesca Cialdai[1], Michele Balsamo[2], Liyana Popova[2], Alessandro Donati[2], Daniele Bani[1], Paolo Romagnoli[1], Jack J.W.A. van Loon[3], Desire' Pantalone[4]

[1]ASAcampus JL, ASA Research Division, Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy
[2]Kayser Italia, Livorno, Italy
[3]Department of Oral and Maxillofacial Surgery/Oral Pathology, Medical Center, VU University Amsterdam, Amsterdam, Netherlands
[4]Department of Surgery and Translational Medicine, Careggi University Hospital, Florence, Italy
**monica.monici@unifi.it*

Repair/regeneration is the process that makes organisms resilient to injuries (trauma, burns, accidental wounds, surgery), allowing survival, restoration of the protective barrier and organism integrity.

Remarkable progress has been achieved in understanding the cellular and molecular mechanisms of tissue repair, but many of them still remain elusive and many questions are unanswered. For example, it remains unexplained why mammals have a tendency for imperfect healing and scarring, rather than full regeneration. Also the causes of failure in tissue repair mechanisms are poorly understood and currently the available therapies are limited [Eming et al., 2014].

It is known that wound healing is a complex set of processes, involving a number of cell lineages. Alterations in one or more of these events, that overlap and intertwine, result in defective repair, ranging from delayed healing and chronic ulcers to scarring and fibrosis. The different steps of tissue repair are regulated by a multitude of biochemical and physical factors, including gravitational/mechanical forces acting at cellular and tissue level. Moreover, several factors related to the state of patients and operative modalities are important for tissue repair and its complications: age, gender, overweight, systemic diseases (e.g. diabetes), wound contamination, non-physiological environment, urgency, emergency care, wound care, suturing materials and techniques.

On Earth, traumatic injury is a major cause of mortality and disability. In addition, millions of surgical wounds are performed annually in the course of routine surgery and medical care. Therefore, defective or delayed wound healing, chronic ulcers and fibrosis are important health and socio-economic problems [Sen et al., 2009]. They are caused by alterations mechanisms underlying repair, such as dysregulated immune function, chronic inflammation, impaired fibroblast function, defective ECM deposition, altered endothelial function, dysregulated apoptosis, etc...

Until now wound healing has not been considered as a major health problem in space because, during the current space missions, the occurrence of injuries, traumatic events and surgical emergencies has been considered unlikely. Despite the current considerations, future space exploration programs require long duration missions beyond LEO and hence health emergencies on board cannot be excluded, while medical evacuation times to Earth might become too long and the communication lag would render useless to guide the crew actions remotely. Therefore, medical care planning for future exploration missions should consider emergency surgery and trauma care, and meanwhile studies on repair processes in space are of paramount importance as wound healing is critical to survive trauma or surgery. Results of our previous studies, in agreement with those of other authors, showed microgravity-induced changes in mechanisms underlying tissue repair [Monici et al., 2011; Cialdai et al., 2017]. Experiments performed in real and simulated microgravity revealed alterations in fibroblast and endothelial cell function, changes in ECM production and dysregulation in apoptosis. Interestingly, in astronauts, deficient immune function, signs of chronic inflammation and insulin-resistance have been observed. These alterations, resembling some features of systemic diseases which impair wound healing on Earth, could affect the body's response to injury and could represent a model to study defective healing mechanisms [Demontis et al., 2017].

The experiment "Wound Healing and Sutures in Unloading Conditions", selected by ESA (ILSRA-2014-0043), aims to study the behavior of "in vitro" sutured wound models in weightlessness (ISS). The findings are expected to increase the knowledge on wound healing in weightlessness, suggest new pathways for tissue engineering, give cues for strategies promoting tissue repair/regeneration and improving the management of defective healing in space and on Earth.

The on-ground activities carried out in preparation of the experiment can be summarized as follows:

1) Standardization of procedures for biopsy collection, model preparation, techniques for model culture and postflight analysis; 2) improvement of culture techniques to ensure tissue survival throughout the experiment; 3) analysis of tissue mechanical properties and development of a device to model physiological tensile strength in the tissue and measure its changes due to suture behavior and the healing process.

The sutured wound models developed for the experiment are based on skin and vein vessel biopsies cultured on dedicated hardware, in conditions that ensure their survival for over 3 weeks and allow to stretch the tissue, mimicking the physiological tensional strength, and monitor tension changes due to suture application and repair process at the wound site. Hence, not only histological and biochemical aspects, but also the biomechanical properties of sutured wounds can be analyzed.

For the in-flight experiment, after collection biopsies are maintained in modified, RPMI-based, organ culture medium at 4°C for storage and travelling to the launch site. Here, linear wounds are performed on skin by a scalpel and then sutured. The vessels are completely divided to perform an end-to-end vascular anastomosis. RPMI-based medium is replaced with modified, DMEM-based, organ culture medium. On the ISS the sutured wound models will be placed at 32°C for activation of repair mechanisms and then frozen at different time-points. During the experiment, the tensile strength in tissues will be monitored and morphological imaging of the sutured wounds is performed. In order to obtain information on the activation of tissue repair mechanism in weightlessness, post-flight analysis is scheduled to study: suture morphology and ultrastructure (histology and electron microscopy); proteomics on membrane microdomains, gene expression profile, extracellular matrix turnover, apoptosis and necrosis, markers of repair mechanism activation, fibroblast behavior, stiffness and strength in sutured tissues.

Beyond the development of the sutured wound models, their survival for a sufficient amount of time (at least 3 weeks) has been identified as a crucial point for the success of the experiment. Focusing on this problem, we developed a tissue culture technique based on tissue stretching and modification of organ culture media by adding

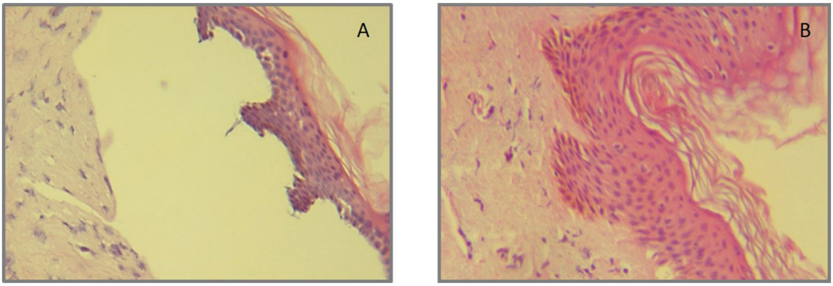

FIGURE 1: Skin samples cultured for over 3 weeks in common culture conditions (**A**) and using the culture technique developed for the in-flight experiment (**B**), which allows a better survival and preservation of the tissue.

proangiogenic substances and peptides. In addition to defining the requirements for the in-flight experiment, the on-ground activity has produced a significant result: this technique, which allows the survival of the tissue biopsies for over 3 weeks (Fig 1), can be applied also on ground in tissue culture and engineering for transplantation and regeneration, studies on tissue mechanical properties, studies for improvement of surgical techniques and materials.

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Keywords: sutures, wound healing, emergency surgery, trauma care, tissue repair and regeneration, microgravity (μg)

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The Coenzyme Q10 as an antiapoptotic countermeasure for retinal lesions onboard the International Space Station

Matteo Lulli[1]*, Francesca Cialdai[2], Leonardo Vignali[2], Monica Monici[2], Sara Luzzi[3], Alessandro Cicconi[3], Stefano Cacchione[3], Alberto Magi[4], Michele Balsamo[5], Marco Vukich[5], Gianluca Neri[5], Alessandro Donati[5], Sergio Capaccioli[6]

[1]*Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Università degli Studi di Firenze, Florence, Italy*

[2]*ASAcampus JL, ASA Research Division, Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy*

[3]*Dipartimento di Biologia e Biotecnologie Charles Darwin, Sapienza Università di Roma, Rome, Italy*

[4]*Department of Experimental and Clinical Medicine, University of Florence, Firenze, Italy*

[5]*Kayser Italia, Livorno, Italy*

[6]*Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università degli Studi di Firenze, Florence, Italy*

**matteo.lulli@unifi.it*

Health of astronauts operating in the International Space Station (ISS) is a major concern for Space Agencies. In addition to a variety of intrinsic environmental issues, the astronauts are exposed for some months to damaging effects of microgravity and low level of high energy solar and cosmic radiation entering the ISS, which ultimate outcome at cellular level is apoptosis (1). Eye, and especially the retina, is one of the most critical and sensitive districts of astronaut organism. Several in vitro and in vivo studies in the retina have shown that microgravity and cosmic radiation generate reactive oxygen species (ROS), which in turn damage retinal cells and induce apoptosis as well as inflammatory response (2-9). Since effective prevention strategies have been scarce so far, we aimed to find pharmacological countermeasures within the CORM project funded by the Italian Space Agency, exploiting the antiapoptotic activity of the Coenzyme Q10 (CoQ10), the unique lipid soluble antioxidant synthesized endogenously. We have previously demonstrated that CoQ10 inhibits apoptosis of corneal keratocytes subjected to excimer laser irradiation (10, 11) and that this was due to its ability to hinder mitochondrial depolarization (12). Successively, we demonstrated that CoQ10 inhibits retinal cells apoptosis induced by radiation both in vitro and in vivo (13) and, if applied as eye drops on the cornea, it reaches the retina protecting the retinal layers from excitotoxicity-induced apoptosis (14). Therefore, we considered CoQ10 as a promising countermeasure to prevent retinal damages elicited by microgravity and cosmic radiation (Figure 1).

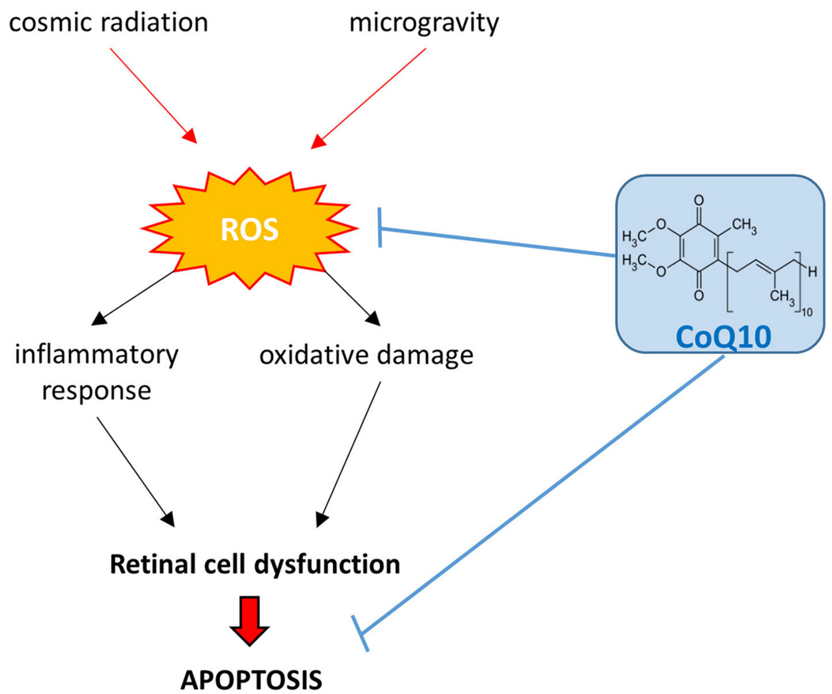

FIGURE 1: Proposed role of CoQ10 in the pathophysiology of cosmic radiation- and microgravity-induced retinal damages.

The CORM project involved two experimental phases. The first was the demonstration of CoQ10 ability to counteract radiation- and simulated microgravity-induced alterations of the human retinal pigment epithelial ARPE-19 and mouse retinal ganglion RGC-5 cells. The second was the execution onboard the ISS in the frame of the ASI VITA mission of an experiment driven by the Italian astronaut of the European Space Agency (ESA) Paolo Nespoli. The experiment has been primed at the Kennedy Space Center (FL, USA) inside specific hardware developed by Kayser Italia integrated in the Kubik incubator of ESA, launched to the ISS by the SpaceX-12 vehicle and recovered to the Earth with the same vehicle. ARPE-19 cells treated and not treated with CoQ10 have been subjected to space environment onboard the ISS for 72h. Once back to the Earth, the following parameters have been evaluated: apoptosis rate, cytoskeleton morphology, telomere length and integrity, RNA and DNA whole sequence.

Experiments on ground revealed that CoQ10 prevents simulated microgravity-induced apoptosis and cytoskeleton alterations in ARPE-19 and RGC-5 cells, and lowers X-radiation-induced accumulation of telomere-induced foci (TIF) as well as senescence promotion in ARPE-19 cells (5).

The results obtained in the CORM project will reveal the damaging effect of the ISS environment on human retinal cells and could qualify CoQ10 as an effective countermeasure. Obviously, this may have a major impact on the Earth to treat different human retinopathies, ranging from glaucoma to age-related macular degeneration, characterized by apoptotic cell death.

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Keywords: apoptosis, retina, antioxidant, ISS, Coenzyme Q10 (CoQ10), microgravity (μg)

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Spaceflight meets geriatrics!

Nandu Goswami

Chair of Physiology Division, Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation, Medical University of Graz, Graz, Austria
Head of Research Unit: "Gravitational Physiology Aging and Medicine", Medical University of Graz, Graz, Austria
nandu.goswami@medunigraz.at

INTRODUCTION

This paper presents a general overview of important physiological effects induced by spaceflight, the aging process in humans on Earth, and important connections between these physiological states. Developing an understanding of links between spaceflight physiology and the physiology of the aging process can provide insights and methods that improve both human health in older persons on Earth and the health and effectiveness of astronauts living in microgravity.

Ever since our ancestors started walking upright, our body has adapted to the impact of gravity. During standing, for example, although the human heart is positioned below the brain, it is able to pump enough blood to the brain against the force of gravity to maintain proper brain function. Furthermore, during standing, gravity induces blood pooling in the legs. This is counteracted by several mechanisms including muscle activity in the lower limbs which together with one way valves in the veins acts as a muscle pump to return blood to the heart which is further supported by the act of breathing. Additionally, the weight-bearing bones and anti-gravity muscles have adapted during evolution to ensure adequate support during standing. Thus humans can stand up without any real problems.

The real importance of gravity on physiological systems is, however, revealed in the reduced gravitational environment (microgravity) encountered during spaceflight. The impact of microgravity can be in many important physiological systems, including the cerebral autoreglatory control system, the cardiovascular and cardipostural systems, as well as sensorimotor and musculoskeletal systems (Blaber et al., 2011; Goswami et al., 2013; Hargens et al., 2013; Morey-Holton et al., 2003; O'Shea et al., 2015; Sandler et al., 1986; Schneider et al., 2009; Vernikos et al., 2010; Waha et al., 2015). For instance, cardiovascular deconditioning remains a persistent problem associated with the time spent in microgravity during spaceflight (Antonutto et al., 2003). The return to to Earth and normal gravity, results in a number of important reactions which can include increased heart rate, dizziness upon standing up (decreased orthostatic tolerance) and a reduction in exercise capacity (Buckey et al., 1996).

Physiological deconditioning as seen in spaceflight also occurs on Earth, especially as a consequence of the aging process and also due to bed-confinement and/ or immobilization. Many conditions and diseases including metabolic or endocrine disorders, cerebral or peripheral vascular disease, cardiac arrhythmias, and autonomic neuropathy, can lead to orthostatic intolerance (dizziness and loss of consciousness upon standing up), especially during changes in posture from lying/ sitting. Furthermore, illness or injury in older persons frequently requires hospitalized based care which often includes bed confinement and immobilization. It is important to observe that immobilization during hospitalization also represents a significant source of deconditioning and decline in physiological function, which in older persons ca be an important additional factor contributing to a continiuing negative spiral of increasing frailty, orthostatic intolerance and greater risk and occurrence of falls (Mühlberg and Sieber, 2004).

Bedrest is a ground-based model for weightlessness that has been applied to investigate the effects of spaceflight on the functioning of human physiological systems as observed during the microgravity of space flight (Goswami et al., 2015; Jost, 2008; Pavy Le Traon et al., 2007). In the bedrest study protocol, subjects are restricted to the supine position over various durations of time which can be days or weeks. The bedrest protocol provides an experimental set up which is highly controllable and allows for investigating changes in physiological function during reduced gravitational stress and has frequently been employed by space research agencies (Arzeno et al., 2013; Cvirn et al., 2015; Oshea et al., 2015). Since older persons in hospital can spend as much as 80% of their time confined to bed, bedrest studies can also help in furthering our understanding of the deconditioning process during hospitalization in older persons (Figure 1).

During spaceflight, astronauts devote a substantial amount of time to physical training with the goal to both reduce deconditioning due to microgravity during flight but also to reduce the problem of orthostatic intolerance after return to normal gravity on Earth after flight (Petersen et al., 2016). They also complement their training regimes with nutrition and other measures to ensure optimal health. Pedersen and colleagues (2016) have reported

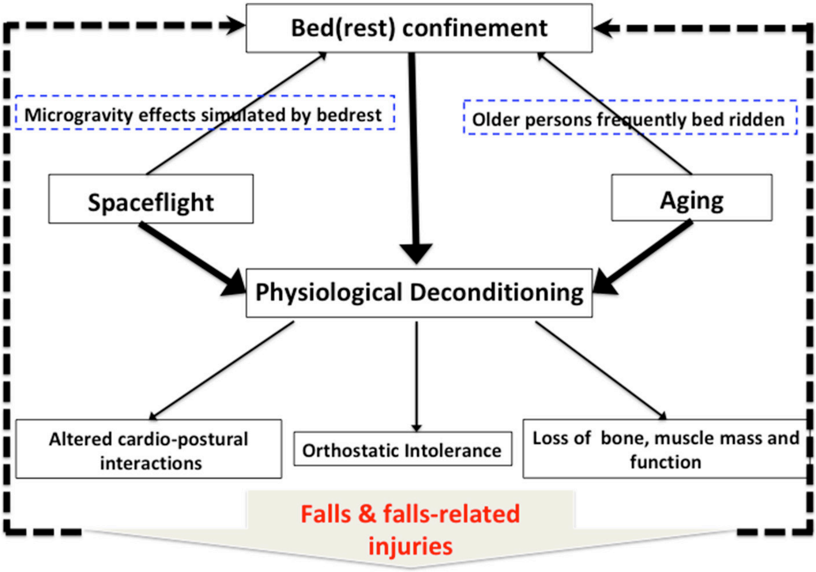

FIGURE 1: Bedrest (confinement), spaceflight and aging induced physiological deconditioning. Shown also are the effects of the deconditioning on cardio-postural interactions, orthostatic tolerance and skeletal muscles thus leading to falls and falls-related injuries.

recently that, following the introduction of the advanced resistive exercise device (ARED), 8 years ago at the ISS, resistance exercise has increased as a component of the planned exercise regimen during space flight.

Could such physical activity programs carried out by astronauts in space be used during bedrest immobilization in older persons to counteract deconditioning as well? Early intervention in bed-confined older persons is vital, as typically, without such intervention, rapid declines in bone and muscle mass as well as functionality are experienced (Singh et al., 2008). Started early enough, remobilization interventions can overcome the decline of physiological function, leading to complete recovery. However, delayed intervention often results in incomplete recovery where patients can be left with reduced physiological functionality. Unfortunately, re-mobilization is started too late in many cases and patients suffer a permanent loss of their functionality, leading as well to a loss of autonomy and the ability to lead an independent life, and resulting to increased risk of mortality (Singh et al., 2008).

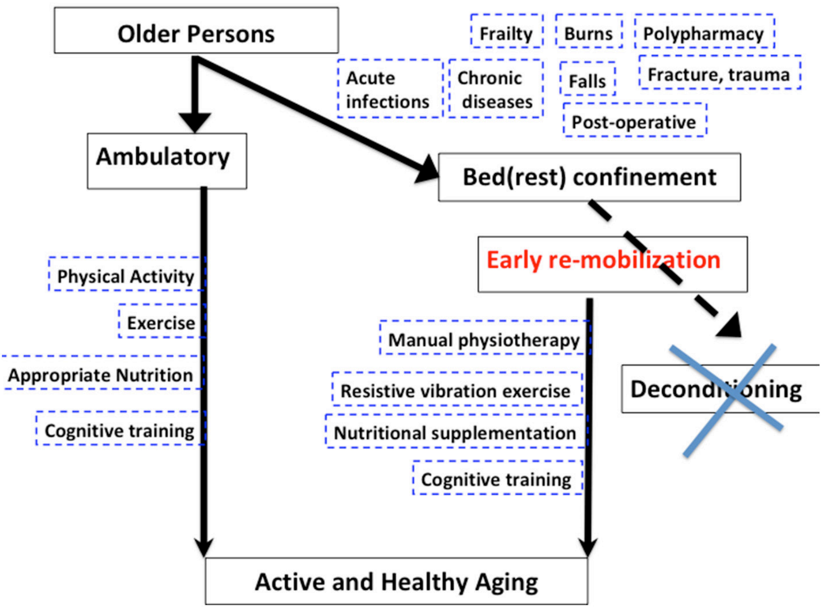

FIGURE 2: Frailty in older persons and how it can contribute to bedrest confinement. The figure also depicts the need to ensure early (re-)mobilization in bed(rest) confined persons via application of effective coutnermeasure to ensure healthy and active aging.

Intervention combinations in older persons confined to bed could incorporate physical exercise and/ or nutrition (Figure 2). While some evidence exists indicting that physical activity is beneficial for maintenance of physical functionality as well as mental health in older persons (Olanrewaju et al., 2016), there is limited literature that has examined the effectiveness of various forms of physical activity in combating the negative consequences of bed-confinement in older persons. Recent data generated from bedrest campaigns related to space research suggest that resistive vibration exercises can maintain muscle strength and function. Even though these data were obtained in young subjects confined to bedrest, resistive vibration exercise could provide an important method to address the problems of deconditioning in bed-confined older persons. This illustrates how data generated from ground based analogs of microgravity could have application in geriatrics.

There is increasing evidence that inadequate food intake and nutrition in general is strongly associated with risk of frailty in older persons (Martone et al., 2013). The consequences of malnutrition include increased morbidity, functional decline, increased/early dependency and institutionalisation, increased re-admission following hospital discharge as well as greater mortality. However, evidence is limited on the degree of benefit that improved nutrition alone can provide in older persons (Muscaritoli et al., 2016). In this regard, Strandberg et al. (1985) found that nutritional therapy, combined with resistive exercise training, can improve muscle mass in older persons. Moreover, another study indicated that a diet high in protein inceases both muscle strength and lean tissue mass in older women when combined with resistive training (Daly et al., 2014). Similarly, spaceflight data have also shown that resistance exercise, together with proper nutrition, including Vitamin D, is effective in maintaining physiological functionality in astronauts during spaceflights of up to six months duration (Smith et al., 2012). These observations indicate that knowledge obtained from space research can provide guidance regarding supplementing and optimizing the effects of physical exercises by including nutritional supplementations and/or pharmacological interventions in an integrated approach to therapy, which could represent key innovations in tackling bed-confined deconditioning, especially in older persons (see Hackney et al., 2015).

The call-out box summarizes the important aspects of this paper:

- Integrating what is known about deconditioning due to minimal gravitation stress in the microgravity of space and deconditioning induced by bed rest, provides an opportunity to develop a more comprehensive understanding of deconditioning (Figure 1) and lead to the development of countermeasures.

- To reduce the deconditioning effects of microgravity, astronauts during spaceflight schedule regular sessions of specialized exercise trainng.

- Could these exercises carried out by astronauts be used to counteract deconditioning in bedconfined older persons?

- Additional countermeasures can incorporate nutrition, and innovative training methods to improve muscle function and strength, as well as cardiovascular and cardio-postural functions (Figure 2).

- Early remobilization after a period of confinement to bed in older persons can prevent orthostatic intolerance, falls and injuries related to falls.

CONCLUSIONS AND FUTURE PERSPECTIVES

Developing an understanding of parallels and sharred aspects between the physiology of humans in reduced gravity and the physiology of aging provides the opportunity of deriving new insights and new strategies of intervenation relevant for both spaceflight physioslogy and the physiology of aging. For example, studies involving bedrest, in addition to simulating spaceflight induced deconditioning, can also provide information on deconditiong during confinement in bed, for example during hospitalization, a common occurrence with special impact in older persons who undergo surgery or must be treated for injury or are subject to debilitating diseases. Indeed the potential exists for a powerful convergence of knowledge between microgravity induced deconditioning and bed rest induced deconditioning in older persons, which could be useful in the development of countermeasures in both conditions.

The parallels between the physiological consequences of aging and microgravity, and strategies that apply spaceflight technology to help life on Earth, especially in relation to bed-confined older persons should be further explored and recommendations developed that incorporate the important aspects of deconditioning and muscle loss, nutrition, and cardiovascular/ cardio-postural functions, which are involved in the aging process and/ or the effects of long term bed confinement.

Keywords: aging, orthostatic intolerance, falls, spaceflight, bedrest, immobilazation

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Microgravity-based modulation of VEGF expression in human thyroid carcinoma cells

Daniela Melnik[1], Marcus Krüger[1*], Sascha Kopp[1], Markus Wehland[1], Johann Bauer[2], Manfred Infanger[1], Daniela Grimm[1,3]

[1]*Clinic for Plastic, Aesthetic and Hand Surgery, Otto von Guericke University Magdeburg, Magdeburg, Germany*
[2]*Information Retrieval Services, Max Planck Institute of Biochemistry, Martinsried, Germany*
[3]*Department of Biomedicine, Aarhus University, Aarhus, Denmark*
**marcus.krueger@med.ovgu.de*

When human thyroid carcinoma cells are exposed to microgravity some adherent cells detach from the bottom of the culture flask and grow into floating multicellular spheroids. It is assumed that this process mimics metastasis. The spheroids generated are more complex than a cell monolayer and exhibit properties of small tumours, which cannot be found in a normal cell culture. Therefore, tumour cell spheroids are important structures in cancer research, for example to find new targets for anticancer therapy.

We investigated the effect of real microgravity on the transcriptome and proteome of FTC-133 human follicular thyroid cancer cells returned from the CellBox-2 Space mission. The cells had been cultured for 5 and 10 days in six automated hardware units on the International Space Station before they were fixed and sent back to Earth. Spheroids were formed in all of the hardware units. They differed from spheroids generated under simulated microgravity on a Random Positioning Machine (RPM) mainly by an enhanced release of the vascular endothelial growth factor (VEGF). VEGF has a key role in the production of tumour vasculature. Its enhanced production may hint at a different angiogenic potential of thyroid cancer cells in space.

INTRODUCTION

Tumour growth and development depend on neovascularization, that is strictly regulated by growth factors binding to receptor tyrosine kinases.

Vascular endothelial growth factor (VEGF) is essential for the formation of new vessels in different developmental processes, pathological conditions, tumour growth, and metastasis (Lin and Chao, 2005). VEGF is up-regulated in thyroid malignancies (Karaca et al., 2011) and initiates an endothelial cell-specific signalling pathway via Flk-1/KDR which is required for blood vessel formation. Inhibition of this pathway has become an important therapeutic target as it blocks pathological angiogenesis in growing tumours, leading to stasis or regression of tumour growth (McMahon, 2000).

Exposure to prolonged microgravity (µ*g*) can influence fundamental processes in human cells including cancer cells. Numerous studies that were performed in the simulated µ*g* (s-µ*g*) of a Random Positioning Machine (RPM) or clinostat have revealed alterations of apoptosis, proliferation, differentiation, growth behaviour, cell adhesion, extracellular matrix, and cytoskeleton (Grimm et al., 2002; Warnke et al., 2014). Moreover, the scaffold-free formation of multicellular spheroids (MCS) was observed for many times (Pietsch et al., 2010; Pietsch et al., 2011; Grosse et al., 2012; Pietsch et al., 2012; Pietsch et al., 2013; Bauer et al., 2017; Sahana et al., 2018).

In 2017, the CellBox-2 experiment was set up to investigate FTC-133 cells in real µ*g* (r-µ*g*) in space during a long-term-experiment of 5 and 10 days. In the current study, we analysed the VEGF gene expression and its release. To evaluate differences of r-µ*g* and s-µ*g*, we performed an additional experiment with an RPM using the protocol and timeline of the CellBox-2 mission.

MATERIALS AND METHODS

Cell culture

The human follicular thyroid cancer cell line FTC-133 were cultured in RPMI-1640 medium supplemented with 10% FCS and 1% penicillin/streptomycin at 37°C and 5% CO_2 until use for the experiments.

Flight preparation and CellBox-2 mission

Cells were sent to the International Space Station (ISS) by a Falcon 9 rocket of SpaceX CRS-13 from Cape Canaveral (USA). 2d before launch, the FTC-133 cells were filled in 6 automated hardware units (FM 1-6, Fig. 1) with a cell suspension containing 1×10^6 cells and were kept at 23°C. Each hardware unit

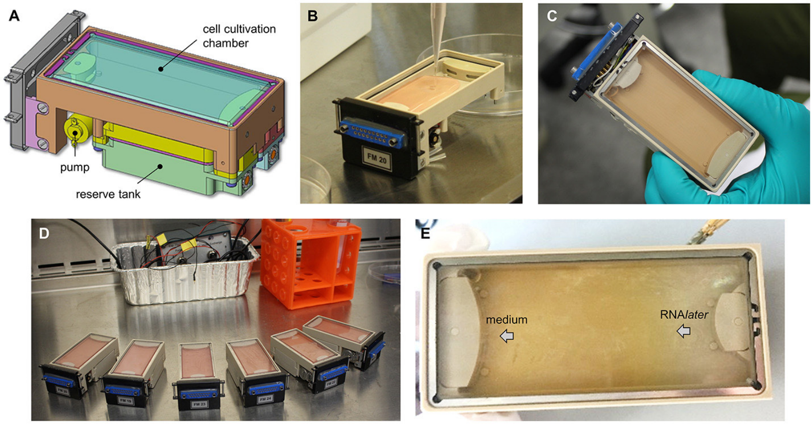

FIGURE 1: Flight hardware for the CellBox-2 experiment. (**A**) Schematic drawing of an automated hardware unit showing the main components. (**B**) Filling of medium and cells into the cell cultivation chamber. (**C**) The assembled hardware unit. (**D**) The complete set of 6 hardware units for the space experiment. (**E**) Fixation of the cells inside the hardware unit after 10d under 1*g* conditions (ground control). The pump is injecting RNAlater (white fluid from the right) in the cell cultivation chamber and simultaneously removing old medium (yellowish, on the left).

consists of a cell cultivation chamber, two reserve tanks for medium and fixative and a pump for fluid exchange. 24h before launch, FM 1-6 were loaded into the rocket. 2d after launch, FM 1-6 were installed on the ISS marking the starting point of our experiment. The FTC-133 cells were stored at 23°C. After 5d in µ*g*, the first medium exchange was performed automatically in all six FMs. Afterwards, the cells of FM 1-3 were fixed automatically with RNAlater. After another 5d, the procedure was repeated with FM 4-6. Finally, all FMs were stored at 4°C until their flight back to Earth.

To investigate the effect of µ*g* the same timeline and handling were used for the 1*g*-control group on ground.

RPM experiment

The RPM experiment was performed as described in (Ma et al., 2014), but with the time and temperature profiles of the space experiment.

RNA extraction and quantitative polymerase chain reaction (qPCR)

RNA extraction and qPCR were performed as recently described in (Kopp et al., 2018). The sequences of the *VEGFA* forward and reverse primers were: 5`-GCGCTGATAGACATCCATGAAC-3` and 5`-CTACCTCCACCATGCCAAGTG-3`.

VEGF release

VEGF release was determined via Multi-Analyte Profiling by Myriad RBM (Austin, USA-TX) using the Human AngiogenesisMAP®.

RESULTS

After the hardware had returned to our laboratory, the evaluation of the samples was started. 5 of the 6 hardware units worked well during the mission. The cells in the last unit were not automatically fixed. Optical analysis confirmed the formation of MCS in all the units. Cells were harvested from the cultivation chambers (Fig. 2A). Supernatants were collected from the reserve tanks to determine protein release (Fig. 2B).

MAP analysis identified a higher release of VEGF in the space samples compared to ground controls. On the RPM VEGF release was reduced after 5 and 10d (Fig. 2C). To determine the source of VEGF release we further investigated gene expression via qPCR. Compared to 1*g* controls *VEGFA* transcription was down-regulated after 5d and strong down-regulated after 10d in r-µ*g*, independently of 3D growth (Fig. 2D). In s-µ*g* *VEGFA* transcription was only minimal reduced (Fig. 2E) with small differences between adherent cells and MCS.

DISCUSSION

The CellBox-2 mission was successful. The cells stayed alive in the flight hardware and we were able to isolate mRNA and protein from adherently growing cells and MCS respectively. With our focus on VEGF gene expression and protein release, because their alteration could trigger two further changes: The paracrine stimulation of angiogenesis, and the autocrine stimulation of the cancer cells themselves (Liu et al., 1995; Lichtenberger et al., 2010). We found a rather constant *VEGFA* expression under s-µ*g* and a down-regulation in r-µ*g*, that seemed not to be a feedback of MCS formation. The hypothesis

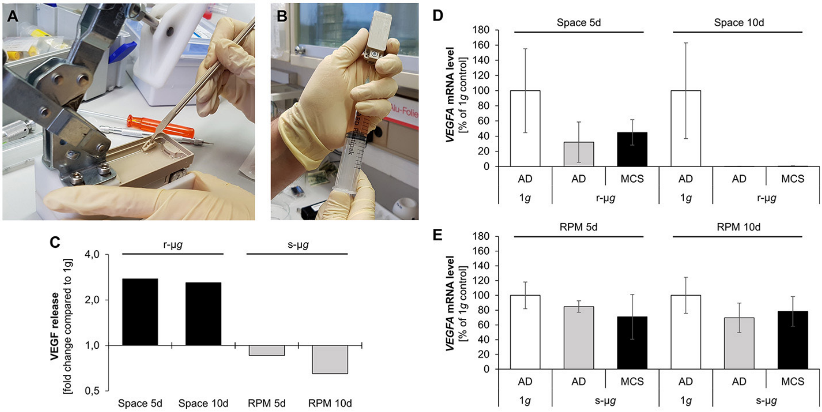

FIGURE 2: VEGF expression of FTC-133 cells cultured in μ*g*. (**A**) Cell harvesting from the cultivation chamber of one hardware unit. (**B**) Collecting the supernatants from the reserve tank. (**C**) Changes in VEGF release after 5 and 10d in μ*g*. (**D**) *VEGFA* expression in cells exposed to r-μ*g* in space or (**E**) exposed to s-μ*g* on the RPM.

is consistent with results of previous long-term studies on the RPM and from the Shenzhou-8/SIMBOX space mission (Ma et al., 2014; Kopp et al., 2015) and may contribute to a less aggressive phenotype of FTC-133 cells cultured in μ*g* (Ma et al., 2014). The MAP result however showed differences in the secretion behaviour. The VEGF release was increased under r-μ*g* and decreased under s-μ*g*. Reasons could be different materials of the cell chambers, as well as effects of shear forces on the RPM or of radiation in space. Overall a higher release of VEGF may indicate a greater angiogenic potential of FTC-133 cells in space.

CONCLUSION & OUTLOOK

During a space experiment onboard the ISS, we found spheroid formation by human FTC-133 cells like during previous experiments using the RPM. Beyond the morphological similarities a comparison of *VEGF* mRNA expression and protein release indicated differences, when human FTC-133 cells were cultured in space or incubated under s-μ*g*. Future genome and proteome analyses may uncover further differences but also similarities.

We expect to detect signalling pathways related to different biological processes, such as spheroid formation *in vitro* and metastasis *in vivo*. In the end, the detection of new gravisensitive proteins may be helpful to find new targets for cancer treatment.

ACKNOWLEDGMENT

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Keywords: cancer, angiogenesis, spaceflight, spheroids, 3D growth

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Alterations of the cytoskeleton in breast cancer cells during microgravity visualised by FLUMIAS live-cell imaging

Mohamed Zakaria Nassef[1], Sascha Kopp[1], Daniela Melnik[1], Marcus Krüger[1]*, Markus Wehland[1], Thomas Juhl Corydon[2], Johann Bauer[3], Manfred Infanger[1], Daniela Grimm[1,2]

[1]*Clinic for Plastic, Aesthetic and Hand Surgery, Otto von Guericke University Magdeburg, Magdeburg, Germany*
[2]*Department of Biomedicine, Aarhus University, Aarhus, Denmark*
[3]*Information Retrieval Services, Max Planck Institute of Biochemistry, Martinsried, Germany*
**marcus.krueger@med.ovgu.de*

The cytoskeleton is a highly dynamic structure playing an important role in graviperception and gravisensitivity of cells exposed to microgravity. The cytoskeletal network is closely connected to many cellular processes and functions. The exact mechanisms are mostly unknown. For the first time, we investigated human MCF-7 breast cancer cells in real microgravity. We were particularly interested in studying early-stage cytoskeletal changes (actin, microtubules) and early mechanisms of tumour spheroid formation. The current study was performed during the TEXUS-54 sounding rocket flight using live-cell imaging of modified MCF-7 cells simultaneously expressing LifeAct-GFP and mCherry-tubulin marker proteins and the FLUMIAS confocal laser spinning disc fluorescence microscope. We detected early alterations of the cytoskeleton during the cells' exposure to microgravity, including disturbance of F-actin bundles and appearance of filopodia- and lamellipodia-like structures. The MCF-7 cells reacted to microgravity within four minutes and showed similar alterations compared to FTC-133 thyroid cancer cells that were investigated before. This might indicate a common graviperception in these cancer cells.

INTRODUCTION

Astronauts, who spend a long time in space, suffer from several side effects such as cardiovascular problems, bone loss and immune system alterations

(White and Averner, 2001). The cause of these side effects are changes at the cellular level such as an altered cellular function and morphology together with activated molecular mechanisms as a response of the cells to microgravity (µ*g*). Space research has also attracted the attention of cancer biologists who search for new targets for therapies (Becker and Souza, 2013). In order to unravel the intracellular modifications that happen in real µ*g* (r-µ*g*), we performed several parabolic flights and sounding rocket missions with human cancer cells (Ma et al., 2014; Corydon et al., 2016; Krüger et al., 2017).

The human breast cancer cell line MCF-7 cells had been extensively studied under simulated µ*g* (s-µ*g*) conditions using the RPM (Kopp et al., 2016; Kopp et al., 2018; Sahana et al., 2018). In addition, these cells are very robust, when cultured under µ*g* especially in space (Vassy et al., 2001) or during a parabolic flight (unpublished results). As the cytoskeleton is assumed to act as a gravisensor in eukaryotic cells (Hughes-Fulford, 2003), we intend to investigate the effect of µ*g* on cytoskeletal rearrangements of human MCF-7 breast cancer cells during the TEXUS ("Technologische Experimente unter Schwerelosigkeit" (TX))-54 sounding rocket flight by means of live-cell imaging.

MATERIALS AND METHODS

Cell culture

MCF-7 cells were cultured as previously described (Kopp et al., 2018). In addition to 10% FCS and 1% penicillin/streptomycin, G418 was added to allow growth of the transfected cells only.

Construction of an expression cassette to visualize F-actin and α-tubulin

The expression construct was produced as published previously (Corydon et al., 2016). It is demonstrated in Fig. 1A.

Generation of MCF-7 cells expressing LifeAct-eGFP-IRES-mCherry-Tubulin

The MCF-7 cell line was stably transfected using a Sleeping Beauty Transposon-based vector containing the LifeAct-eGFP-IRES-mCherry-Tubulin (LAGICT) expression cassette for the visualization of F-actin and α-tubulin (Aronovich et al., 2011; Corydon et al., 2016).

TEXUS-54 sounding rocket mission

The sounding rocket mission was performed as previously described (Corydon et al., 2016).

Live-cell imaging by the FLUMIAS Microscope

Approximately 7000 MCF-7 cells were seeded into one channel of an Ibidi ibiTreat µ-slide VI 0.4 (Fig. 1B). The slide was temperature controlled and loaded into the FLUMIAS microscope (Corydon et al., 2016) shortly before the launch (steps of hardware assembly shown in Fig. 1C-F). Five minutes prior to launch three z-stacks were obtained from pre-selected cells as a ground control. About 75s after launch the µ*g* phase was reached, and the microscope started recording the pre-selected cells Three z-stacks were taken every one minute with 125ms exposure time. The thickness of the z-stack was 21µm with 0.5µm step size. The procedure was repeated four times with a total number of five active phases covering 6min of µ*g*.

After recovery of the image data, a single image was extracted from each z-stack taken during µ*g*. The extracted images were deconvolved by Huygens Essential Scientific Volume Imaging software and compared to a control image taken on ground.

RESULTS

To increase the current knowledge about cytoskeletal alterations of human breast cancer cells exposed to r-µ*g* conditions, live-cell imaging was performed using MCF-7 cells during a sounding rocket mission. The TX-54 mission was performed in May 2018 at Esrange Space Center, Kiruna, Sweden. We investigated MCF-7 cells which were transfected with LAGICT expression cassette for visualization of F-actin and α-tubulin. The observation was performed and recorded using the FLUMIAS microscope onboard the TX sounding rocket. The microscopic images were analysed to evaluate the effect of µ*g* on the MCF-7 cytoskeleton. We detected F-actin changes such as disturbances of F-actin bundles and the appearance of filopodia- and lamellipodia-like structures (Fig. 2, white and yellow arrows). In breast cancer cells no morphological signs of programmed cell death were detectable.

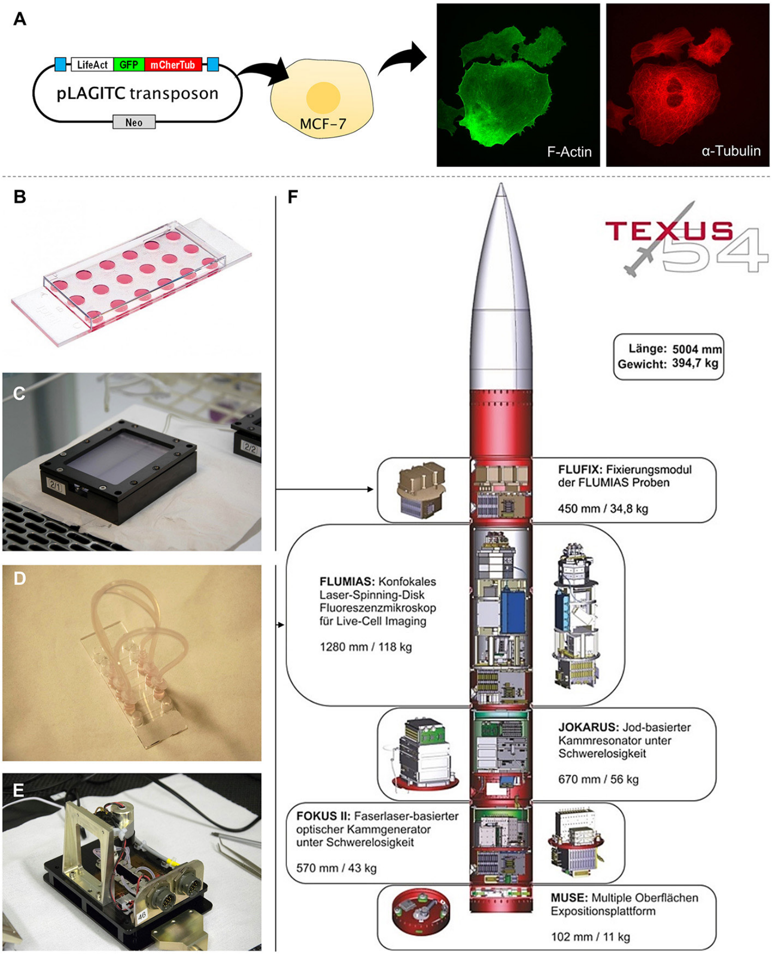

FIGURE 1: (**A**) Diagram showing the transfection of MCF-7 cells with LifeAct-eGFP-IRES-mCherry-Tubulin (LAGICT) expression cassette. Microscopic images showing visualization of F-Actin (488 laser) and α-tubulin (568 laser). (**B**) 18-well Ibidi slides used inside the FLUFIX (image from ibidi.com). (**C**) FLUFIX used to incubate 18-well Ibidi slides to be fixed during the TX-54 mission. (**D**) Ibidi μ-slide VI 0.4 ibiTreat prepared for the FLUMIAS microscope. (**E**) The late access and fixation unit after installing the Ibidi slide. (**F**) Image of TX-54 sounding rocket showing the accommodation of FLUFIX and FLUMIAS inside the rocket (courtesy of Airbus Defence & Space).

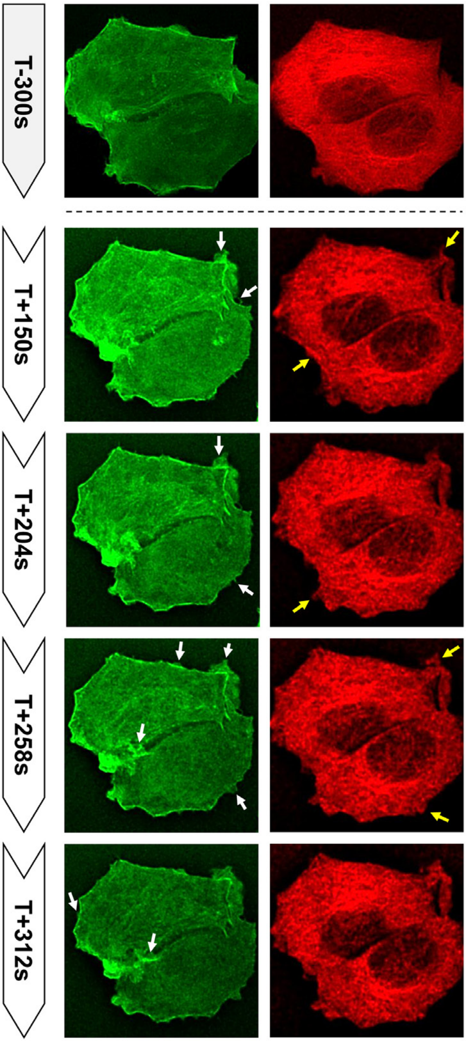

FIGURE 2: Timeline and images obtained by FLUMIAS showing the MCF-7 cells 5 min before the launch (T-300s) of the rocket and during the µ*g* phase (T+150s–T+312s). White arrows indicate the changes occurring to the F-actin and yellow arrows indicate the changes occurring to the α-tubulin.

DISCUSSION

For the first time, we were able to show the effect of r-μ*g* on the cytoskeletal structure of breast cancer cells during a sounding rocket mission in real time. The images obtained from the FLUMIAS microscope during the TX-54 mission were compared to those from the TX-52 mission (Corydon et al., 2016), which was performed with FTC-133 follicular thyroid cancer cells. Both missions showed similar effects of r-μ*g* on the two different cell lines. This is a further prove that the cytoskeleton is affected by μ*g* very early (Grosse et al., 2012) and enforces the suggestion that the cytoskeleton directly senses μ*g* (Vorselen et al., 2014).

CONCLUSION & OUTLOOK

In order to further confirm the hypothesis of direct sensing of μ*g* by the cytoskeleton, our next step is to investigate the gene activation status of MCF-7 after short time exposure of the cells to r-μ*g*. In this way we intend to uncover signalling pathways that are connected with extracellular matrix, cell adhesion, cytoskeleton, cytokines, growth factors, cell cycle and apoptosis. This should enable us to learn the molecular mechanisms for initial sensing and adaptation to μ*g* of breast cancer cells.

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Keywords: cytoskeleton, tubulin, cancer, actin, microgravity, live-cell imaging, sounding rocket

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Growing tissues in space

Sascha Kopp[1]*, Marcus Krüger[1], Markus Wehland[1], Johann Bauer[2], Anita Dittrich[3], Manfred Infanger[1], Daniela Grimm[1,3]

[1]*Clinic for Plastic, Aesthetic and Hand Surgery, Otto von Guericke University Magdeburg, Magdeburg, Germany*

[2]*Department of Biomedicine, Aarhus University, Aarhus, Denmark*

[3]*Information Retrieval Services, Max Planck Institute of Biochemistry, Martinsried, Germany*

**sascha.kopp@med.ovgu.de*

Despite the fact that astronauts have various health problems in space, the unique culture condition of microgravity (μ*g*) bears the possibility to grow human cells in a three-dimensional (3D) way without scaffolds or matrices. Using facilities like the International Space Station (ISS) or unmanned satellites, we showed that human thyroid cancer cells, as well as human endothelial cells formed 3D aggregates or spheroids after μ*g*-exposure for up to 14 days. Comparable results were obtained when using ground-based facilities like the Random Positioning Machine (RPM) and fast rotating clinostat (FRC). During spheroid formation, factors involved in angiogenesis, proliferation, cell adhesion, migration, extracellular matrix signalling and others seem to play a crucial role in concert with the initial cell density. These findings will help to engineer human tissues which might be used for transplantation or drug testing. The following proceeding should give a condensed overview of the results obtained so far in real μ*g* (r-μ*g*) and simulated μ*g* (s-μ*g*).

INTRODUCTION

Tissue engineering is a fast-developing research field. Even though a lot of improvement was achieved during the last years, the use of scaffolds and matrices is still a critical point to be solved (Grimm et al., 2014). In addition, nutrient and waste transport in the engineered tissue is still a problem because the tissues will become necrotic with growing size and culture time (Grimm et al., 2014).

The majority of the available techniques to engineer tissues scaffold free are forcing the cells to stick to each other, like the hanging drop or the liquid overlay method. The use of µ*g* allows the cells to form spheroids naturally without any force (Aleshcheva et al., 2016). Ingram et al. had investigated several different human carcinoma cell lines cultivated in a NASA rotating bioreactor, which was developed to simulate aspects of µ*g*. The authors found that each cell line formed 3D spheroids, when kept from sedimentation (Ingram et al., 1997). These experiments, however, started with suspended cells floating in the medium. In 2002, Grimm et al. exposed adherent ML-1 thyroid cancer cells to an RPM to check for its possible usability in cancer studies. They found that these cells from multicellular spheroids aside adherently growing cells suggesting that during clinorotation some cells detached from the substrate (Grimm et al., 2002). How and why the cells detach and accumulate into spheroids is still under investigation. Therefore, the objective of this proceeding is to condense recent findings in the field, which were observed when different cell types (thyroid cancer cells and endothelial cells) were exposed to r-µ*g* in space or to s-µ*g* using ground-based facilities.

METHODS

Follicular thyroid cancer cells (Pietsch et al., 2013; Ma et al., 2014a; Riwaldt et al., 2015) and endothelial cells (Pietsch et al., 2017) were investigated during international spaceflights in orbit or on the International Space Station (ISS) and were cultured in newly developed automated hardware for several days in space. Post-flight the cells were analysed by histological and molecular biological methods (proteomics, gene array, quantitative rtPCR, multianalyte profiling). In addition, cell biological experiments using the 3D RPM or the 2D FRC were performed.

RESULTS AND DISCUSSION

Exposing the cells to r-µ*g* and s-µ*g* revealed the formation of two phenotypes: one part of the cells of both investigated cell types detached from the culture flask bottom and grew in form of 3D multicellular spheroids (MCS; Figure 1), the other one continued growing as a 2D monolayer (Pietsch et al., 2013; Ma et al., 2014a; Warnke et al., 2014; Kopp et al., 2015; Pietsch et al., 2017). Interestingly, this 3D formation occurred scaffold-free. MCS formed by thyroid cancer cells were more round aggregates (Fig. 1 A) (Kopp et al., 2015), whereas

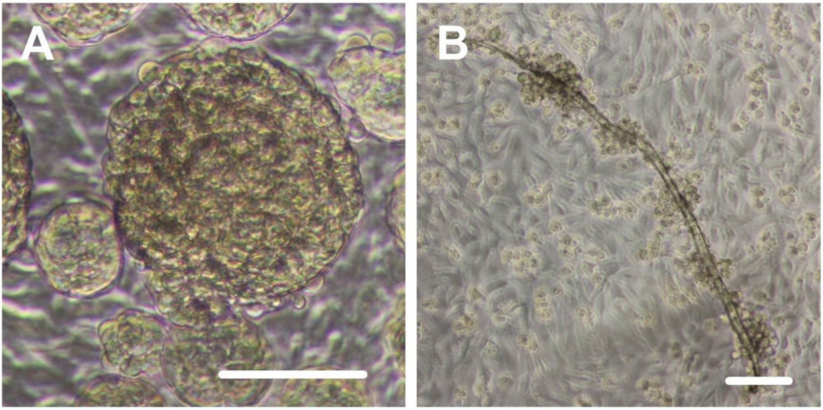

FIGURE 1: Cells build up 3D spheres when exposed to the Random Positioning Machine. While FTC-133 thyroid cancer cells produce round spheres (**A**) (here after a 7-day-exposure), endothelial cells grow in form of tubular structures resembling the inner layer (intima) of blood vessels (**B**) (here after a 23-day-exposure). Scale bars: 100 μm.

endothelial cells exhibited a tube formation (intima constructs) in addition to 3D MCS (Fig. 1 B) (Ma et al., 2013; Ma et al., 2014b). The density of the monolayers exposed to microgravity revealed an impact on the results as during *CELLBOX1* the cells were overgrown before launch and no spheroids were formed (Riwaldt et al., 2015). Genomic and proteomic alterations were induced by altered gravity conditions (Pietsch et al., 2010; Pietsch et al., 2011; 2012). Biological processes such as proliferation, migration, the composition of the extracellular matrix proteins (ECM), cell adhesion, focal adhesions, and apoptosis are influencing 3D growth under μ*g* conditions (Pietsch et al., 2013; Ma et al., 2014a; Riwaldt et al., 2015). Growth factors, cell adhesion molecules and cytokines such as vascular endothelial growth factor A (VEGFA), epidermal growth factor (EGF), connective tissue growth factor (CTGF), fibronectin, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), interleukin-6 (IL6), IL8, caveolin-1 (CAV1), monocyte chemoattractant protein-1 (MCP1) and intercellular adhesion molecule 1 (ICAM1) and others have shown to be involved in spheroid formation in μ*g* in follicular thyroid cancer cells and normal thyrocytes (Riwaldt et al., 2015; Warnke et al., 2017). Human follicular epithelial thyroid cells (Nthy-ori 3-1) exposed to the RPM formed MCS within 24h. Cytokines and focal adhesion proteins play a key role in the early phase of MCS formation (Warnke et al., 2017).

Moreover, RPM-exposed FTC-133 monolayer cells or MCS incorporate vinculin, paxillin, focal adhesion kinase 1, and adenine diphosphate (ADP)-ribosylation factor 6 in different ways into the focal adhesion complex (Bauer et al., 2017).

Further studies have to be performed to investigate how these factors exert their effect on cell detachment and aggregation in adherent human cells.

CONCLUSION

Cultivation of human cells in space or on a µ*g*-simulator induced 3D growth (Fig. 2). Different phenotypes occurred and MCS showed a different gene expression profile involving important biological processes compared to monolayer cells. Tissue engineering under r-µ*g* and s-µ*g* conditions represents a new technology in gravitational biology and translational regenerative

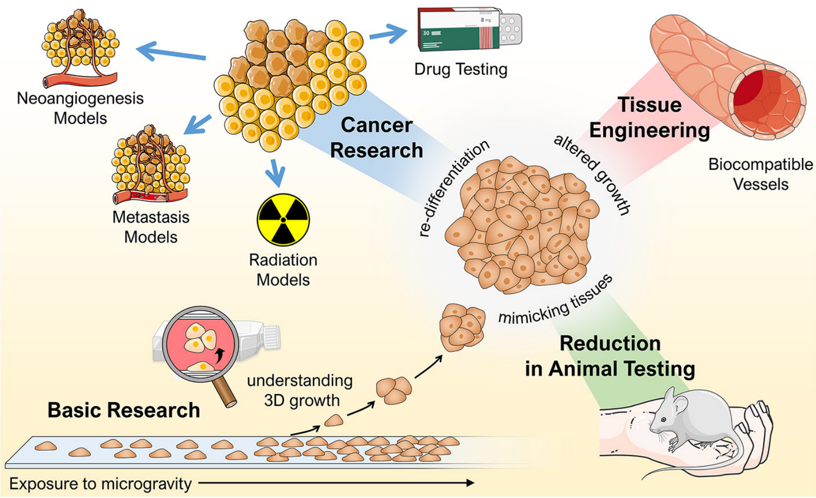

FIGURE 2: In microgravity, cells of various tissue origin are detaching from their substrate and start floating in the supernatant. While being suspended, they start forming 3D spheres which resemble tissue-specific features without the need of adding scaffolds or matrices. They can be used for neoangiogenesis, metastasis and radiation models. In addition, this new technique can benefit tissue engineering as well as drug screening and can reduce the need of lab animals erasing the black box of artificial species-reactivity.

medicine which can be beneficial in cancer research and drug screening as well as tissue engineering and can reduce the use of laboratory animals (Fig. 2).

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Keywords: tissue engineering, 3D growth, vascular constructs, microgravity (μg), spheroids culture

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Weightlessness and fluctuation of mass values, as interrogation factors of systemic properties of the carcinogenic transformation: Development of human breast cancer cells models for a systemic study

Serhiy Souchelnytskyi

College of Medicine, Qatar University, Doha, Qatar
serhiy@qu.edu.qa

Cancer is a systemic disease, which involves a network of hundreds of molecules (nodes) and interactions (edges). Systemic properties of tumorigenesis are characterized by a fragile robustness. It means that the tumor cells may be resistant to some treatments, but may be sensitive to other inputs (1). Successful cancer treatments target nodes of such fragility. Blocking of these nodes would lead to a collapse of a cancer-specific signaling network and subsequently, to the death of tumor cells. The challenge is to find fragility nodes.

Human cells are not wired to be robust towards weightlessness and changes in the gravitational forces. Therefore, an interrogation of tumorigenesis in the space may provide a fast track to identification of the fragility nodes (Figure 1). Such interrogation requires development of dedicated cell-based models.

We established models of a 3-dimensional growth and proliferation of human breast and renal cancer cells. These models mimic formation of microtumors. The term "microtumors" defines spheroids and clusters of tumor cells, which proliferate in a 3-dimensional culture and show features of the cellular carcinogenic transformation. Formation of microtumors in a human body precedes formation of larger tumors with a stroma and vasculature. Clusters formed by cells representing non-aggressive (MCF7) and aggressive and metastatic (MDA-MB-231) human breast cancers are shown in Figure 2. Microtumors of MCF7 and MDA-MB-231 cells may differ in shapes and growth patterns, confirming a different initial status of their carcinogenic transformation. Interrogation of the cells by treating them with different drugs alone and/or in combinations showed additive, cooperative, simulating or inhibiting effects. These effects could be explained only by interactions of nodes in

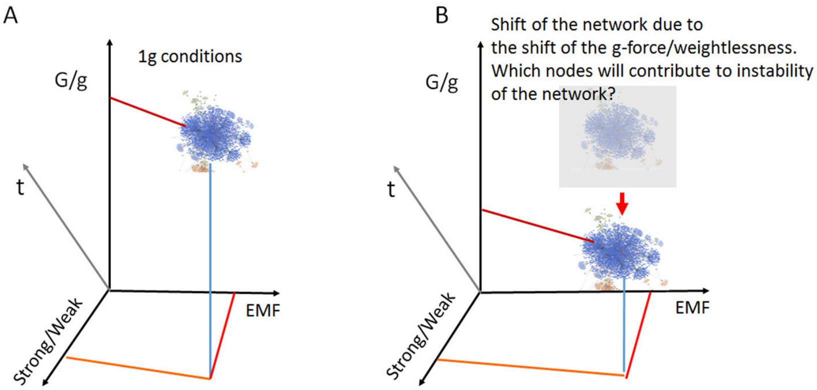

FIGURE 1: Interrogation of a cancer regulatory network in weightlessness may expose fragility nodes. (**A**) Regulatory processes in tumor cells are adapted to the forces acting on the Earth, e.g. gravity (G/g), electro-magnetic (EMF), strong and weak forces. In weightlessness (**B**), the cells are exposed to non-familiar to them changes in weightlessness and gravity. This may enhance fragility of the network and facilitate collapse of this network. Nodes involved in the collapse would be the candidates for key markers, and subsequently, would be targets for treatment of cancer. We expect that it would be a number of such fragility nodes, i.e. a pattern signature. The shown network was built with proteins affected in cancer cells of a real patient (7).

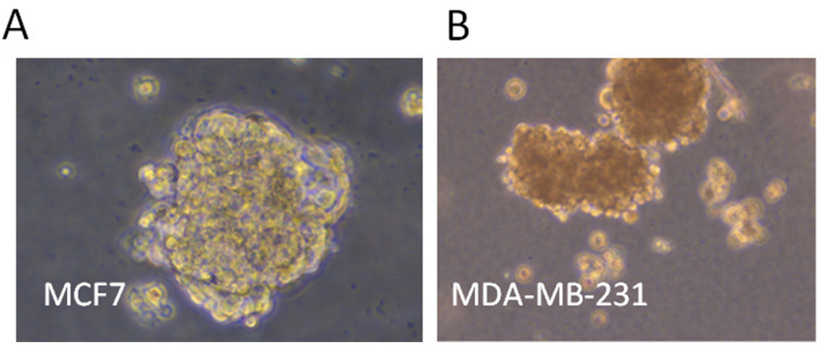

FIGURE 2: Clusters formed by MCF7 (**A**) and MDA-MB-231 (**B**) cells are shown. Formation of cellular clusters correlates with ability of the cells to form microtumors in the body, and is one of the features typical for transformed tumorigenic cells. The cells clusters may be formed with the most of human solid tumor cells.

the cancer cells regulatory networks (1). This confirms systemic character of responses. We believe that these models are ready for interrogation in the weightlessness condition.

Weightlessness affects distribution of molecules inside and outside of cells, changes morphology of cells and affects regulatory processes (reviewed in 2-4). Tumorigenesis studies in weightlessness have generated a significant volume of data (2-4). However, systemic approach has been missing. Therefore, the searches for a single "silver bullet" have not yet been successful in a clinical applicability.

We have developed cellular models and approaches to use these models in the space. We will do an unbiased analysis of the space-related changes, in parallel with similar interrogations at 1g and 10g conditions. Unbiased approach is dictated due to the variety of regulatory effects, which may involve signaling, transport, energy processing, enzymatic reactions, formations of molecular complexes, changes in composition of the cytosol and membranes and interactions between cells. Narrow focusing would exclude potentially important effects. Systemic analysis of data obtained under weightlessness, at 1g and 10g for cells of non-aggressive tumorigenic and aggressive-metastatic types, would allow to extract changes predominantly driving tumorigenesis. Proteomics will be the main tool of profiling of the cells, as it delivers clinically-relevant networks (5-7). Multiple network analyses will be used, e.g. functions-dependent fusions, cross-sections and deductions of the networks built with different databases (8).

The cells in the space would be exposed not only to weightlessness, but also to radiation of different types and intensity, changes of gravitational forces and to mechanical impacts during a flight. An impact of the radiation exposure will be evaluated by monitoring of a DNA damage and radiation-induced post-translational modifications of proteins. Mechanical impacts would be possible to mimic in the laboratory, by reproducing changes in g-forces (in a centrifuge) and by a mechanical shaking.

Regarding the gravitational forces, we observed that the mass of biological molecules fluctuates depending on positions of the Earth, the Sun and the Moon. This fluctuation is higher than predicted due to the calculated gravitational forces of the planets. In large biological molecules, an accumulated level of the mass fluctuations may reach values, which may affect functions of these molecules. An example of a smaller molecules, water and deuterium oxide (heavy water), illustrates a strong impact of a heavy water on the cell physiology (9). Mass fluctuations due to positions of the celestial bodies are predicted to be too small (e.g. few ppm only), as compared to the isotope-dependent changes of masses. However, our data show that in biological peptides, mass variability is tenth times higher as compared to the predicted values. Such high level of the mass fluctuations may affect mechanisms of enzymatic reactions. Thus, mass fluctuations may be another interrogation factor of the systemic mechanisms of carcinogenesis.

Why weightlessness and gravity have a chance to expose fragility nodes of the cancer signaling? An answer to this question defines whether this project will deliver practical solutions, or will be just another academic exercise. We believe that the success is based on the facts that weightlessness and change of the gravitational force represent non-familiar to the cells inputs. Selected by us proteome profiling and systemic analysis have been proven to deliver clinically relevant markers and drug targets, meaning that it should be no methodological hurdles. Therefore, weightlessness and gravitational forces would add an important trigger for extraction of key nodes and edges essential for elimination of tumor cells.

AUTHOR CONTRIBUTION

This is a single author work.

CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interests.

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Keywords: cells, weightlessness, cancer, gravity, carcinogenic transformation

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The SERiSM project: Preliminary data on human stem cell reprogramming in microgravity

Monica Bari[1], Natalia Battista[2], Giulia Merlini[1], Marina Fava[4], Cristina Ruggiero[1], Sara Piccirillo[3], Giovanni Valentini[3], Gabriele Mascetti[3], Alessandra Gambacurta[1]*, Mauro Maccarrone[4]*

[1]*Department of Experimental Medicine and Surgery, NAST Centre, Tor Vergata University of Rome, Rome, Italy*
[2]*Faculty of Bioscience and Technology for Food, Agriculture and Environment, Università degli Studi di Teramo, Teramo, Italy*
[3]*Faculty of Bioscience, Agenzia Spaziale Italiana, Rome, Italy*
[4]*Department of Medicine, European Center for Brain Research, IRCCS Santa Lucia Foundation, Università Campus Bio-Medico, Rome, Italy*
**gambacur@uniroma2.it; * m.maccarrone@unicampus.it*

Bone loss is a major thread to astronauts' health during Space missions, and thus the identification of novel biomarkers to be exploited in bone regeneration is a main focus of Space research. The "SERiSM" (Role of the Endocannabinoid System in Reprogramming Human Pluripotent Stem Cells under Microgravity) project has been selected by the Italian Space Agency (ASI) following the 2012 Life Science Research Announcement (ASI DC-MIC-2012-024), in order to evaluate the osteogenic differentiation under real microgravity and to investigate the involvement of the endocannabinoid system in this process. SERiSM was approved by the National Aeronautics and Space Administration (NASA) and was launched to the International Space Station (ISS) on board the Falcon rocket from Cape Canaveral, Florida (USA), on August 14th, 2017, in the frame of the VITA mission of ASI.

Accumulated evidence has recently demonstrated that endocannabinoid (eCB) signaling is modulated by weightlessness (Chouker et al., 2010; Strewe et al., 2012). eCBs are lipid mediators with manifold pathophysiological roles both in the central nervous system (Chiurchiù et al., 2018), where they control also neuronal cell programming (Maccarrone et al., 2014), and at the periphery of our body (Maccarrone et al., 2015). Of note, eCB signaling seems to be a key for bone homeostasis (Bab and Zimmer, 2008; Deis et al., 2018).

The SERiSM project aimed at interrogating alterations of eCBs that could be possibly linked to bone loss in Space. To this end, human Blood-Derived Stem Cells (hBDSCs) were used as an innovative and easily accessible stem cell model derived from peripheral blood (Marfe et al., 2012). Indeed, the hBDSC model is autologous and possesses a remarkable proliferative and differentiative capacity, thus leading to different cell types with a remarkable therapeutic potential (Marfe et al., 2012a and 2012b; Alaimo et al., 2013). Moreover, hBDSCs start osteogenic differentiation when exposed to a single agent (rapamycin), in the presence of the bone scaffold BioOss (Carpentieri et al., 2016; 2017). The SERiSM rationale and preliminary data showing eCB system expression and osteogenic induction during the on ground experiments and the early phases of differentiation in the preparatory Experiment Sequence Test (EST) and aboard the ISS, are presented here.

hBDSCs were isolated from whole blood, withdrawn from a human healthy donor, and were purified by repetitive centrifugation in Phosphate Buffer Saline (PBS) containing macrophage colony-stimulating factor (M-CSF, 50 nM) and gentamicin sulfate (5 μM), and were incubated for 72 h at 37°C. One day before launch, cells were resuspended in DMEM/F-12 medium containing L-glutamine (300 mg/L), gentamicin sulfate (50 mg/L), HEPES (40 mM), penicillin/streptomycin (10 mL/L), fetal bovine serum (100 mg/L) and BioOss granulate, and were used for simulate microgravity experiments or loaded into a specific hardware developed by Kayser Italia for EST and ISS experiments. The latter was composed of 8 experimental units (EUs), each fitting into a KIC-SL container. Once aboard the ISS, the SERiSM experiment was installed into the KUBIK facility, pre-conditioned at 37°C, and was activated in the Columbus module. During inflight operations, hBDSCs were activated automatically for different times (0, 48 and 72 hrs), by adding rapamycin (1 μM) to each culture chamber (Carpentieri et al., 2017). Then, cells were fixed with RNAlater (900 μl/culture chamber; Ambion, Austin, TX, USA), and were immediately moved by the astronauts into the MELFI (minus 80°C laboratory freezer for ISS) facility, where they were stored until download with the Space Capsule Dragon.

In the on-ground experiments, the viability of hBDSCs stained with Hoechst was tested every day by flow cytometry, to verify cell survival during the same time-window as the in-flight experiment. Our results demonstrated that cell viability remained > 90% of the controls for up to 10 days, which is the maximum planned time for SERiSM experiment in Space; then, viability decreased to ~80% on day 11, and down to ~30% on day 14 (Fig. 1a). Furthermore, osteogenic differentiation from hBDSCs was documented by the formation of calcium deposits stained by Alizarin Red, showing that metabolic and phenotypic changes towards bone formation took place already

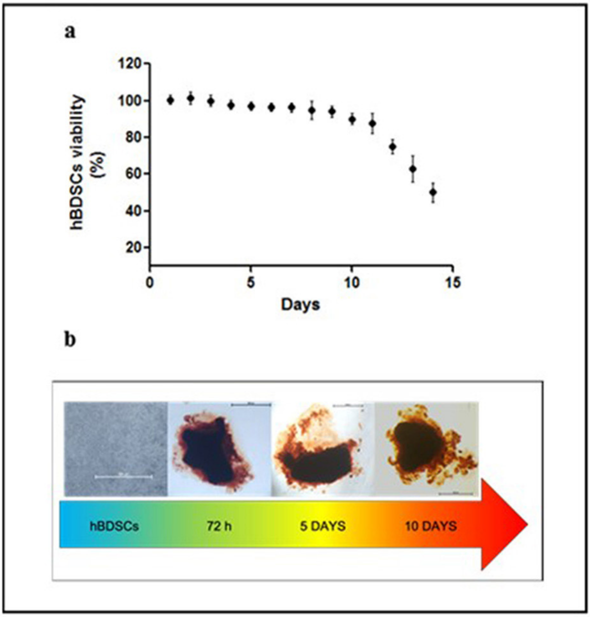

FIGURE 1: (**a**) hBDSCs viability during 15 days after isolation. (**b**) Staining with Alizarin Red to highlight bone formation after rapamycin treatment and incubation with BioOss scaffold.

within 72 h (Fig. 1b). Therefore, all subsequent experiments on ground and on the ISS were carried out at t0 (control) and t72 (i.e., after 72h of osteogenic differentiation). The expression of the main eCB-binding type-1 and type-2 cannabinoid receptors (CB1 and CB2) was analyzed by Western blot, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as housekeeping control (Fig. 2). CB1 and CB2 proteins were expressed at both t0 and t72, and showed opposite changes along the differentiation processes: CB1 increased, whereas CB2 decreased (Fig. 2). The samples recovered from the EUs at the end of the EST, and those flown aboard the ISS, showed an increased amount of cells at t72 than at t0 (Fig. 3a), suggesting a proper cell growth in the EUs. Moreover, electrophoretic analysis performed on the same samples, in order to evaluate the protein pattern of hBDSCs after addition of RNAlater and subsequent freezing, showed variations in the low molecular weight proteins that are typical of transcription factors (Fig. 3b and c).

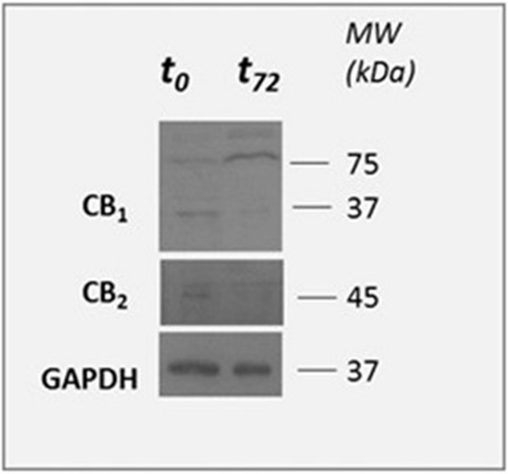

FIGURE 2: Expression of CB1 and CB2 receptors in hBDSCs at t0 and t72.

In order to ascertain the integrity of the samples and to standardize the experimental procedure for further analysis of ISS samples, Western blot was performed on t0 and t72 samples to measure lactate dehydrogenase (LDH) A and B (Fig 3d and e). Of note, LDH A is highly expressed in cells with a glycolytic metabolism that is typical of stem cells, whereas LDH B is more active in cells with oxidative metabolism that is typical of differentiated cells. The samples recovered from the EUs flown aboard the ISS showed a more marked reduction of LDH A than of LDH B at t72 compared to t0 and t72 ground experiments (Fig 3f and g), supporting the concept that stem cells were less abundant and that commitment towards differentiation took place (Shyh-Chang et al., 2013).

Taken together, our preliminary data on endocannabinoid system, cellular proliferation and metabolic switch provide the basis for the ongoing experiments aimed at disclosing the potential of endocannabinoid signalling in bone remodeling and osteogenesis modulation under authentic microgravity conditions.

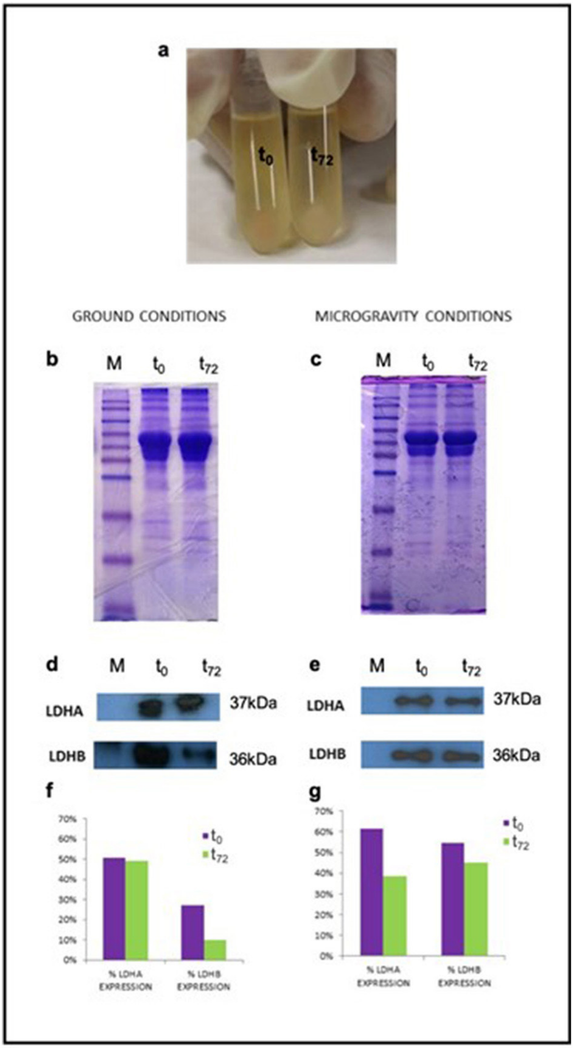

FIGURE 3: Ground control (EST) and microgravity (ISS) experiment samples. (**a**) Sample pellets; (**b, c**) Gel Coomassie staining; (**d, e**) LDH A and LDH B protein expression in hBDSCs at t0 and t72; (**f, g**) densitometric analysis of LDH A/LDH B expression.

ACKNOWLEDGEMENT

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Keywords: osteogenesis, differentiation, microgravity, endocannabinoid system, human blood-derived Stem Cells

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Ribosomal S6 kinase phosphorylation and nuclear–cytoplasmic traffic of class IIa histone deacetylases in rat soleus muscle at the early stage of gravitational unloading

Natalia Vilchinskaya*, Boris Shenkman

IInstitute of Biomedical Problems (RAS), Myology laboratory, Moscow, Russia
**vilchinskayanatalia@gmail.com*

INTRODUCTION

In a number of human and animal studies, it has been shown that gravitational unloading results in a significant atrophy of mammalian postural muscles such as soleus, decrease of their contractile properties and slow-to-fast shift in myosin heavy chain expression [Baldwin et al., 1990]. To date little is known about changes in signaling pathways regulating protein synthesis in the rodent soleus muscle during early stages of gravitational unloading. Previously we showed that AMPK phosphorylation is significantly decreased in rat soleus at the early stage of mechanical unloading [Mirzoev et al., 2016]. It is well-known that histone deacetylases 4 and 5 (HDAC4/HDAC5) are the targets of the AMPK and protein kinase D (PKD). We hypothesized that at the early stage of the gravitational unloading alterations in AMPK phosphorylation level may affect mTORC1 signaling pathway and nuclear-cytoplasmic traffic of class II histone deacetylases.

MATERIALS AND METHODS

To verify the hypothesis, we used administration of AMPK activator, AICAR, before (for 6 days) and during 24-h hindlimb suspension (HS). The content of p-p70S6K, p-AMPK, p-ACC, p-PKD, HDAC4, HDAC5 in rat soleus was determined by Western-blotting. All data are expressed as means ± SEM. Significant differences between groups were analyzed by Kruskal–Wallis test.

RESULTS AND DISCUSSION

It is known that AMPK suppresses mTORC1 activity [Inoki et al., 2003]. Mirzoev et al. (2016) have shown a significant increase in mTORc1 target p70S6K phosphorylation after 24-h HS. We suggested that it is a decrease in AMPK activity to provide the elevation of p70S6K phosphorylation at the early stage of gravitational unloading. We found that in the HS group phosphorylation of p70S6K was increased, but in the HS+AICAR group phosphorylation of p70S6K didn't differ from the control level (Fig.1C). We can conclude that p70S6K phosphorylation increase at the early stage of gravitational unloading could be due to reduced AMPK activity. It was also assumed that a decrease in AMPK activity would lead to a decrease in the phosphorylation of HDAC4/HDAC5 and their accumulation in nuclei after the 1 day of the hindlimb suspension. In fact we have observed the accumulation of only HDAC4 in the nuclear fraction after 24-h HS. In the presence of AICAR nuclear accumulation of HDAC4 didn't occur (Fig.2A). These data correlate well with the AMPK phosphorylation (Fig.1A). After 10 days of immobilization accumulation of HDAC4 in the nuclei of rat

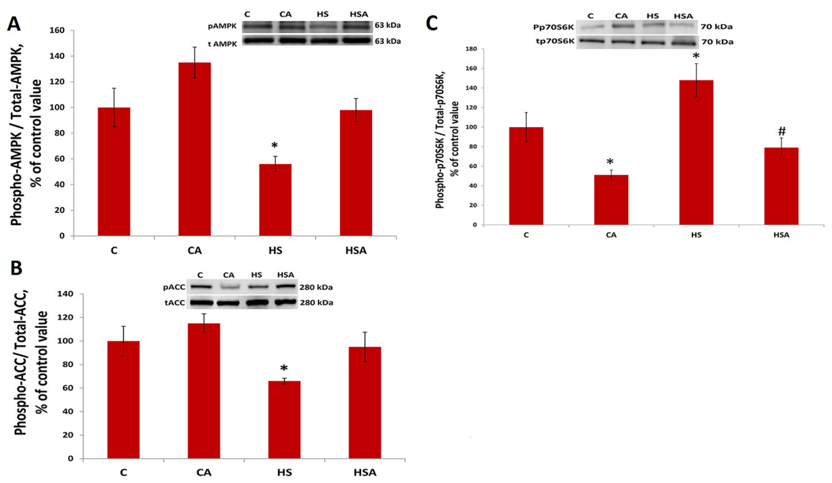

FIGURE 1: Quantification of p-AMPK (Thr 172) (**A**), p-ACC (Ser 79) (**B**) and p-p70S6K(Thr389) (**C**) in the rat soleus expressed relative (%) to control. C - control, CA – control + AICAR; HS - hindlimb suspension for 24-h, HSA - HS + AICAR. * - significant difference from C (p<0.05), # - significant difference from HS (p<0.05).

gastrocnemius muscle was shown. This nuclear accumulation of HDAC4 was accompanied by a decrease in AMPK phosphorylation level [Yoshihara T. et al., 2016], which is in line with our results.

On the other hand, we have found a decrease in the nuclear HDAC5 level after HS in all experimental groups including the groups where AMPK activity was stimulated (Fig.2B). Reduced level of nuclear HDAC5 after HS can be explained by HDAC5 phosphorylation and its export from nuclei or HDAC5 degradation. It is known that HDAC5 is a target not only for AMPK but for PKD as well. It was shown that a decline in AMPK activity leads to an increase in PKD phosphorylation level [McGee S.L., et al., 2014]. Indeed, HS resulted in a significant increase in the PKD phosphorylation level (Fig.2D). In the HS + AICAR group PKD phosphorylation level didn't differ from control group (Fig.2D). We found that after HS the level of negative AMPK phosphorylation (Ser485/491) was increased (Fig.2C). It is known that PKD can phosphorylate AMPK on Ser485/491 [Coughlan K.A. et al., 2016]. But AMPK activation with AICAR in the HS group led to a decrease in the negative AMPK phosphorylation, probably due to a decrease in PKD phosphorylation (Fig.2C).

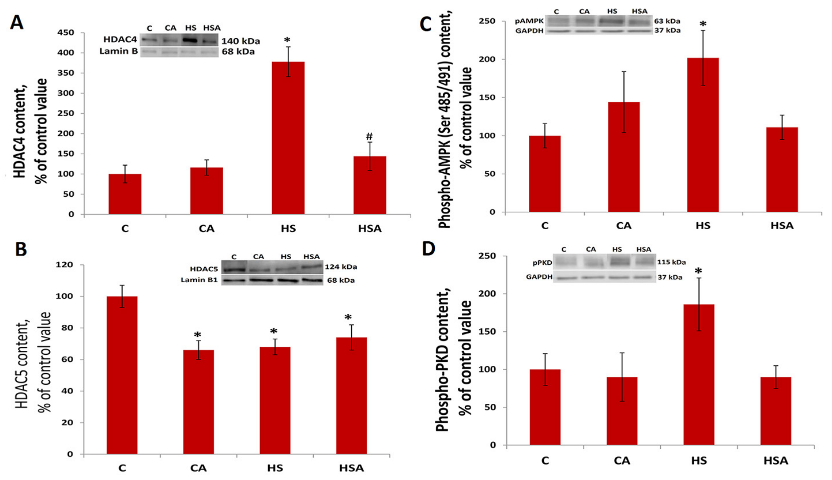

FIGURE 2: Quantification of HDAC4 (**A**), HDAC5 (**B**), p-AMPK (Ser 485/491) (**C**) and p-PKD (Ser 916) (**D**) in the rat soleus expressed relative (%) to control. C - control, CA – control + AICAR; HS - hindlimb suspension for 24-h, HSA - HS + AICAR. * - significant difference from C (p<0.05), # - significant difference from HS (p<0.05).

The results of the study indicate that AMPK dephosphorylation after 24-h HS had a significant impact on nuclear-cytoplasmic traffic of class II histone deacetylases. It was shown that at the early stage of gravitational unloading, HDAC4 nuclear import is associated with a decrease in AMPK activity. We first showed the reciprocal relations between AMPK/HDAC4 and PKD/HDAC5 pathways in a skeletal muscle at the early stage of gravitational unloading. The study was supported by Russian Science Foundation grant #17-75-20152.

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Keywords: hindlimb suspension, AMPK, P70S6K, gravitational unloading, HDAC4/5

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Locomotion on the earth after long-duration space flights as step to locomotion on other celestial bodies

Nataliya Lysova[1]*, Vladimir Kitov[1], Ilya Vyacheslavovich Rukavishnikov[1], Igor S Kofman[2], Elena S. Tomilovskaya[1], Millard Freeman Reschke[2], Inessa Kozlovskaya[1], Marissa Rosenberg[2], Nikolay Osetsky[1], Elena Fomina[1]

[1] *Institute of Biomedical Problems (RAS), Myology laboratory, Moscow, Russia*
[2] *Neuroscience Laboratories, National Aeronautics and Space Administration Johnson Space Center, Houston, TX, United States*
**cehbr@list.ru*

BACKGROUND

It's well known that long term exposure to microgravity leads to the deep changes in the activities of sensory systems: semicircular canals, otoliths (Kornilova, 2006), proprioception (Edgerton, 2000) and support afferentation (Kozlovskaya, 2017). These changes affect motor control system and characteristics of voluntary movements: eye -head coordination, space orientation (Lipshits, 1993), posture and locomotion (Baroni, 1985). In the future humans will have to stay and work on the surface of other celestial bodies for example Moon or Mars, which may present a challenge due to the adverse effects of long-duration exposure to microgravity and the success of an interplanetary mission will depend on the ability of crew members to perform operations on the surface of space objects.

The goal of our study was to gain an in-depth understanding of gravity-related changes in motor control of crewmembers after long-duration space flights (SF) using the Stepping Over Obstacle test.

METHODS

Our paper presents biomechanical characteristics of locomotion of five Russian cosmonauts (men, age 42±5 years; mass: 81± 5 kg) that performed space missions of 115 – 196 days in duration aboard the International Space Station. We used two difference systems in our experiment: video motion analysis «Videoanalyzer Biosoft 3D hardware/software» («NMF BIOSOFT», Russia) and the movement monitoring system of Opal miniature inertial measurement units (IMUs) by APDM, Inc. (Portland, OR). During the test each cosmonaut had to walk at a self-selected speed and stepped over the obstacles of 5, 10, 15 and 30 cm high. We calculated toe-obstacle clearance, the length of the step (defined as the horizontal distance between toe raising and heel strike leading limb) and joint motion in the hip, knee and ankle joints. The angular amplitude of joints was calculated as the difference between the maximum and the minimum of the angle The data were collected 60-30 days before SF and on post-flight during first hour after landing and day 3, 4, 7 and 12 after SF accomplishment.

RESULTS

The toe-obstacle clearance for the 5 cm obstacle during the first hour after the landing decreased significantly compared with the preflight results ($p \leq 0,05$). However this parameter didn't change compared with BDC on day 3, 4, 7 and 12 after SF (fig. 1).For the 10 and 15 cm obstacle, the results were the same.

DISCUSSION

In our research it was shown that the first hours of recovery after SF are characterized by the changes in the motor task Stepping Over Obstacle characteristics. The toe-obstacle clearance and the length of step decreased significantly compared with pre-flight results for the obstacle 5, 10 and 15 cm. We hypothesize that these changes can be induced by vestibular and sensorimotor disorders and muscle atrophy. No significant changes in the toe-obstacle clearance on the day 3 after landing compared with pre-flight level for all the obstacles. The length of step was significantly decreased only for the 5 cm obstacle on the day 4 after landing. These alterations of the toe-obstacle clearance and length of step in recovered almost fully on the day 3-4 after SF.

Our study revealed that the range of motion of every joint examined at the above 3 obstacle heights was reduced after long-duration spaceflights.

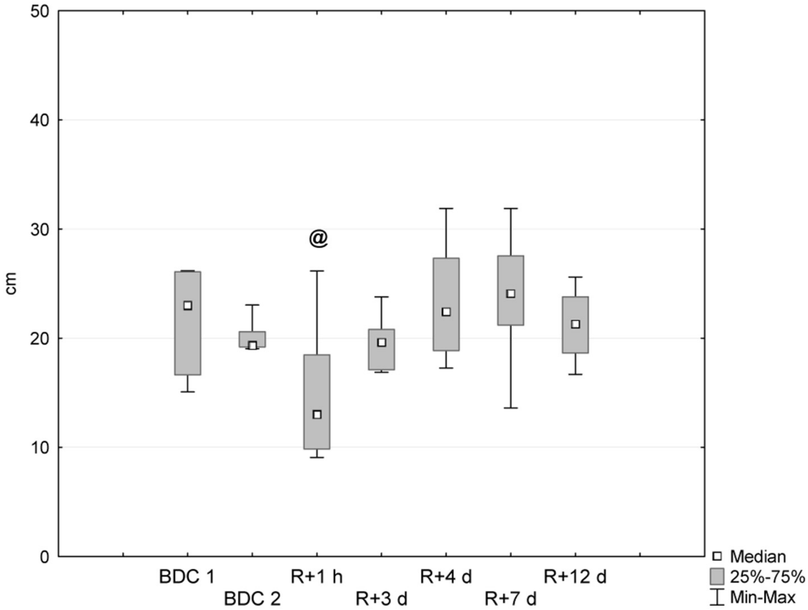

FIGURE 1: The step length decreased significantly that during the first hour after the landing compared with the preflight results for the 5, 10, 15 cm obstacle (p≤0,03). On day 4 this parameter decreased significant only for the 5 cm obstacle (p≤0,05).On day 7 and 12 the step length did not change (fig. 2).

This corresponds to previous observations indicating a decrease in the angular amplitude of joints during walking (Shpakov, 2013). We hypothesize that these changes can be induced by lower muscle contractility (LeBlanc, 1995).

Another factor responsible for motion range changes can be a decline in muscle tone following long-duration space missions. It was reported that after exposure to real or simulated microgravity of muscle tone, particularly of shin extensors, was reduced (Kozlovskaya, 2017).

It can be concluded that changes in multi -sensory integration following long-duration exposure to microgravity produced a significant effect in the test of Stepping Over Obstacle performance during the first hour after landing.

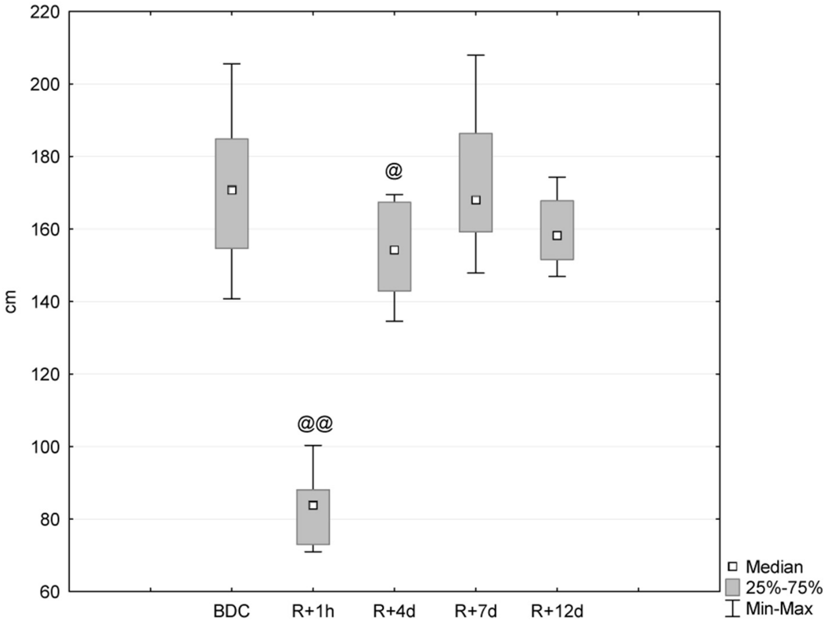

FIGURE 2: Angular amplitude in the hip joint on the day 3 after landing significantly decreased compared with the pre-flight level for the 5 (19,4±11,9%), 10 (15,3±7%), 30 (8,3±2,9%) cm obstacle. The same changes were registered for the knee joint for 5 cm obstacle angular amplitude decreased by 12,7±8,7%, for the 10 cm obstacle – 6,4±3%, for 30 cm obstacle – 8,8±5,1%. For the ankle joint the results were also the same. For 5 cm obstacle angular amplitude decreased by 11,4±8,2%, for the 10 cm obstacle – 11,5±10,4%, for 30 cm obstacle – 12,5±9,1%.

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Keywords: locomotion, space flight, motor control, stepping over an obstacle, microgravity (μg)

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Role of axial and support unloading in development of hypogravitational motor syndrome

Elena S. Tomilovskaya[1]*, Ilya V. Rukavishnikov[2], Tatiana A. Shigueva[1], Tatyana B. Kukoba[1], Inna S. Sosnina[1], Lyubov E. Amirova[1], Inessa B. Kozlovskaya[1]

[1]*Department of sensory-motor physiology and countermeasures, Institute of Biomedical Problems (RAS), Moscow, Russia*
[2]*Department of operative control of space flights' medical support, Institute of Biomedical Problems (RAS), Moscow, Russia*
**finegold@yandex.ru*

Dry Immersion (DI) studies that were performed for years at the Institute of Biomedical Problems have shown that support withdrawal is followed by the development of hypogravitational motor syndrome signs (HMS), similar to those observed after space flights: postural muscle atonia and atrophy, hyper-reflexia of spinal reflexes, voluntary movement discoordination, postural and locomotor alterations, back pain etc. (Kozlovskaya et al., 1988; 2007; Miller et al., 2004; Shenkman et al., 2017). Daily mechanical stimulation of the soles during DI eliminates the mentioned effects almost to full extent (Grigoriev et al., 2004; Kozlovskaya et al., 2007). Basing on these results it was concluded that support afferentation plays the role of trigger in development of HMS. However it is necessary to take into account that withdrawal of weight in weightlessness and DI is followed not only by supportlessness but also by axial unloading. So the aim of this work was to study the effects of supportlessness coupled with axial loading to define the role of axial load on HMS development.

Twenty healthy volunteers divided into 2 groups took part in the study. No influences except 5-days DI was used in the group which served as a control. The subjects of experimental group in DI every day for 4 hours were wearing "Penguin" axial loading suit that provided the 16-18 kg axial load on the body – from the shoulders to the ankles. Parameters of the postural and locomotor activities, the spinal stretch reflexes, the accuracy of voluntary movements, intervertebral discs height, back pain intensity, tone and force-velocity properties of leg and back muscles have been studied. All the mentioned signs of HMS were observed in the control group: the tone of postural muscles sharply decreased, hypereflexia of stretch reflexes was observed, muscle strength and endurance decreased, postural stability was altered, voluntary movement coordination declined. Results of the experiments confirmed our previous suggestion that muscular atonia provides the development of movement disorders, back pain and other phenomena such as increase of height, elongation of the spine and height of intervertebral disks, etc. (Rukavishnikov et al., 2016; Treffel et al., 2016). Back extensor muscles atonia and significant increase in the intervertebral disk height were registered in the lumbar spine projection; at the same localization all test subjects reported the symptoms of back pain. In the experimental group the intensity of back pain was significantly lower ($p=0.0028$ for DI1, $p=0.006$ for DI2 and $p=0.0004$ for DI3 – by ANOVA repeated measures with Bonferroni correction) (Fig.1).

Height increase and the decline of force-velocity properties of back and neck muscles were also significantly lower than in the group without countermeasures ($p<0.05$). However for the leg muscles the changes were similar to the control group. Spinal reflex facilitation (Fig.2), accuracy of hand voluntary movements' decrease and other HMS signs were not significant in this group. Slight tendency to H-reflex threshold decrease was registered in this group, however the changes were not significant ($p=0.062$).

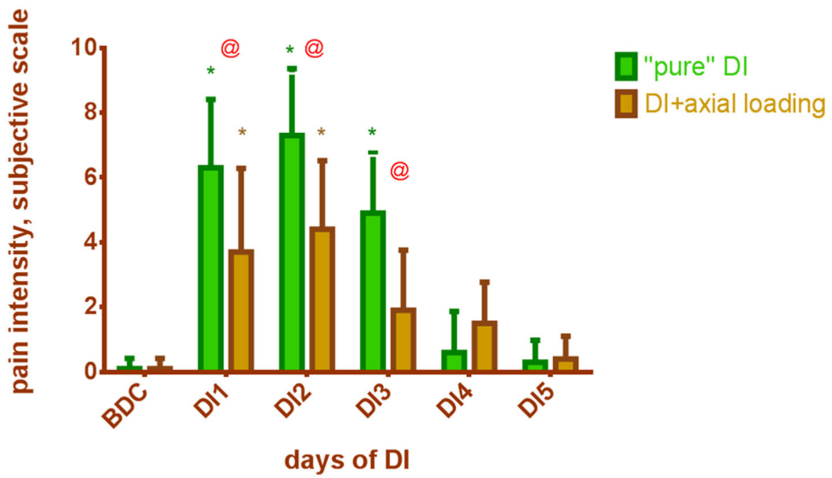

FIGURE 1: Back pain intensity in the course of Dry Immersion in 2 groups. * - significant difference compared to the baseline; @ - significant difference between groups (p<0.01).

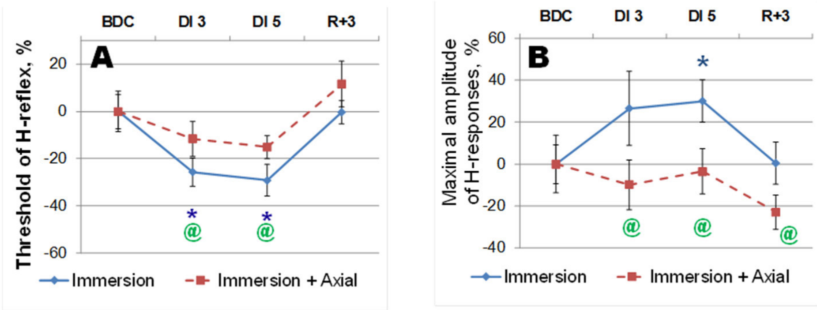

FIGURE 2: Changes of spinal reflex characteristics in 2 groups in the course of Dry Immesion. (**A**) – Changes in the threshold of H-reflex; (**B**) – Changes in peak amplitude of H-response. Blue lines – the group of "pure" immersion with no countermeasures; red dotted lines – the group of 4 hours daily axial loading in the course of Dry immersion. * - $p<0.05$ compared to baseline values; @ - significant difference between group ($p<0.05$).

There were not revealed significant difference between groups in parameters of postural and locomotor coordination. The results of the study have shown that axial unloading in microgravity plays significant role in development of HMS. However it reveals more tight connections of avail loading to muscles of the body when the support reactions are involved to a great extent in the control of leg muscles state and locomotor and postural functions.

ACKNOWLEDGEMENT

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Keywords: Back Pain, H-Reflex, microgravity, axial loading, dry immersion, hypogravitational motor syndrome, support unloading

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Effects of gravitational hindlimb unloading and plantar mechanical stimulation on calcineurin/NFATc1 signaling pathway and slow myosin expression in rat soleus muscle

Christina Sharlo*, Olga Turtikova, Inna Paramonova, Boris Shenkman

Institute of Biomedical Problems (RAS), Myology laboratory, Moscow, Russia
**lubimowa.cristina@yandex.ru*

INTRODUCTION

It was observed that gravitational unloading during space missions and simulated microgravity in ground-based studies leads to the slow MyHCIbeta expression decrease in the postural soleus muscle. After the first 24 hours of unloading it was observed a significant decrease of MyHCI(β) expression (Giger et al, 2009), although after 3 days of unloading there was no further decline of MyHC I (β) expression (Lomonosova et al, 2016). Further decrease of MyHC I (β) expression occured only after 7 days of unloading (Fauteck et al, 1995). Calcineurin/NFATc1 signaling system is believed to be the most important cascade that promotes the expression of slow MyHCI(β). However, the time-course of NFATc1 nuclear translocation while unloading is still unexplored. So the first aim of our work was to analyze the time-course of NFATc1 myonuclear translocation at the first week of unloading and to compare it with MyHC I(β) expression time-course.

Gravitational unloading also withdraw ground support reaction forces perceived by specific cutaneous receptors that transmit the stimuli to the nervous system. NFAT transcriptional activity acts as a nerve activity sensor in skeletal muscle. It was demonstrated, that increasing sensory input by performing plantar mechanical stimulation (PMS) to the soles of the feet results in an increase in neuromuscular activation of the lower limb muscles (Grigoriev et al., 2004). The second aim of our work was to test the effect of PMS on NFATc1 myonuclei accumulation and slow MyHCI(β) expression.

MATERIALS AND METHODS

Male Wistar rats were randomly assigned to vivarium control (C), 1-day unloading (1HS), 1-day unloading with PFS (1HS+PFS), 3-day unloading (3HS), 3-day unloading with PFS (3HS+PFS) and 7-day unloading (7HS) groups. The hindlimb unloading was performed by Morey-Holton method. The plantar stimulation protocol mimicked the normal animal walking (104 mmHg pressure, 4 Hz frequency and 250 ms signal duration) for a total of 20 min followed by a 10-min rest interval for 4 hours. Nuclear extract of soleus muscle tissue was prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents. The NFATc1 content in rat soleus nuclear extract was determined by Western-blotting. Total RNA was extracted and purificated by Qiagen RNeasy Micro kit followed by RT-PCR. MyHCI(β) expression was determined by real-time PCR. All results are given as box-and-whiskers diagram. Significant differences between groups were analyzed by Kruskal–Wallis test.

RESULTS AND DISCUSSION

After the first 24-hours of unloading NFATc1 content in nuclear extract of soleus muscle declined by 49% compared to control group. After 3 days of unloading there were no significant differences between unloaded group and control ($p=0.12$), although 30% NFATc1 nuclear content decrease was observed, and after 7-days unloading nuclear NFATc1 declined by 53% (Fig.1 A). So, time-course of nuclear NFATc1 content aligns with previously observed MyHCI(β) expression dynamics. This NFATc1 partial recovery after the 3 days of unloading may be caused by calcium ions accumulation in unloaded soleus muscle starting after the second day of unloading (Ingalls, 1999) leading to re-activation of calcineurin. The observed NFATc1 nuclear decrease compared to control corresponds to the data by Salanova et al., 2008, but is in variance with the data obtained by Dupont-Versteegden et al., 2002; after 14-days unloading. However, it may be possible that at some stages of unloading NFATc1 nuclear content may increase up to control values and MyHCI(β) expression decline may be partially regulated by other mechanisms, such as HDAC4 and MEF-2D pathways.

Plantar mechanical stimulation was found to prevent from both nuclear NFATc1 decrease (Fig.1 B) and MyHC I(β) expression decrease after 24 hours of unloading (data not shown). These data correspond to previous results that plantar mechanical stimulation prevented slow-to-fast fiber shift while 7-day

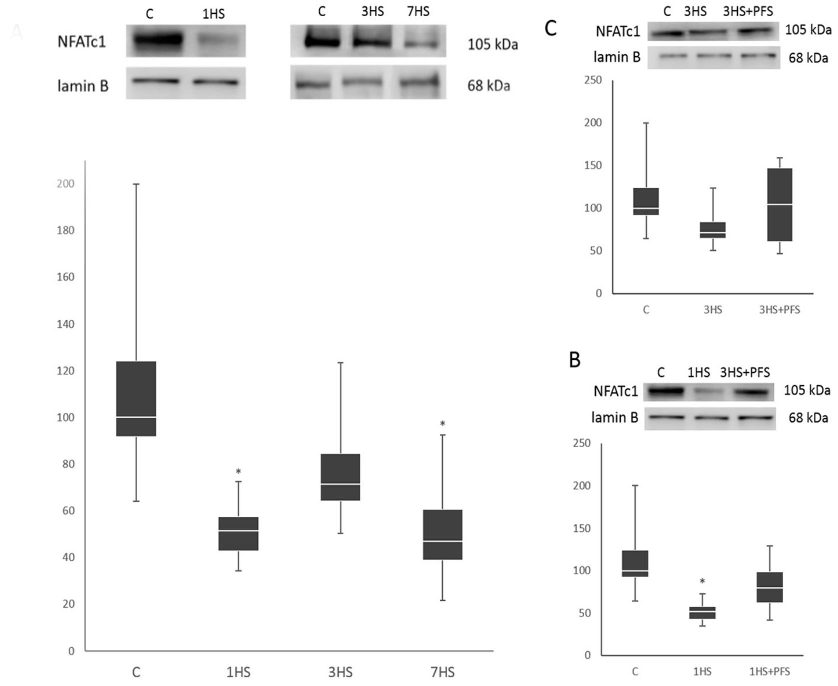

FIGURE 1: (**A**) – Western-blot analysis of NFATc1 content in soleus muscles nuclear fraction after 1 day (1HS), 3 days (3HS) and 7 days (7HS) of hindlimb unloading. (**B**) - Western-blot analysis of NFATc1 content in soleus muscles nuclear fraction control group (C), 1-day hindlimb-suspended group (1HS), and 1-day hindlimb suspended group with plantar foot stimulation (1HS+PFS). (**C**) – Western-blot analysis of NFATc1 content in soleus muscles nuclear fraction control group (C) - 3-day hindlimb-suspended group (3HS), and 3-day hindlimb suspended group with plantar foot stimulation (3HS+PFS). Data shown as % of control group by box-whisker-plots (interquartils range, maximum and minimum). *Significant difference from the control group (p<0.05).

exposure to dry immersion (Shenkman et al., 2004). At the same time after the 3-days unloading there were no significant differences between nuclear NFATc1 content in C, 3HS and 3HS+PMS groups (Fig 1 C), although the MyHC I(β) expression in 3HS+PMS group was 90% higher than in control group. It should be noticed that in this study we could not detect previously observed MyHCI(β) expression recovery after 3-days unloading. These discrepancies between NFAT nuclear content and MyHCI(β) expression may be explained by a time-lag between NFAT nuclear accumulation and MyHCI(β) mRNA accumulation.

So the obtained results show that the time-course of NFATc1 nuclear content aligned with previously described time-course of MyHCI(β) expression during the first week of unloading, and it may be possible that 3-day MyHCI(β) expression recovery is caused by NFATc1 nuclear content recovery. Plantar mechanical stimulation prevented NFATc1 nuclear content decrease after 1-day unloading and prevented MyHCI(β) expression decrease after 1-day and 3-day unloading.

ACKNOWLEDGEMENT

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Keywords: skeletal muscle, Calcineurin/NFAT signaling, myosin I, NFATc1, nuclear factor of activated T-cells cytoplasmic, hindlimb unloading model

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Alterations of eccentrically evoked protein synthesis in isolated rat soleus following gravitational unloading: Role of fak

Sergey Tyganov*, Timur Mirzoev, Sergey Rozhkov, Boris Shenkman

Institute of Biomedical Problems (RAS), Myology laboratory, Moscow, Russia
**sentackle@yandex.ru*

INTRODUCTION

Skeletal muscle mass is critical for muscle function and can vary depending on the load, activity or pathological conditions. Prolonged periods of muscle inactivity as a result of gravitational unloading can lead to muscle disuse atrophy, a complex process, which is characterized by a decrease in muscle fiber cross-sectional area, protein content, muscle strength and an increase in muscle fatigue (Thomason and Booth 1990, Zhang et al. 2007). Precise mechanisms by which mechanical load transduce to anabolic and catabolic response (mechanotransduction) remain undefined, despite of fundamental dependence of its development and maintenance from mechanical stimuli (Hornberger 2011). mTORC1 is a key regulator of mRNA translation and, consequently, protein synthesis. Accordingly, mTORC1 is able to influence both the translational efficiency (the rate of mRNA translation) and the translational capacity (rate of ribosome biogenesis) (Mahoney et al. 2009). Noteworthy that, mTORC1 activation by a different types of mechanical stimuli was shown in a skeletal muscle using various models (cell-culture, whole muscle in vivo and ex vivo models) (Goodman 2014). There are also different molecules that can be proposed as skeletal muscle mechanosensor – stretch-activated channels (SAC) (McBride 2003), focal adhesion-integrin system (Fluck et al. 1999), sarcomeric proteins (Gautel 2011) and others. Focal adhesion kinase can be interconnected with a phosphoinositide 3-kinase (PI3K) – FAK tyr398 phosphorylation leads to its binding with SH2 domain of 85kDa PI3K subunit, and this, in turn, to PI3K activation, and, therefore, to the whole PI3K, AKT, p70S6k pathway activation (Chen et al. 1996). In the present study we analyzed an impact of FAK inhibition on realization of mechanical signal in unloaded skeletal muscle after eccentric contraction.

MATERIALS AND METHODS

Unloading of the hindlimbs was induced by using a standard rodent hindlimb suspension/unloading model (Morey-Holton and Globus 2002). Wistar rats weighing 220±5 g were randomly divided into the following groups: Control (C), Hindlimb Suspension for 7 days (HS) and HS plus FAK inhibitor – PD562,271 (HS+PD) (Roberts et al. 2008). HS was followed by ex vivo bout of Eccentric Exercise (ECC). Isolated rat soleus muscle was placed in Krebs–Henseleit solution with constant perfusion of carbogen (95% O_2 + 5% CO_2) and maintaining of the temperature at 37°C. One end of the muscle was connected to a dynamometer/stress generator by silk threads through tendons and another to a fixed retainer. The muscle was then stretched to the optimum length (L_0). Eccentric contractions were performed using electric field stimulation (80 V, 50 Hz for 3 s). The muscle was stretched to 15% of L_0 during stimulation (the time of stretching and returning to the previous length was 100 ms) (Burry et al. 2007). Upon completion of the EC, muscles were subjected to Western blot analyses in order to determine the content of phosphorylated forms of the key anabolic markers. Phosphorylation status as a proxy of activation of the signaling proteins was expressed relative to the total amount of each protein. The rate of the protein synthesis was analyzed using SUnSET (surface sensing of translation) technique (Goodman et al. 2011). Intact muscle was compared to a muscle after ECC. The significance of the differences between groups was determined by analysis of the variance (ANOVA method).

RESULTS AND DISCUSSION

Western blot analysis revealed a significant decrease in phosphorylated/total ratios of p70s6k (marker of mTORC1 activity) and its substrate S6rp after 7-day HS. SUnSET measurements also showed a significant 40% decrease in the protein synthesis rate following 7 days of HS ($p<0.05$). We didn't observe any significant differences in the HS group compared to the HS+PD group. An increase in protein synthesis after ECC was significantly higher in the C group compared to the HS group (+127%), and didn't differ from that of the HS+PD group (Fig.1). We also observed similar effect for p70s6k and S6rp (Fig.2) phosphorylated/total ratios. Earlier we showed that inhibition of SAC in the unloaded rat soleus can lead to attenuated anabolic response to eccentric

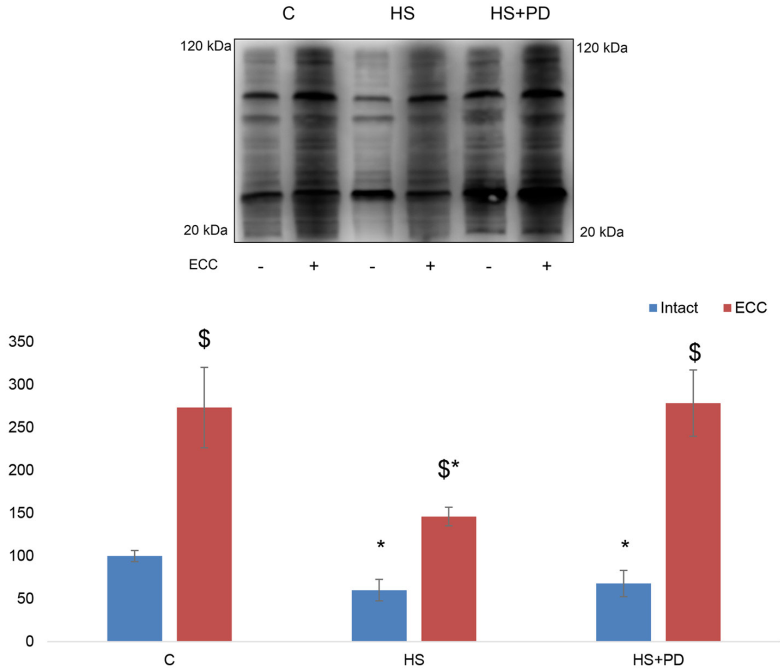

FIGURE 1: The rate of protein synthesis, % of control. C – control; HS – 7-day hindlimb suspension; HS+PD – 7-day hindlimb suspension plus FAK inhibitor injection. Blue bars – intact muscle; red bars – muscle after eccentric contractions. * – significant difference from control; $ - significant difference between intact muscle and muscle after eccentric contractions (p<0.05).

contractions (Mirzoev et al. 2018). Nevertheless, SAC inhibition did not lead to a complete AKT/mTOR pathway inactivation. This fact suggests that there are other mechanosensors which can detect and transduce mechanical signals. One of such mechanosensors is believed to be FAK. In the present study, FAK inhibition in the unloaded rat soleus muscle prevented the attenuated anabolic response following ECC. We speculate that this effect could be associated with the disturbance of possible reciprocal relationships between the two mechanosensors (SAC and FAK).

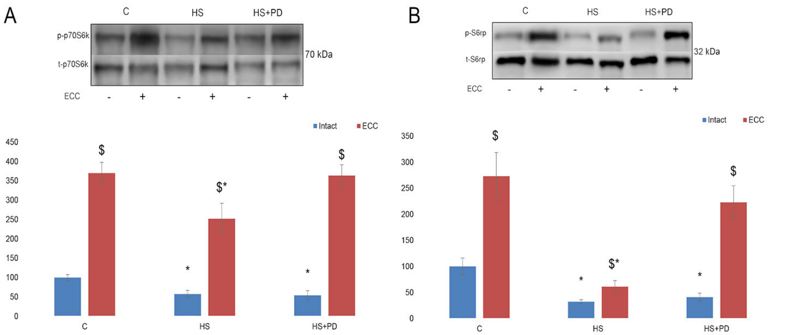

FIGURE 2: Phosphorylation status of the p70s6k (Thr 389) (**A**) and S6rp (Ser 240/244) (**B**), % of control. C – control; HS – 7-day hindlimb suspension; HS+PD – 7-day hindlimb suspension plus FAK inhibitor injection. Blue bars – intact muscle; red bars – muscle after eccentric contractions. * – significant difference from control; $ -significant difference between intact muscle and muscle after eccentric contractions (p<0.05).

CONCLUSION

In summary, the results of the study indicate that inhibition of FAK activity in the unloaded rat soleus muscle may prevent a diminished anabolic response to mechanical stimuli.

ACKNOWLEDGEMENT

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Keywords: mechanotransduction, mTORC1, hindlimb suspension (unloading), FAK, Eccentric contraction (ECC), soleus muscle

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Effects of antioxidants on bone turnover markers in 6° head-down tilt bed rest

Katharina Austermann[1], Natalie Baecker[1], Sara R Zwart[2], Scott M Smith[3], Martina Heer[1]*

[1]Ernährungsphysiologie, Institut für Ernährungs- und Lebensmittelwissenschaften, Universität Bonn, Bonn, Germany
[2]Department of Preventive Medicine and Community Health, The University of Texas Medical Branch at Galveston, Galveston, United States
[3]Human Health and Performance Directorate, National Aeronautics and Space Administration, Houston, United States
drmheer@aol.com

BACKGROUND

Inactivity during space flight and in ground-based analogs, such as 6° head-down tilt bed rest (HDBR), is associated with bone loss. This bone loss is mainly induced by decreased mechanical loading [1,2]. Immobilization is associated with an increase of oxidative stress [3,4], resulting from excessive formation of reactive oxygen species (ROS) or dysfunction of antioxidant defense systems [5]. Oxidative stress also leads to increased activity of bone resorption processes [5]. Thus, antioxidants like polyphenols, omega-3 fatty acids, vitamins, and micronutrients may mitigate the damaging effects of ROS on bone turnover [6,7].

HYPOTHESIS

We hypothesized that antioxidant supplementation during 60-d HDBR would reduce bone resorption and increase bone formation compared to non-supplemented controls.

MATERIALS AND METHODS

A randomized, controlled, single-blind intervention study in a parallel design was conducted at the Institute for Space Medicine and Physiology, Toulouse, France with 20 healthy male volunteers (age 34 $\pm$ 8 y, weight 74 $\pm$ 6 kg). The study was implemented in two campaigns; each consisted of a 14-d adaptation, a 60-d HDBR, and a 14-d recovery phase. Ten volunteers participated in each campaign. In both campaigns, five volunteers were randomly allocated to the intervention group and five volunteers to the control group. In the intervention group volunteers received an antioxidant-cocktail, consisting of 741 mg polyphenols, 2.1 g omega-3 fatty acids, 168 mg vitamin E and 80 µg selenium. In the control group volunteers received no supplement. All volunteers received an individually tailored and strictly controlled diet. For the calculation of energy intake the resting metabolic rate of volunteers was measured. During adaptation- and recovery period a physical activity level of 1.5 was used for energy intake calculation. During HDBR a physical activity level of 1.2 was considered, because of reduced physical activity. Protein intake was between 1 and 1.2 g/kg body weight/d. Fat intake was estimated between 30-35 energy percent/d and remaining energy percent/d were consumed as carbohydrates. The protein intake was kept constant during the whole study period and the energy reduction during HDBR was achieved by reducing fat- and carbohydrate intake. To ensure an adequate micronutrient supply vitamins and minerals were kept constant during the whole study period, according to ESA bed rest standards, based on the dietary reference intakes (DRI) [8,9]. Serum calcium, parathyroid hormone (PTH), and bone formation marker aminoterminal propeptide of type I collagen (P1NP) were analyzed, along with urinary calcium and bone resorption marker C-telopeptide of type I collagen (CTX). Presented results comprise pre-bed rest values (baseline) and values at HDBR-d 60.

STATISTICAL ANALYSIS

Data presented are means $\pm$ standard deviation. The statistical analysis was performed with IBM SPSS statistics 25.0. Data was tested for normal distribution with Saphiro-Wilk test. For the analysis of an inner-group comparison paired t-test was performed for normal distributed data and Wilcoxon test for non-normal distributed data. The inter-group comparison was detected by an unpaired t-test for normal distributed data and Mann-Whitney-U test for non-normal distributed data. $P< 0.05$ was considered as significant.

RESULTS

For all parameters a significant time effect was observed. Urinary calcium excretion was increased at HDBR-d 60 compared to baseline (pre-bed rest) with an increase of 35 $\pm$ 20 % ($P<0.001$) for the intervention group and

32 ± 26 % (P=0.003) for the control group. Serum calcium increased in both groups (4 ± 3 % for intervention group, P=0.02; 3 ± 2% in control group, P=0.007). The decrease of PTH was significant for the intervention group only (-21 ± 13%, P=0.002 in intervention group vs. -12 ± 17% in control group, P=0.07). Serum P1NP was increased at HDBR-d 60 with +23 ± 22 % in the intervention group (P=0.012) and +16 ± 13 % in the control group (P=0.004). Urinary CTX increased in both groups at the end of bed rest: 77 ± 42 % in the intervention group (P<0.001) and 103 ± 89 % in the control group (P<0.001). For all parameters no antioxidant supplement effect was found.

DISCUSSION

Our preliminary results indicate that the applied antioxidant-cocktail did not affect calcium homeostasis, bone resorption- and -formation markers. Other human studies, however, showed positive results on bone turnover after consumption of antioxidant rich foods, such as dried plums [10], green tea [11] and soy products [12]. These findings might be attributed to differences in the type of antioxidant administration (extract vs. food). Other food compounds, such as lipids, proteins, vitamins, minerals and/or other polyphenols affect antioxidant bioavailability and therefore may affect their efficacy [13]. Apart from these food related factors, bioavailability also depends on internal factors such as gender, age and colonic microflora [14]. In the present study healthy male volunteers (age 34 ± 8 y) were included, whereas previous studies investigated the effects of antioxidants in postmenopausal women [10–12]. Thus, differences in gender and age may be another explanation for the contradicting results.

CONCLUSION

The antioxidant supplement applied as a countermeasure in this study did not reduce the deleterious effects induced by 60 days of HDBR on bone turnover.

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Keywords: antioxidants, bed rest, polyphenols, bone turnover, immobility

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Bone remodelling study using strontium enriched hydroxyapatite nanoparticles

Angela Maria Rizzo[1]*, Gaetano Campi[2], Francesco Cristofaro[3], Giuseppe Pani[1], Paola Antonia Corsetto[1], Barbara Pascucci[2], Giuseppina Rea[2], Livia Visai[3]

[1]*Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy*

[2]*Institute of Crystallography, Italian National Research Council, Rome, Italy*

[3]*Department of Molecular Medicine, University of Pavia, Pavia, Italy*

*angelamaria.rizzo@unimi.it

INTRODUCTION

Microgravity (MG) significantly modifies the metabolism of bone leading to site-specific alterations in remodeling of the bone tissue. A decrement in bone formation and an increase in bone resorption determine a significant loss of bone mass causing bone fragility and therefore a greater risk of fractures. The proposed study is focused on the development of the countermeasures to be taken in order to reduce the process of bone demineralization, while promoting a greater deposition of bone matrix by using a nanotherapeutic approach. Strontium (Sr) is present in the mineral phase of bone, in particular in regions with high metabolic activity turnover. Recently, both in vitro and in vivo studies of Sr effects showed the reduction of bone resorption and the promotion of bone formation. The mineralization process is crucial to the load-bearing characteristics of the bone extracellular matrix. In a previous study (Frasinelli et al., 2017), we produced stable and biocompatible suspensions of calcium (Ca100) and Strontium (Sr100) hydroxyapatite nanoparticles (nHAps) to be potentially used to deliver Ca or Sr to bone cells. In the work presented here, we have studied the role exerted by the addition of exogenous Ca100-nHAp or Sr100-nHAp as countermeasure to MG induced osteoporosis, by using a model of human bone marrow mesenchymal stem cells (hBMSCs) differentiated in simulated MG (Random Positioning Machine, RPM). Further, to deeper investigate the mechanism on bone formation, we studied the spatiotemporal dynamics of mineral deposition by hBMSCs differentiating toward osteoblasts promoted by the presence of exogenous hydroxyapatite nanoparticles, using scanning micro X-ray diffraction and scanning micro X-ray fluorescence (Campi et al., 2017).

MATERIALS AND METHODS

Human bone marrow mesenchymal stem cells were differentiated for 8 or 28 days as previously described (Campi et al., 2017) but cultured in simulated MG using a RPM (Fokker space) at 37°C. Ground controls (GC ctrl) were performed in the same room on the RPM frame to simulate the effect of instrument vibration (Fig. 1 blue line). Cells were untreated (RPM ctrl, red line) or treated with Ca100-nHAp (RPM Ca100, Fig. 1 green line) or Sr100-nHAp (RPM Sr100, Fig. 1 yellow line) produced and characterized as previously described (Frasnelli et al., 2017). At 28 days of cell differentiation, samples were analyzed for bone markers using confocal laser scanning microscopy (CLSM) and Elisa techniques.

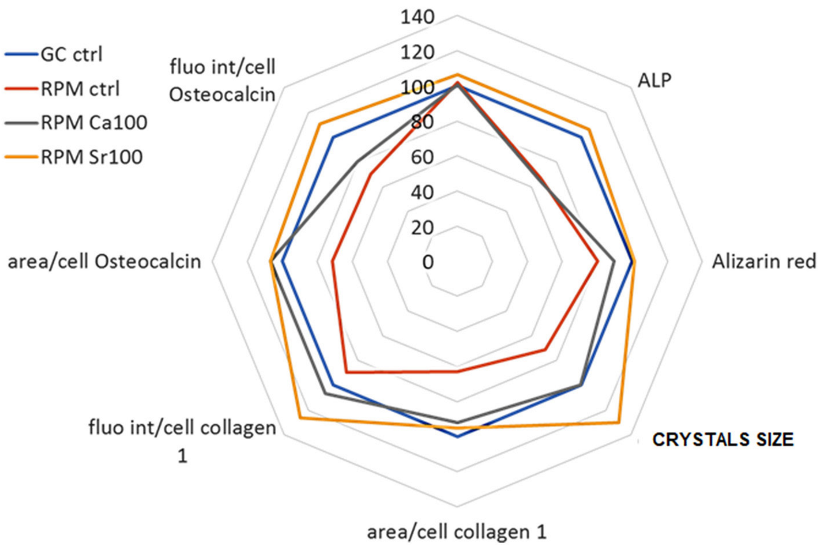

FIGURE 1: Effects of simulated MG (28 days) on hBMSCs untreated or treated with Ca and Sr enriched nHAPs. Bone markers expression (ALP, alkaline phosphatase activity, Ca crystals size, Collagen area and florescence intensity, Osteocalcin area and fluorescence intensity) is shown to be reverted by the addition in the culture media of Ca100-nHAps (green line) or, at higher extent, by Sr100-nHAps (yellow line), in comparison with untreated cells (red line). In blue line are indicated the cells cultivated in ground control, not exposed to RPM.

Moreover, samples were fixed to be further analyzed at the European Synchrotron Radiation Facility (ESRF) in Grenoble, (France) using scanning micro X-ray diffraction and scanning micro X-ray fluorescence.

RESULTS

Simulated MG strongly affects hBMSCs differentiation at day 28; bone markers are strongly modified in RPM exposed cells with a reduction of crystal size, a decrease in calcium deposition measured by alizarin Red, collagen 1, osteocalcin and ALP expression. Data obtained in our study are resumed in figure 1 and expressed as percent of control: in comparison with RPM control cells (red line), the RPM samples treated with Ca100-nHAps (green line) or Sr100-nHAps (yellow line) were able to strongly revert the effect of MG. In particular, this effect was higher in the presence of Sr100-nHAps in comparison to Ca100-nHAps.

At the molecular level, the added nanoparticles positively modulated the expression of bone-specific markers and enhanced calcified matrix deposition during osteogenic differentiation when analyzed both at early and late stages of differentiation (8 and 28 days), increasing matrix protein deposition in comparison with 1 g treated samples. The nucleation, growth and spatial arrangement of newly deposited hydroxyapatite nanocrystals were evaluated using scanning micro X-ray diffraction and scanning micro X-ray fluorescence (data not shown). As leading results, we have found the emergence of a complex scenario where the spatial organization and temporal evolution of the process exhibit heterogeneous and self-organizing dynamics.

CONCLUSIONS

The possibility of controlling the differentiation kinetics, through the addition of synthetic nanoparticles, paves the way to empower the generation of more structured bone scaffolds in tissue engineering and to design new drugs in regenerative medicine, useful during long term space flights.

ACKNOWLEDGEMENT

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Keywords: strontium, microgravity, Human bone marrow mesenchymal stem cell, nanop article, osteporosis

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Ceramide enriched membrane domains in rat skeletal muscle exposed to short-term hypogravitational unloading

Irina G. Bryndina[1]*, Vladimir A. Protopopov[1], Valery G. Sergeev[2], Maria N. Shalagina[1], Sergey V. Ovechkin[2], Alexey A. Yakovlev[1]

[1]Department of Pathophysiology and Immunology, Izhevsk State Medical Academy, Izhevsk, Russia
[2]Department of Physiology, Cell Biology and Biotechnology, Udmurt State University, Izhevsk, Russia
**i_bryndina@mail.ru*

INTRODUCTION

Since the 90th, many studies confirmed the important role of sphingolipid messengers including ceramide in regulation of skeletal muscle function. We have previously found that ceramide accumulates in disused soleus muscle of rodents subjected to 12 h, 4, 14 and 30 days of hindlimb unloading (HU), and one of the possible mechanisms of this effect is acid sphingomyelinase (aSMase) upregulation (Bryndina et al., 2014, 2017). We also revealed that aSMase inhibitor, clomipramine partially prevented membrane lipid raft disassembly (Bryndina et al., 2018), shown previously to be induced by 6-12 h HU (Petrov et al., 2017). In the present work we studied the impact of short-term HU on the amount and distribution of ceramide in rat soleus muscle.

MATERIALS AND METHODS

Adult male Wistar rats (n=12) were subjected to 4-day hindlimb unloading (HU) by tail-suspension method of Novikov & Il'in in Morey-Holton modification. Some of these rats were clomipramine treated (1.25 mg/g/day, intramuscularly, for 5 days before HU and every other day during HU, n=6), another part of the animals (n=6) was treated with vehicle (0.9% saline solution). Intact rats were used as a control group (n=6).

Ceramide amount and distribution in soleus muscle fibers were studied biochemically and immunohistochemically. Biochemical analysis was performed by thin layer chromatography of detergent resistant membrane fraction (DRM) isolated from muscle homogenates by ultracentrifugation in sucrose gradient (Radeva, Sharom, 2004). Lipids extracted from the DRM by the Folch reagent were developed on HPTLC Silica gel 60 F254 plates (Merck) according to Boath et al. (2008) together with a standard chloroform solution of ceramide (Avanti). Spots were visualized by iodine vapor. Quantitative evaluation of ceramide amount was performed with Sorbfil UV Videodensitometer (Sorbpolymer). Western-blot analysis (Laemmli, 1970) was applied to detect the amount of aSMase and nSMase proteins in DRM.

For immunohistochemical staining of longitudinal and transverse muscle cryosections, anti-ceramide antibodies (murine IgG, 1: 300, Abcam) and anti-mouse biotinylated antibodies (goat IgG, 1: 200, Abcam) were used. Analysis of the images obtained with the Nikon Epsilon E200 was performed using the Image-Pro Plus 6.0 and Image-Pro Insight programs. The expression of immunoreactive Cer was estimated by the fluorescence intensity of the test section on a standard area (0.1mm2). Each 5th section was taken and 10 measurements of intensity were made (at least 150 measurements per animal).

Statistical analysis was performed using SPSS 6.0 program. Depending on the distribution, one-way ANOVA with post hoc test or Mann-Whitney U test were used, the differences between groups considered significant at $p<0.05$.

RESULTS

Biochemical study of DRM revealed the increase of ceramide and sphingomyelinases amount after 4d HU. Ceramide was higher 4-fold ($p<0.05$) after HU, but did not differ significantly from the control level in rats suspended with clomipramine treatment. The levels of aSMase and nSMase proteins were increased by $75.2 \pm 9.4\%$ and $161.6 \pm 27.3\%$ respectively ($p<0.05$). Clomipramine treatment completely abolished aSMase increase and did not restore nSMase content.

Immunoreactive labeling of Cer in m. soleus fibers showed a substantial rearrangement of its distribution in unloaded muscle compared with the intact one. The anti-Cer staining of intact muscle fibers revealed very weak Cer labeling. Immunoreactive Cer looked like a diffuse fluorescence of an adjucent to membrane sarcoplasm, a low amount of lightly colored small size granules on the surface of the cytoplasmic membrane (Fig. 1A-C), and small fibrillar and globular structures within the fibers (not shown). Even in the visual assessment, a significant increase in both the

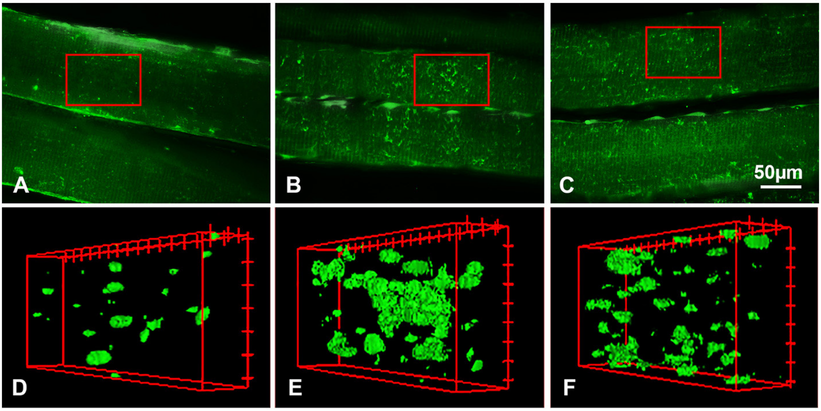

FIGURE 1: Clomipramine reduced the formation of large ceramide membrane domains in unloaded rat soleus muscle. Muscle fibers labeled with anti-ceramide antibodies (upper line); 3D reconstruction (lower line): (**A, D**) – control; (**B, E**) – 4-day hindlimb unloading; (**C, F**) - 4-day unloading with clomipramine treatment.

number and volume of Cer immunoreactive structures were observed after 4 days of HU. Unloading was accompanied by the increase in total intensity of immunoreactive Cer staining, which became higher by 84.6 $\pm$ 12.1% ($p<0.05$) in the sarcolemma and by 55.7 $\pm$ 12.7% ($p<0.05$) in sarcoplasmic compartment. In addition, we observed a significant rearrangement of the immunopositive to Cer surface structures which looked like intensely luminescent fused large granules and irregular fibrils that formed a kind of big clusters or platforms. Clomipramine pretreatment decreased the overall luminescent immunoreactivity to Cer (by 14.3 $\pm$ 4.5%, $p<0.05$), but its value was still significantly higher than in the control animals ($p<0.01$).

3D reconstruction of Cer membrane domains allowed us to evaluate their volumes (fig.1D-F). It has been shown that, in the membrane of control animals, there are two principal populations of Cer structures, "small" and "medium" in size. Unloading leads to the emergence of two additional groups of clusters: "large" and "very large," and a number of such clusters becomes more than "small" and "medium" ones. Clomipramine treatment abolished the population of "very large" clusters, significantly reduced the amount of "large" and "medium" ones, associated with the parallel formation of the group "intermediate" in size. Although the number and volume of Cer immunopositive clusters reduced their amount still kept higher than in control animals.

DISCUSSION

The present data confirm our previous findings of the intensive Cer formation in postural muscle disused with unloading (Bryndina et al., 2014), and expand the knowledge about the membrane effects of Cer in short-term disuse (Bryndina et al., 2018). We demonstrated for the first time that ceramide forms the peculiar clusters in the plasma membrane of unloaded soleus muscle, and we believe that these clusters are similar to the previously described Cer enriched membrane domains (Grassme et al., 2001). Cer microdomaines can spontaneously merge into large clusters known to serve as signaling platforms for a number of receptors, channels and other proteins. The main mechanism of their formation is heightened sphingomyelinase hydrolysis activated in response to some environmental stimuli (Grassme et al., 2001) including muscle disuse (Bryndina et al., 2014, 2017). In the present study we have demonstrated the increase of aSMase and nSMase availability in DRM of soleus muscle, and this is the main mechanism of Cer accumulation in the plasma membrane of unloaded muscle. Clomipramine attenuates Cer and aSMase upregulation without affecting nSMase. New results obtained in the present study support our previous data about efficacy of clomipramine pretreatment in the prevention of lipid raft disorders caused by 12h disuse (Bryndina et al., 2018).

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Disuse skeletal muscle atrophy in humans. Proteomic and molecular adaptations

Lorenza Brocca[1]*, Monica Canepari[1], Jörn Rittweger[2], Marco Vincenzo Narici[3], Maria Antonietta Pellegrino[1], Roberto Bottinelli[1]

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy
[2]Deutsches Zentrum für Luft- und Raumfahrt, Helmholtz-Gemeinschaft Deutscher Forschungszentren (HZ), Cologne, Germany
[3]Dipartimento di Scienze Biomediche, Università degli Studi di Padova, Padova, Italy
**lorenza.brocca@unipv.it*

Skeletal muscle atrophy is a multifactorial process common in different catabolic conditions. It can be a consequence of many diseases, e.g., cancers, AIDS, metabolic diseases, sepsis, burn injury, organ failures, and respiratory diseases. However, unloading of skeletal muscles is one of the most frequent and relevant causes of muscle atrophy, being observed in conditions such as limb casting following trauma, limb suspension and bed rest, . Finally, atrophy is a major consequence of muscle unloading in microgravity. Muscle atrophy is characterized by reduced fiber size, loss of force and power and decreased myosin concentration.

Muscle atrophy is due to an imbalance between protein synthesis and protein degradation. Most studies carried out on animal models of disuse showed that an initial decrease in protein synthesis is followed by a likely predominant increase in protein breakdown (Pellegrino et al. 2011; Powers et al. 2005; Thomason & Booth, 1990). On the contrary, several human studies seem to indicate that increased protein breakdown, as evidenced by increased expression of the atrogenes, is a transient phenomenon mostly explaining the first few days (˜4 days) of immobilisation and thereafter (Suetta et al. 2012) a decline in muscle protein synthesis (MPS) is the predominant mechanism (Phillips et al. 2014).

However, the mechanisms underlying skeletal muscle atrophy likely vary through species and in the same species through different models and muscles (Pellegrino et al. 2011).

DISUSE MUSCLE ATROPHY IN MICE

The hindlimb-unloading is one of the most studied mouse models of disuse atrophy. In this model, mitochondrial dysfunction indicated by several phenomena such as downregulation of mitochondrial enzymes, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and mitochondrial profusion proteins (Mitofusin 1, Mitofusin 2 and OPA1) have been observed. In particular, downregulation of PGC1α, a master controller of mitochondrial biogenesis, in a slow muscle as Soleus (Cannavino et al. 2014) and a downregulation of mitofusins in a fast muscle as Gastrocnemius (Cannavino et al. 2015) have been indicated as a major cause of disuse atrophy. On the contrary, the data did not support a major role of redox imbalance in triggering the phenomenon (Pellegrino et al. 2011) in contrast to what was previously reported (Powers et al. 2005). The role of a metabolic program causing disuse muscle atrophy is supported by the observations that high PGC-1α levels prevent the detrimental effects of FoxO3, a transcription factor activating the ubiquitin proteasome system, on muscle mass. Consistent with this, skeletal muscles from unloaded transgenic mice overexpressing PGC1α did not show upregulation of catabolic systems (Ubiquitin proteasome system and autophagy system) and downregulation of pro-fusion proteins protecting from disuse muscle atrophy (Cannavino et al. 2014; Cannavino et al. 2015). The same mechanism was observed in other disuse models like denervation and fasting (Sandri et al. 2006).

DISUSE HUMAN MODELS

In humans, the mechanisms and effectors underlying skeletal disuse atrophy are not fully understood. We studied three different human models, Bed Rest (BR), unilateral lower limb suspension (ULLS) and Space Flight (SP). Healthy young man (age = 18–25 years) were enrolled in BR campaigns and in ULLS program and muscle investigations were carried out on biopsies from vastus lateralis muscle. Moreover, the adaptations of skeletal muscle were studied in soleus muscle from 2 astronauts that have been in the International Space Station for 6 months.

All disuse models were associated with single muscle fiber size decrease with BR and ULLS showing similar loss of mass (approximately 25%) while in SP, one astronaut showed atrophy degree comparable to BR and ULLS and

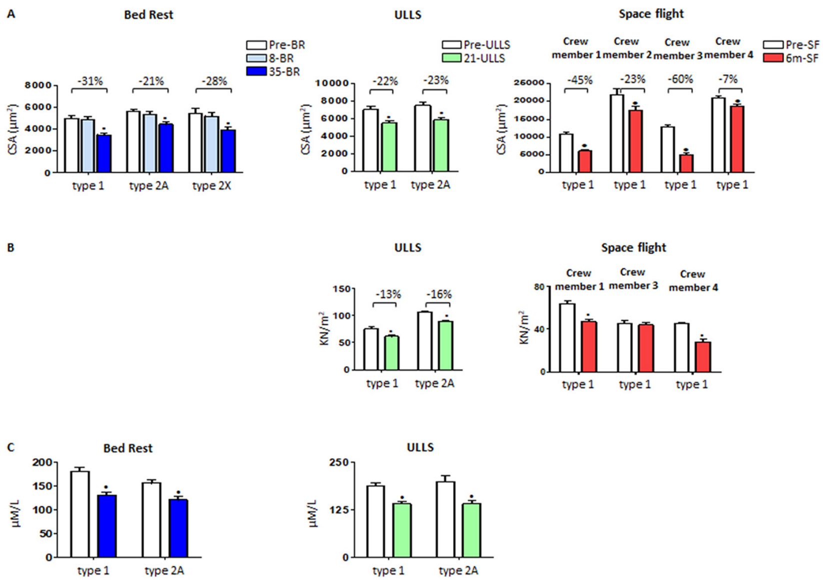


FIGURE 1: Effect of BR, ULLS and SF on muscle fibre size, specific force an d myosin concentration. (**A**), cross sectional area (CSA) of single muscle fibres, identified on the basis of MHC isoform content, pre-BR, post 8 days of BR (8-BR) and post 35 days of BR (35-BR), pre-ULLS and post 21 days of ULLS (21-ULLS), pre-SF and post 6 months of SF (6m-SF). (**B**), specific force measured in single muscle fibres, type-1 and type-2A. (**C**), myosin concentration evaluated on single muscle fibres, type-1 and type-2A. CSA is expressed in μm^2, Po/CSA is expressed in kN/m^2 and myosin concentration is expressed in μM/L. • $P < 0.05$, significantly different from Pre-BR or Pre-ULLS or Pre-SF. Data are the mean ± SEM.

the other one 45% of fiber size reduction (Brocca et al. 2012; Brocca et al. 2015; Rittweger et al. 2018) (Fig. 1A).

Moreover, all disuse models were characterized by loss of specific force of single muscle fibres (Trappe et al. 2004; Brocca et al. 2015; Rittweger et al. 2018) (Fig. 1B). In BR and ULLS the latter phenomenon could be ascribed to a significant decrease of myosin concentration in single muscle fibres (Borina et al. 2010; Brocca et al. 2015) (Fig. 1C).

Notwithstanding the similar adaptations observed in muscle mass and function in all disuse models, the underlying molecular mechanisms were somewhat different.

Bed rest. Following 24 days of BR, the increase of polyubiquitinated protein level, Beclin1, P62 and LC3B after 24 days suggested an activation of Ubiquitin proteasome system (UPS) and autophagy (Fig. 2A). On the contrary, the

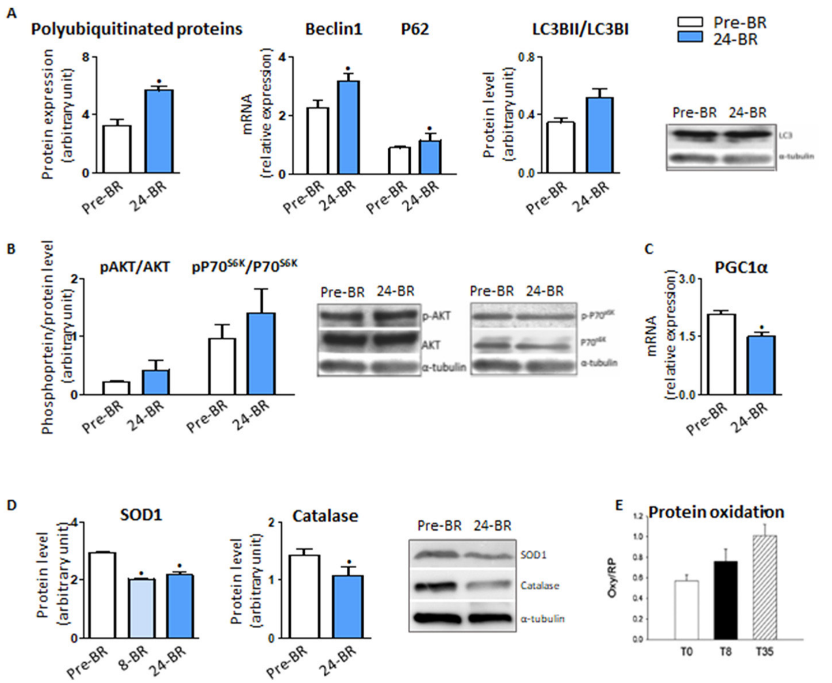


FIGURE 2: Effect of 24 days of Bed Rest on catabolic and anabolic pathways, metabolism and oxidative stress. (**A**), relative content of polyubiquitinated proteins; Beclin-1and p62 mRNA expression evaluated by quantitative RT-PCR; ratio between the content in II and I forms of LC3 by Western blotting and relative representative blot. (**B**), pAKT/AKT and $pP70^{SGK}$/ $P70^{SGK}$ protein level assessed by Western blot and relative representative blot. (**C**), PGC1α mRNAexpression level evaluated by quantitative RT-PCR; (**D**), SOD1 and Catalase protein level assessed by Western blot and relative representat ive blot. (**E**), Carbonylated protein levels evaluated after 8 and 35 days of BR. • $P < 0.05$, significantly different from Pre-BR. Data are the mean± SEM.

unchanged levels of markers involved in protein synthesis pathways (AKT and P70S6K) did not support a role of lower protein synthesis in muscle mass loss (Brocca et al. 2012) (Fig 2B).

Regarding the triggers of the adaptations in intracellular signaling pathways, both mitochondrial dysfunction and the reactive oxygen species (ROS) production can activate degradation pathways and inhibit protein synthesis pathways. In BR, the lower mRNA levels of PGC-1α suggest the presence of mitochondrial impairment (Brocca et al. 2012) (Fig. 2C). Moreover, a significant reduction of Superoxide dismutase 1 (SOD1) and Catalase was found after 8 and 24 days of BR (Brocca et al. 2012) (Fig. 2D). The alterations of antioxidant defense systems occurring in the early phase of disuse lead to protein carbonylation in the last phase of disuse (35 days) (Dalla Libera et al. 2009) (Fig. 2E) indicating the presence of redox imbalance and oxidative stress. Therefore, in BR both mitochondrial impairment and oxidative stress could be potential triggers of the activation of degradation pathways.

ULLS. In ULLS (21 days) no changes in UPS and autophagy (Fig. 3A), but a significant reduction of phosphorylated form of AKT, S6 and 4EBP1 (proteins involved in IGF1/AKT/mTOR pathway) were found (Brocca et al. 2015) (Fig. 3B). Indeed, FoxO pathways did not appear activated and even their potential triggers were not induced. In fact, the mRNA levels of PGC-1α were unchanged (Fig. 3C), SOD1 and Catalase were upregulated and no alterations were observed in carbonylated protein level (Brocca et al. 2015) (Fig. 3D). The results suggest that a decreased activation of the protein synthesis pathway could play a major role in muscle mass loss and that oxidatve stress is unlikely to be a major trigger of muscle atrophy. Accordingly, several findings have underlined the role of impaired protein synthesis on human disuse atrophy (Rudrappa et al. 2016).

Space flight. 6 months of SF induced an increase of Atrogin1 and Beclin1 in two astronauts and an increase of MurF1and P62 in one astronaut (Fig. 4A). Moreover, a decrease of FAK and FRNK level (–60 and –44% respectively) in two crew members and a reduction of FAK-pY397 (-92%) in one crew member was observed (Rittweger et al. 2018) (Fig. 4B). Importantly, the adaptations in the FAK pathway indicated a reduction in protein synthesis since FAK can modulate the anabolic IGF1/Akt/mTOR pathway linking muscle atrophy to the imbalance between protein synthesis and degradation (de Boer et al. 2007; Graham et al. 2015, Klossner et al. 2009).

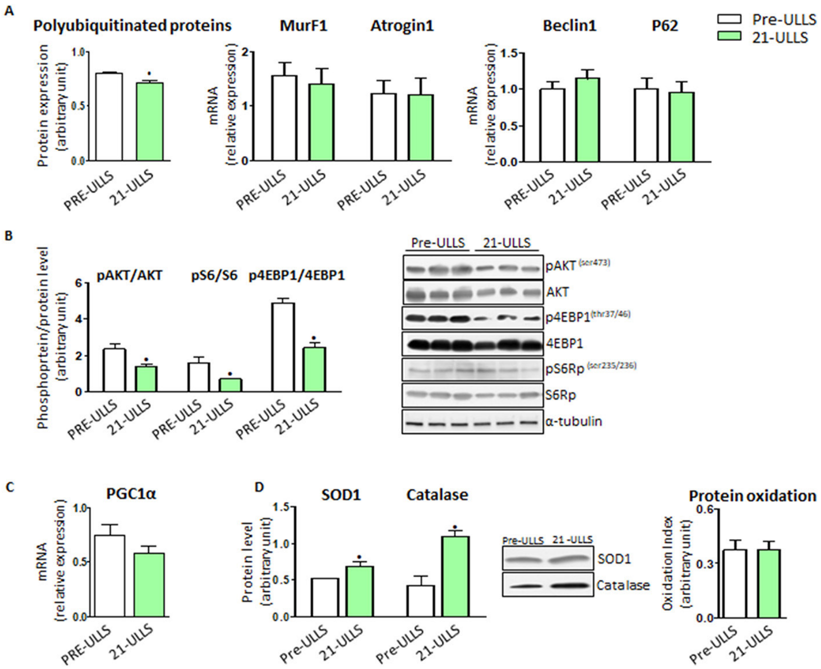
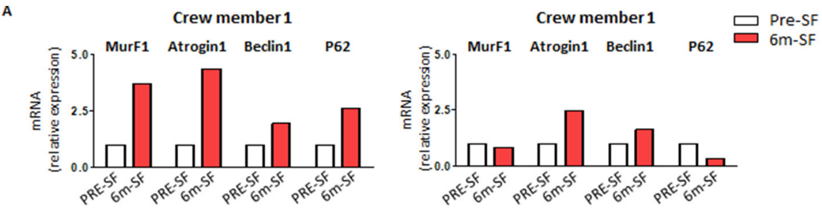


FIGURE 3: Effect of 21 days of Unilateral lower limb suspension on catabolic and anabolic pathways, metabolism and oxidative stress. (**A**), relative content of polyubiquitinated proteins; MurF1, Atrogin1, Bedin-1and p62 mRNA expression evaluated by quantitative RT-PCR. (**B**), pAKT/AKT, pS6/PS6 and p4EBP1/4EBP1 protein level assessed by Western blot and relative representative blot. (**C**), PGC1α mRNA expression level evaluated by quantitative RTPCR; (**D**), SOD1 and Catalase protein level assessed by Western blot and relative representative blot and Carbonylated protein levels. • $P < 0.05$, significantly different from Pre-ULLS. Data are the mean ±SEM.

The present data support a significant contribution of the protein degradation and protein synthesis pathways in muscle atrophy progression in SF, although the number of subjects did not enable to definitely settle the issue.

A



B

	PF-1	
Parameter	Crew member 1	Crew member 2
FAK-pY397	-92%	11%
FAK	-60%	-44%
FRNK	-60%	-67%

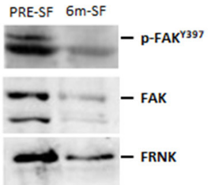


FIGURE 4: Effect of 6 months of Space Flight on catabolic and anabolic pathways. (**A**), MurF1, Atrogin1, Beclin-1and p62 mRNA expression evaluated by quantitative RT-PCR in crew member 1 and crew member 2. (**B**), p-FAKY^{391}, FAK and FRNK percentage of expression assessed by Western blot and relative representative blot.

CONCLUSIONS

The commune features in all human disuse appear to be: 1) atrophy of single muscle fibres, 2) loss of specific force and 3) decrease of myosin concentration (at least for BR and ULLS).

Notwithstanding the similar adaptations found in all three models, the mechanisms underlying muscle atrophy seem different.

Collectively the data suggest that the atrophy may be related to protein degradation in BR and linked to a down-regulation of protein synthesis in ULLS. In SF both processes could play an important role in muscle mass loss although more subjects are needed to settle such issue.

However, although In BR protein synthesis does not seem to play a major role, it should be noted that the phosphorylation of proteins belonging to IGF1/Akt/mTOR pathway might not reflect the actual rate of synthesis in vivo (Glover et al. 2008). Indeed, in humans, dissociation between phosphorylation of signaling proteins and protein turnover has been shown (Crossland et al. 2018).

Moreover, in BR, both mitochondrial dysfunction and redox imbalance appear as potential triggers of degradation pathways. However, in hindlimb unloaded mice, although the adaptations were found to be similar as those observed in human BR, a careful analysis of the relative role of mitochondrial dysfunction and redox imbalance indicated a major role for mitochondrial dysfunction and a minor role for redox imbalance. More work is, therefore, needed to understand which of the two conditions actually triggers atrophy, such as in human BR where disuse atrophy progresses slowly.

ACKNOWLEDGEMENT

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Keywords: catabolic pathways, muscle atrophy, synthetic pathway, human disuse, myosin loss

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Differences between left and right ventricular cardiac output during (simulated) hyper-to micro-gravity transitions

Lutz Thieschäfer[1]*, Jessica Koschate[1], Uwe Drescher[1], Andreas Werner[2,3], Daniel Dumitrescu[4], Uwe Hoffmann[1]

[1]*Institute of Physiology and Anatomy, German Sport University Cologne, Cologne, Germany*
[2]*Deutsches Zentrum für Luft- und Raumfahrt, Helmholtz-Gemeinschaft Deutscher Forschungszentren (HZ), Cologne, Germany*
[3]*Center for Space Medicine and Extreme Environments, Institute of Physiology, Berlin, Germany*
[4]*Abteilung für Kardiologie, Kölner Herzzentrum, Universitätsklinikum Köln, Cologne, Germany*
**l.thieschaefer@dshs-koeln.de*

PURPOSE

Alterations in gravity or body position impact direction and magnitude of hydrostatic pressure gradients acting inside the human body. Thus, pulmonary and systemic circulation are influenced which may lead to temporary asymmetries between left (Q'LV) and right ventricular cardiac output (Q'RV).

METHODOLOGY

Eight healthy male subjects (32 ± 3 yr, 182 ± 7 cm, 82 ± 6 kg) were tested during parabolic flight (PF) and also in an analog experiment in a long arm human centrifuge (laHC) and on a tilt seat (TS). Exposure to posGz in laHC and tilting angles on TS were chronologically matched to the PF protocol with amplitudes ranging from 1.2 posGz to 2.1 posGz and -6° to 90° respectively. During the (simulated) µ-G phases subjects performed one of four maneuvers in randomized order: inactivity, intermittent exerted exhalation (IEE), muscle contraction (MC) or intermittent exerted exhalation and muscle contraction combined (IEE-MC). Q'LV was measured beat-to-beat using a finger cuff and applying the Modelflow® algorithm. Pulmonary oxygen uptake was recorded breath-by-breath which allowed Q'RV calculation via the Fick principle assuming a temporary constant arteriovenous oxygen concentration difference.

RESULTS

The mean accumulated differences in Q'RV and Q'LV (ΣΔQ'RV-LV) during the initial 22 s of μ-G phase for all subjects represent the blood volume shift between systemic and pulmonary circulation. For the inactivity maneuver, ΣΔQ'RV-LV increases up to 0.42 L during PF and up to 1.45 L on TS. MC amplifies ΣΔQ'RV-LV with 0.64 L during PF and 1.72 L on TS. Whereas IEE switches the blood volume shift in opposite direction with ΣΔQ'RV-LV of -0.93 L during PF and -0.42 L on TS. IEE-MC shows almost equivalent effects like IEE. The consequences for ΣΔQ'RV-LV in laHC condition are negligible.

CONCLUSIONS

Reduction in gravity during PF as well as tilting the gravity angle from caudal to cranial direction on TS raises ΣΔQ'RV-LV. This is due to increased venous return to the right ventricle resulting in asymmetrical filling pressure compared to the left ventricle. Hence, a pooling of blood volume in pulmonary vessels can be assumed. MC enhances this effect inducing an even pronounced ΣΔQ'RV-LV. Contrary observations have been made regarding the IEE and IEE-MC maneuver. Intrathoracic pressure overcomes above mentioned effects and forces blood out of the pulmonary circulation into the left ventricle and reduces venous return. These responses should be considered in hemodynamic experiments comprising variations in gravity or body position.

ACKNOWLEDGEMENT

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Keywords: cardiac output, hemodynamics, hydrostatic pressure, pulmonary circulation, hyper-gravity, micro-gravity, parabolic flight, tilt table, systemic circulation, filling pressure, venous return, human centrifuge, blood pooling, blood volume shift

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Time- and frequency-domain parameters of heart rate variability and blood pressure in Parkinson's disease patients under dry immersion

Liudmila Gerasimova-Meigal*, Alexander Meigal

Department of Human and Animal Physiology, Pathophysiology, Petrozavodsk State University, Petrozavodsk, Russian Federation
**gerasimova@petrsu.ru*

"Dry" immersion (DI) is used as ground-based analogue of microgravity since it mimics some spaceflight features, such as supportlessness, centralization of body fluids, confinement, immobilization, and hypokinesia (Navasiolava et al., 2011; Watenpaugh, 2016). Besides merely space applications, DI is ever more extensively used in health care. In our earlier study, DI program exerted notable rehabilitation effect in Parkinson's disease (PD) patients (Meigal et al, 2018). Adaptation of hemodynamics to microgravity is rather complex and its mechanisms are not completely understood. DI is known to strongly affect body fluid distribution and circulation (Watenpaugh, 2016). Though DI less markedly effects on hemodynamics in comparison to other ground-based spaceflight simulations, in older adults or/and patients with chronic diseases one should carefully monitor circulation during DI to prevent its side effects, such as acute hypotension and orthostatic intolerance.

This study was focused on the evaluation of cardiovascular reactions of PD patients during DI using time- and frequency-domain characteristics of heart rate variability (HRV).

Eighteen PD patients (11 m, 7 f) aged 49-66 years, H&Y staged 1-3 volunteered to pass through DI program. Ten young healthy controls (HC, 6 m, 4 f) aged 19-26 years formed the reference group. During DI procedure, subjects were immersed in head-out supine position in thermo-neutral water for 45 min (Meigal A et al, 2018). Blood pressure (BP), heart rate (HR) and ECG in the standard lead II were monitored at rest (after the subject was lying for 15 min before DI procedure), on the 15th, 30th and 40th min of DI. HRV was analyzed on 5-min ECG–samples from subjects without arrhythmias. Intra-group differences were estimated by non-parametric criteria.

The investigated groups had slightly different BP and HR before DI. In HC group systolic and diastolic BP were 101-122 and 56-64 mm Hg, respectively, HR was 58-72 min-1. In PD patients systolic and diastolic BP were 111-132 and 73-82 mm Hg, respectively, HR was 65-79 min-1. During DI in PD patients we observed the decrease of diastolic BP to 62-73 mm Hg (p=0,003), and HR to 60-65 min-1 (p=0,006) in average while in HC these parameters did not significantly change.

Time- and frequency-domain HRV characteristics are presented on figures 1 and 2. In HC group, at rest condition, before DI, the autonomic

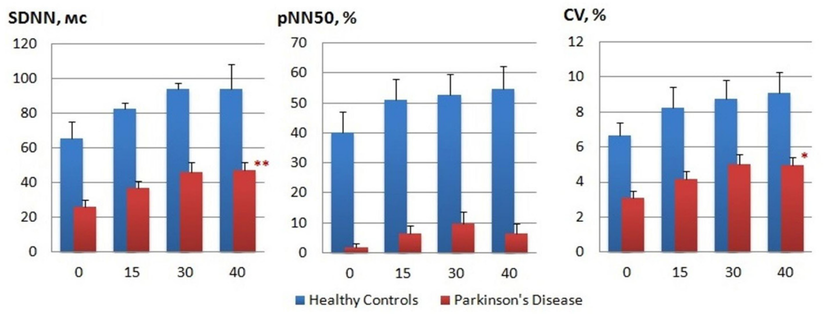

FIGURE 1: Effect of "dry" immersion on time-domain HRV characteristics.

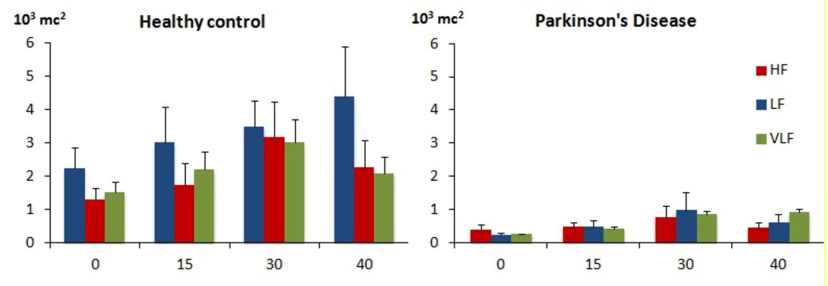

FIGURE 2: Effect of "dry" immersion on frequency-domain HRV characteristics.

regulation was characterized by marked variability of HR and prevalence of parasympathetic activity seen as high values of time-domain (SDNN - 39-101 ms, pNN50 - 28-60 %) and frequency-domain (TP 1540-9730 ms2, HF=46%, LF=25%, VLF=29%) parameters of HRV. DI in HC caused notable autonomic response, both parasympathetic and sympathetic. On the 30-40th min of DI we observed the increase of time-domain (SDNN - 58-132 ms, pNN50 - 43-74), and frequency-domain (TP - 2710-13560 ms2, HF=44%, LF=30%, VLF=26%) parameters of HRV. Stress index (SI) before DI was 15-140, and after DI - 10-71.

By contrast, PD patients before DI had low variability of HR (SDNN 17-30 ms2, pNN50 0-1 %), lower TP (310-1200 ms2), HF (36%), LF (25%), and higher VLF (39%). These time- and frequency-domain HRV characteristics correspond with finding that the affection of the autonomic nervous system in PD can be detected at early stages of PD and did not substantially change with disease duration (Maetzler et al., 2015). During DI in this group we observed the increase of time-domain (SDNN -36-58 ms, pNN50 - 1,7-6,6 %) parameters of HRV in respect with the rest condition ($p<0,01$) as well as frequency-domain (TP - 1010-2160 ms2) parameters of HRV, while the proportion of spectral bands shifted in the direction of lower frequencies (HF=26%, LF=28%, VLF=46%). SI before DI was 236-575, and after DI - 48-167. Thus, during DI parameters of HRV in PD patients tended to converge with the ones of HC group. In both groups, presented HRV parameters before and during DI proved significantly different.

In conclusion, time- and frequency-domain HRV parameters in PD patients, both before and during DI, can be explained by the deficiency of neurogenic (HF, LF) and prevalence of metabolic (VLF) factors in the control of hemodynamics. The tendency of HRV-parameters of PD patients under DI to shift in the direction of HC are in line with our earlier results on the temporary improvement of some motor symptoms and depression signs in PD after the DI program (Meigal et al, 2018). Nonetheless, one should consider the limited adaptation of cardiovascular system in PD patients because of autonomic dysfunction and to carefully monitor BP, ECG and to strictly follow the inclusion criteria in respect with DI.

FUNDING

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Keywords: heart rate (HR), dry immersion, Parkinsion's disease (PD), heart rate variability, blood pressure

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Evaluation of combined effects of lunar gravity simulation and the altered magnetic field on cardiovascular system of healthy volunteers

Yury I Gurfinkel[1,2], Mikhail V Baranov[3], Andrey L Vasin[4], Roman Y Pishchalnikov[5]*

[1]Medical Research and Education Center, Lomonosov Moscow State University, Moscow, Russia
[2]Space Research Institute (RAS), Moscow, Russia
[3]Federal Medical & Biological Agency of Russia, Moscow, Russia
[4]Institute of Biomedical Problems (RAS), Moscow, Russia
[5] Wave research center, Prokhorov General Physics Institute (RAS), Moscow, Russia
**rpishchal@kapella.gpi.ru*

The aim of this study is to evaluate the combined effects of a lunar bed rest analog and altered magnetic field, on cardiovascular system of healthy volunteers by means of the prerecorded geomagnetic storm and static magnetic field exposure.

MATERIALS AND METHODS

Eight healthy volunteers (26,1 $\pm$ 5,5 yr.) were enrolled in the blind study after previous examination of their health. The body mass index was 23.9 $\pm$ 3.9 kg / m^2. Blood pressure and pulse at rest were within the normal range. Subjects did not take any medications, as well as coffee or caffeine-containing beverages.

For the sake of comparison each person has been exposed twice: under geomagnetic storm (a Storm mode) and static magnetic conditions (a Quiet mode). During experiments, we did not provide for participants the information about the type of exposure to avoid possible influence of this information on the results of study. All volunteers gave the informed consent to take part in the experiment approved by the Committee on Biomedical Ethics.

The experimental facility for magnetic field exposure named "Faraday" allows providing different types of magnetic fields exposure for a couple of days. The bed for subjects is located in the center of a Helmholtz coil system

(2.5 x 2.5 x 2.5m), to obtain the maximally homogenous magnetic field during exposure. A natural geomagnetic storm has been initially recorded (k-index=7) by using 3-axis fluxgate magnetic field sensor (FL3-100, Stefan Mayer Instruments). In Storm mode, the most intensive 6-hour part of the record is repeated four times in a row. In the Quiet mode, the static magnetic field has the average induction of 49 μT. During the entire experiment, the subjects stay in the lying position. To simulate a lunar loading environment, the plane of the working surface of the bed was set at an angle of 9.6 degrees to the horizontal. The 3D picture of the facility and scheme of the experiments are shown in Fig. 1.

Physiological studies include only noninvasive techniques. Heart rhythm and heart rate (HR) were recorded using the monitoring system "Astrocard" (Russia) which allows a simultaneous viewing of the ECG on the screen of a mobile

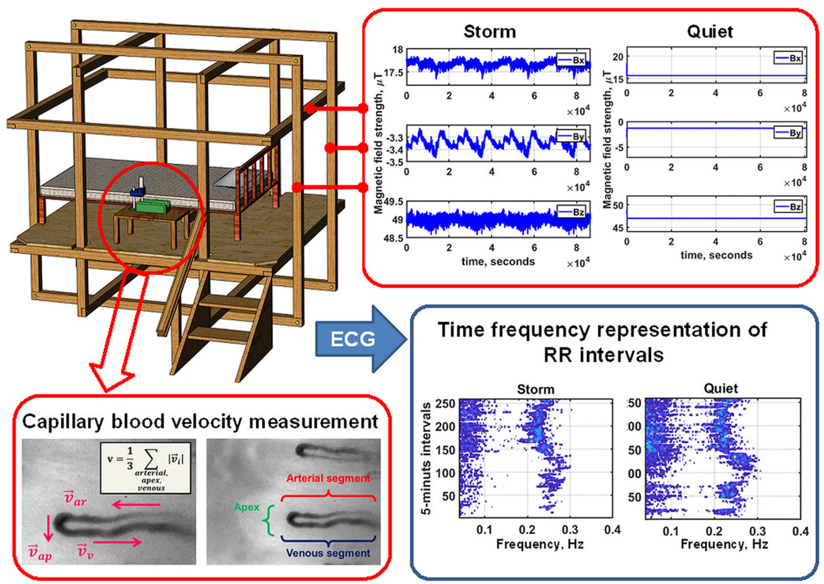

FIGURE 1: The magnetic field exposure facility. A screenshot from the capillaroscope CCD camera is at the bottom. The upper plots show dynamics of Bx, By, and Bz components for two modes of exposure: Storm and Quiet. The lower plots present the results of ECG processing: RR intervals series transformed to TRF with 5-minute time window.

device and storing the results obtained in the study (1). The capillary blood velocity was measured by the digital capillaroscope equipped with a CCD camera every hour of the study. Each of the volunteers carried out loading veloergometry tests before the beginning of the experimental conditions and the morning of the next day after their termination.

RESULTS AND CONCLUSION

Veloergometry tests showed a decrease in the working capacity of the subjects relative to the initial value after being in Storm, compared with Quiet mode.

The analysis of heart rate variability revealed a significant decrease in the duration of cardio intervals during the entire study when exposed in a magnetic storm compared to the conditions of a quiet magnetic condition. During the Storm exposure, 24-hour ECG monitoring of a particular volunteer has revealed episodes of cardiac arrhythmias (supraventricular and ventricular extrasystoles). A second-degree atrioventricular bloc (2 episodes) was found after 24-hour ECG monitoring in other individual during the Storm conditions.

In order to detect the effect of lunar gravity simulation, the RR interval series of our previous study (2), have been used. This study has been conducted under the same exposure conditions but with the horizontal bed. To compare RR intervals of both studies, we have transformed the time series to time-frequency representation (TRF). Such representation gives an opportunity to trace the time evolution of certain HF and LF frequencies in the power spectra of RR intervals (Fig. 2). The differences in LF and HF spectral regions of both studies allow us to reveal peculiarities typical for lunar gravity simulation.

ACKNOWLEDGEMENT

The ECG data processing was carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University.

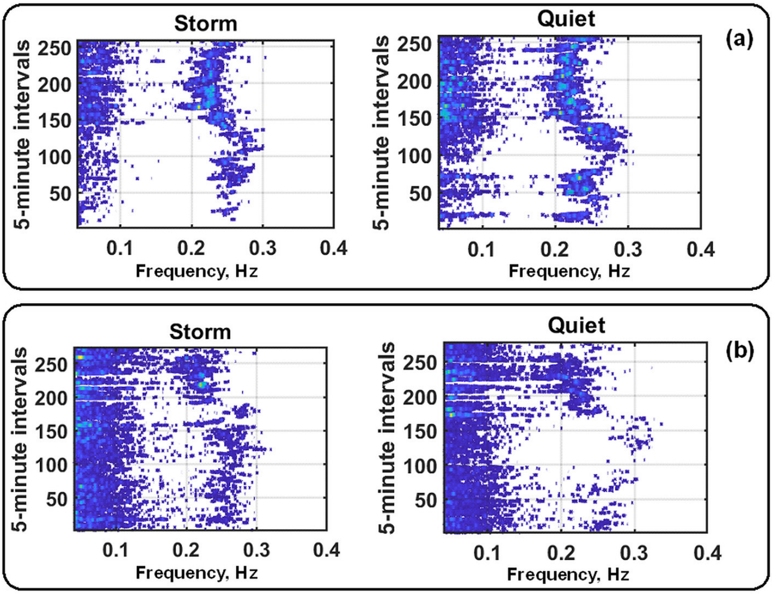

FIGURE 2: Daily time evolution of the RR interval power spectra at LF (0.04-0.15 Hz) and HF (0.15-0.4 Hz) frequencies is shown. The results of experiments with tilted (**a**) and horizontal (**b**) bed under different modes of exposure are shown for comparison.

Keywords: geomagnetic storm, lunar gravity, time frequency representation, ECG processing, capillaroscopie

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A comprehensive molecular and morphological study of the effects of space flight on human capillary endothelial cells: Sample quality assessment and preliminary results

Ivana Barravecchia[1,2], Chiara De Cesari[1], Olga V. Pyankova[1], Francesca Scebba[1], Mario Enrico Pè[1], Mattia Forcato[3], Silvio Bicciato[3], Helen A. Foster[4], Joanna Mary Bridger[5], Debora Angeloni[1]*

[1]*Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy*
[2]*Department of Pharmacology, University of Pisa, Pisa, Italy*
[3]*Center for Genome Research, Department of Life Science, University of Modena and Reggio Emilia, Modena, Italy*
[4]*Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, United Kingdom*
[5]*Department of Life Sciences, Brunel University London, Uxbridge, United Kingdom*
**angeloni@ifc.cnr.it*

Human subjects and experimental animal models returning from Space show health issues related to the endothelium, a diffuse organ fundamental in maintaining body homeostasis. There are striking similarities between the effects of Space Flight (SF) on astronauts, and the consequences of old age, degenerative diseases and leading a sedentary life on Earth. Therefore, understanding the pathophysiological processes caused by SF might significantly advance prevention and treatment of frequent pathologies associated with ageing and lack of exercise.

Up to now, several cell types were characterized, although partially, for their response to SF, except endothelial cells (ECs) of capillary origin, a fundamental part of the endothelium. Therefore, we launched a human EC line from dermal capillary (HMEC-1, human Microvascular Endothelial Cells 1, CDC, USA) to the International Space Station (ISS): 'Endothelial cells' project, http://blogs.esa.int/iriss/2015/08/25/how-do-blood-vessel-cells-react-to-weightlessness/ (Soyuz 44S-42S launch campaign, September 2015) and undertook a cell and molecular biology study that included analysis of protein markers, of the transcriptome (RNASeq) and the methylome. Cells were seeded onto Cyclic Olefin Copolymer coverslips (IBIDI, Germany) coated with 2% pork skin gelatine, in MCDB131 medium supplemented with 20 mM HEPES (all reagents: Sigma-Aldrich, USA). Cell cultures were placed into micro-incubators (Experimental Units, EUs, Kayser Italia, Livorno, Italy) that, when connected to power, are able to fulfill under electronic control and without human intervention a scientific protocol of cell culture and fixation 2. We prepared 40 EUs: 24, were integrated on the Soyuz rocket and reached the ISS, 16 others remained on Earth to serve as ground controls (GC), (Figure 1, Table 1). The study required six-days of culturing in space, within KUBIK onboard ISS, with two cell growth medium changes, and a final fixation step. The short-duration mission foresaw a 6-hours rendez-vous flight to the ISS that instead, for unpredicted reasons, lasted 48 hours. Only afterwards, the samples were integrated into KUBIK. In parallel, we activated the GC EUs within the KISS facility in Pisa. Due to the delay, the medium change within KUBIK and KISS was still activated twice but after two instead of three days each time. At the end of space mission, the science material was returned via a commercial flight to Pisa, traveling in the ESA Thermocase B5 (Yellow Box). Cell culturing procedures, fixation methods and feasibility of planned experiments had been extensively tested during the experiment definition and experiment sequence test phases of the project 2,3. However, due to the operational complexity of the whole study, when the samples arrived at Pisa, before undertaking the molecular analyses, we performed a quality assessment of fixed samples whose results we present here with some preliminary data.

Initially, we evaluated the cell general morphology with light-microscopy observation. All coverslips (except GM03, GM08, FM05, FM18, that were somewhat less satisfactory) were found covered with cells (about 100% confluent) adherent and elongated (examples in Figure 2A). DNA (Figure 2B) and RNA were extracted and quantified. Each sample type, i.e. microgravity exposed (μ–g), centrifuge exposed (space-1g), and GCs, yielded in average about 500 ng total RNA, and about 1 μg genomic DNA.

Eight samples (Table 1) were used for whole-transcriptome RNASeq analysis (Applied Genomics Institute, IGA, Udine, Italy). Libraries were prepared with TruSeq Stranded Total RNA with Ribo-Zero Library Prep Kit (Illumina, San Diego, CA) and sequenced with Illumina HiSeq 2500 platform; CASAVA v1.8.2 was used for base calling. Quality of 2X125bp reads was assessed with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Raw reads were aligned using STAR v2.5.3 4 to build version hg38 of the GENCODE human genome release 25. All samples had at least 44 millions PE reads uniquely aligned to the genome (Figure 2C). Counts for GENCODE

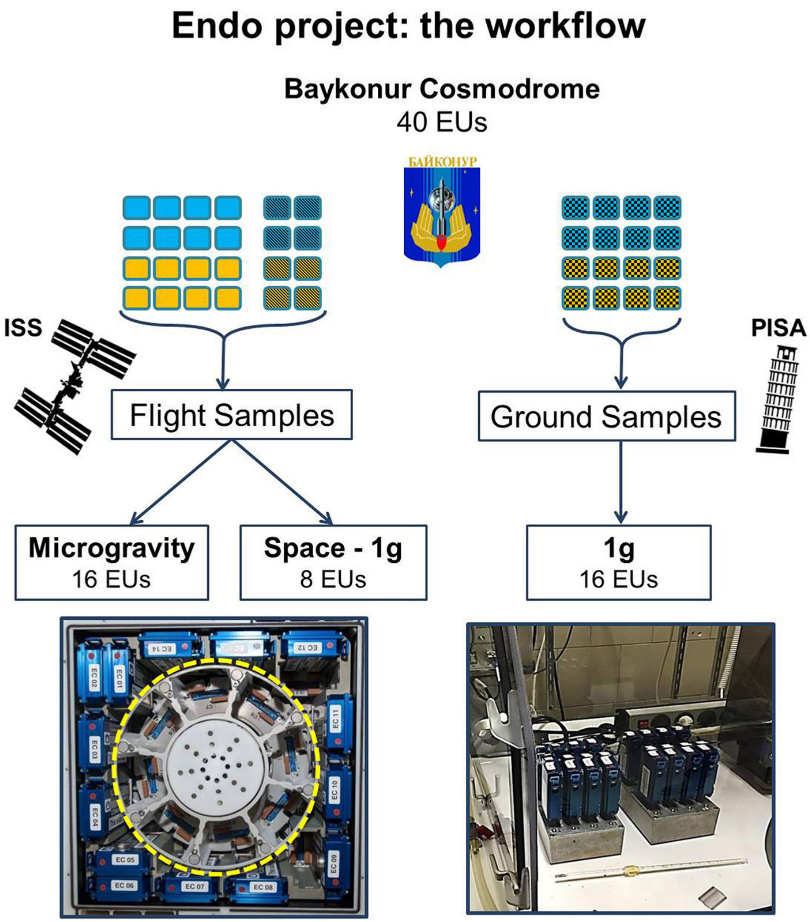

FIGURE 1: Workflow of the 'Endothelial Cells' (ENDO) study. The centrifuge (circled in yellow) within KUBIK could accommodate 8 EUs maximum. Blue: samples prepared for nucleic acid extraction. They were fixed in RNAprotect Cell Reagent (Qiagen, Germantown MD, USA) 1:6 in 1X PBS. Yellow: samples prepared for cell imaging analysis, fixed in NOTOXHisto (EarthSafe Industries, IL USA) 1:2 in 1X PBS. Diagonal stripes: samples to be exposed to 1g in space. Squares: GCs.

Table 1: The eight samples used for whole-transcriptome RNASeq analysis

Ground Control EU name	Analysis	Microgravity EU name	Analysis	Space-1g EU name	Analysis
GM1	Transcriptome	FM1	Transcriptome	FM17	Methylome
GM2	Methylome	FM2	Transcriptome	FM18	Transcriptome
GM4	Transcriptome	FM3	Transcriptome	FM19	Methylome
GM5	Methylome	FM6	Methylome	FM20	Transcriptome
GM6	Methylome	FM7	Methylome	FM21	Morphology
GM7	Transcriptome	FM8	Methylome	FM22	Morphology
GM9	Morphology	FM9	Morphology	FM23	Morphology
GM10	Morphology	FM10	Morphology	FM24	Morphology
GM11	Morphology	FM11	Morphology		
GM12	Morphology	FM12	Morphology		
GM13	Morphology	FM13	Morphology		
GM14	Morphology	FM14	Morphology		
GM15	Morphology	FM15	Morphology		
GM16	Morphology	FM16	Morphology		

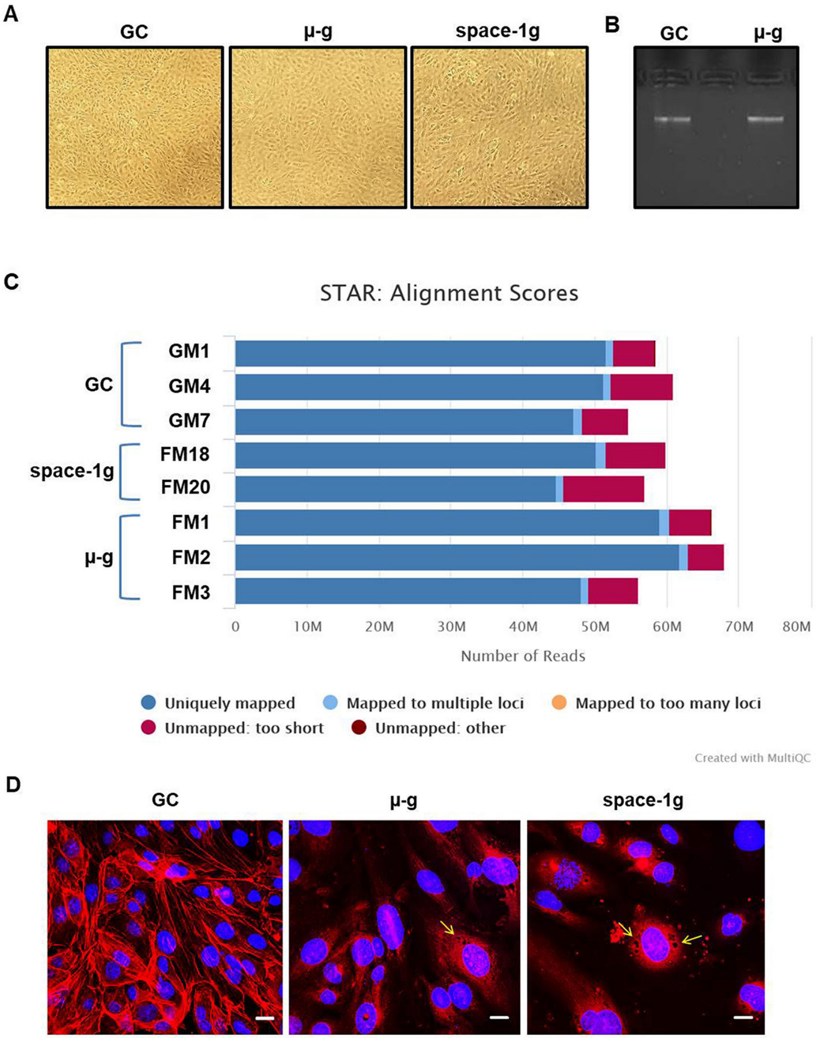

FIGURE 2: (A): Light-microscopy inspection of cell cultures returned from space. Microscope: Leica DM IL Led. Magnification: 10X. **(B):** Representative case of gel electrophoresis of gDNA from GC and μ-g samples. **(C):** Statistics for STAR alignment of the eight samples used for whole-transcriptome RNASeq analysis. Plot generated using MultiQC 5. **(D):** HMEC-1 stained for F-actin (red, phalloidin Atto 550, Sigma-Aldrich, USA) and nuclei (blue, DAPI, Sigma-Aldrich, USA). Confocal laser scanning microscope Nikon C2s, 40X. Scale bar: 20 μm. Yellow arrow: putative stress granules.

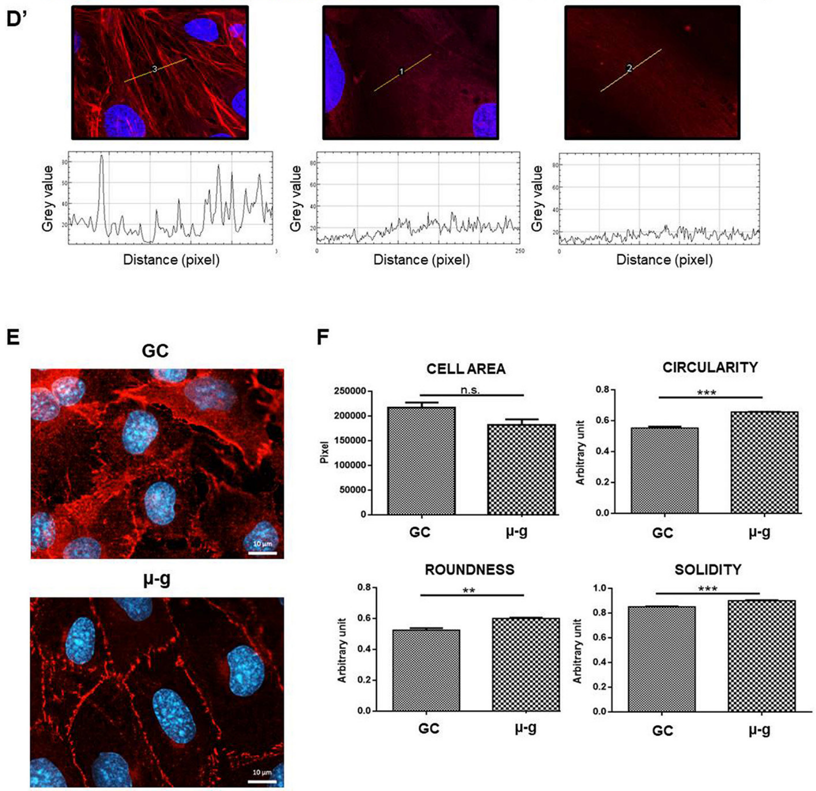

FIGURE 2: (Continued) (D'): Analysis of intensity of fluorescent peaks (ImageJ 1.50i software). **(E):** HMEC-1 stained with beta-catenin Monoclonal Antibody (Thermo Scientific) and DAPI (Sigma-Aldrich, USA). Microscope: Zeiss PALM MicroBeam, 100x objective. Scale bar: 10 μm. **(F):** To gain quantitative data about cell morphology, cell area and cell-shape descriptors (circularity, roundness and solidity) were calculated with ImageJ 1.50i software as in 6. We analyzed cells stained for beta-catenin. There were not statistically significant changes in cell area between GC and μ-g samples. However, circularity, roundness and solidity were significantly higher in μ-g cells than in GCs. At least 20 cells were analyzed by three independent operators.

basic annotated genes were calculated from the aligned reads using featureCounts function of the Rsubread R package 7. Normalization was carried out using edgeR R package 8. Raw counts were normalized according to library size to obtain counts per millions (CPM). Only genes with CPM greater than 1 in at least two samples were retained for subsequent analysis. Differential analysis was performed with voomWithQualityWeights function of limma R package 9, to down-weight outlier samples. Gene Set Enrichment Analysis (GSEA) 10 is in progress to determine whether the set of genes transcriptionally affected by microgravity or cosmic radiation was enriched in molecular pathways.

Eight samples (Table 1) were reserved for methylome analysis, currently in progress.

To evaluate cell morphology we began with F-actin staining because several reports describe the effect of microgravity 11–16, either real or simulated, on this protein in several cell types, so as to compare our own original results regarding microvascular ECs in real SF.

GCs showed well-defined F-actin stress and peripheral fibers whereas μ–g and space-1g cells showed collapsed F-actin networks (Figure 2D), with fluorescence intensity peaks considerably decreased (Figure 2D'). Also, stress granules (cytoplasmic structures that appear upon different stress stimuli 17,18) of different size were found in all space samples (Figure 2D, yellow arrow). These similarities in both space sample types were possibly caused by the long rendez-vous flight, and/or other space-related factors, e.g. cosmic radiation, that were not counterbalanced by four days at 1g. The distribution of beta-catenin appeared less cytoplasmic and more in structured and oriented adherens junctions of μ-g cells compared to GCs (Figure 2E). HMEC-1 cells showed statistically significant change of morphology, measured with descriptors as circularity, roundness and solidity (Figure 2F) 19.

Altogether, these preliminary observations confirm that SF stresses ECs, damaging basic assets of cytoskeleton and transcriptome activity, whose dynamics are known to be strongly intertwined. The disorganization of F-actin seems counterbalanced by well-structured adherens junctions that preserve the endothelial sheet in SF.

For the first time, this work attempts at an integrated study of morphological, protein and transcriptome markers on a same biological system, specifically microvascular ECs to describe as integrally as possible their response to SF. On-going analysis will uncover further molecular details regarding the adaptive response of this fundamental component of physiological homeostasis.

ACKNOWLEDGEMENT

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Keywords: microgravity, International Space Station (ISS), Soyuz, capillary endothelium, RNA sequencing (RNAseq), F-actin = filamentous actin, Illumina HiSeq 2500, human microvascular endothelial cells-1 HMEC-1

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Magnitude of cardiovascular system response is dependent on the dose of applied external pressure in lower body negative and positive pressure devices

Elizabeth Bird[1]*, Alan R Hargens[1], Lonnie Grove Petersen[1,2]

[1] *Clinical Physiology Laboratory, University of California, San Diego, San Diego, United States*
[2] *Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark*
**embird@ucsd.edu*

BACKGROUND

Environmental and tissue pressures can have large effects on arterial and venous blood flows. For example, application of positive pressures in the range of 20 to 40 mmHg to a small area of the lower leg can increase local muscle, skin and bone microvascular flows, while negative pressures between -20 and -40 mmHg have been shown to decrease microvascular blood flow.[5-7, 9, 10] Additionally, large LBNP devices create foot-ward venous fluid shifts and decrease stroke volume and blood pressure at -20 and -40 mmHg.[2,3] Diverse patient populations benefit from these phenomena, including astronauts and individuals with chronic wounds. [2,3,8,13] Astronauts experience cardiovascular and skeletal muscle deconditioning, worsening vision, cerebral changes, and bone loss during long space flight due to the head-ward distribution of the venous blood volume and musculoskeletal unloading. [1-4,11,12] Large, Lower Body Negative Pressure (LBNP) devices can mitigate these changes by altering the macrovascular hemodynamics and returning more venous volume to the lower limbs, and creating a ground reaction force under the feet that loads the musculoskeletal system. [2,3] Patients with chronic wounds, such as venous stasis ulcers or diabetic ulcers, have underlying microvascular changes that increase the difficulty of wound healing. Compression bandages and negative pressure wound therapies both apply external pressures over small areas, leading to decreased wound healing time and increase microvascular blood flow. [8,13]

But in one population that requires an external pressure driven macrovascular effect and another that requires a microvascular effect, how do you ensure that adverse microvascular macrovascular effects are limited, respectively? Additionally, what is the dose threshold needed to achieve a negative pressure driven venous fluid shift in astronauts or the dose threshold needed to achieve microvascular flow increase in patients with chronic wounds? Despite extensive literature on the many effects of external pressure on microvascular or macrovascular blood flow, no study exists to examine the relationship between the blood flow in the cardiovascular system, bone microvasculature, skin microvasculature and muscle microvasculature and the dose of applied external pressure. The purpose of this trial is to examine the effect of different doses of external pressure exposure, defined in terms of pressure exposure magnitude and area, on the bone, skin and muscle microvasculature and systemic blood flow. This abstract deals with the preliminary cardiovascular data of this trial.

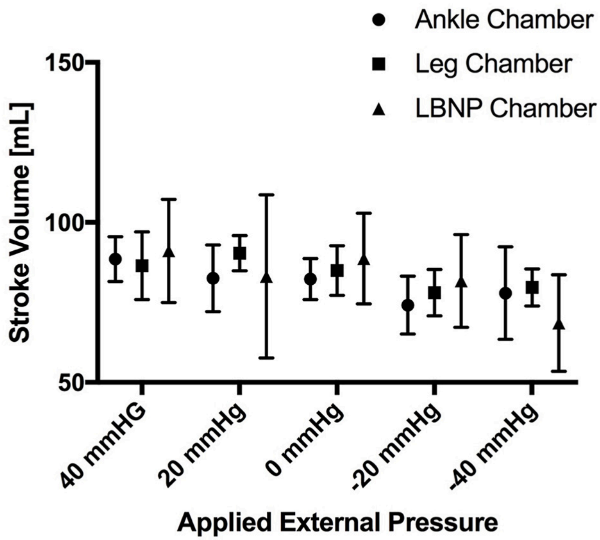

FIGURE 1: Stroke volume (n = 3) is affected by the magnitude of the applied pressure. Data are presented as means ± SD.

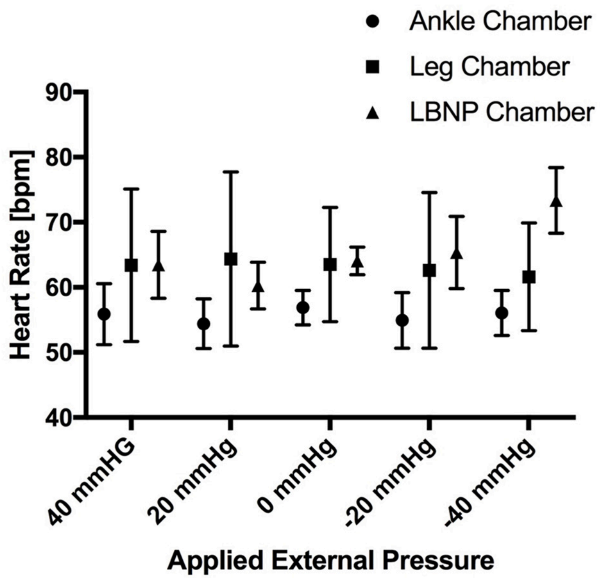

FIGURE 2: Heart rate (n = 3) is significantly affected by the interaction between the magnitude and area of exposure of applied external pressures. Heart rate seems to increase most in the LBNP pressure chamber at – 40mmHg. Data are presented as mean ± SD.

HYPOTHESIS

Altering the exposure area and magnitude of external, pneumatic pressure will affect stroke volume, mean arterial pressure, heart rate and total peripheral resistance of healthy individuals. It is expected the largest exposure area and most extreme pressure magnitudes will lead to the largest change in systemic cardiovascular parameters relative to baseline.

METHODS

Healthy adults (n=3, all female, ages 21 to 26) had different areas (unilateral below the ankle/"Ankle Chamber", unilateral above the knee/"Leg Chamber" , and bilateral below the iliac crest/"LBNP chamber") exposed to varying magnitudes of external pressure (+ 40, + 20, and 0 mmHg). Pressure exposure was maintained for 5 minutes, while heart rate, blood pressure, stroke volume and systemic vascular resistance were measured during the last minute of exposure using a finger sphygmomanometer and finger arterial pulse contour analysis system (Nexfin Blood Pressure Monitor, BMEYE,USA). Skin, muscle and bone microvascular blood flow were measured with a photoplethysmography device over the tibialis anterior muscle and tibia bone (Data not discussed here). There was a 5 minute rest period between pressure exposures of different magnitudes to allow subjects to return to their baseline hemodynamics. Only one area exposure was tested in a given day. Subjects were tested on 3 different occasions to obtain cardiovascular and microvascular measures for each exposure area and magnitude. A 2-way ANOVA was used to analyze the presented cardiovascular data.

RESULTS

Data for n = 3 subjects suggest that stroke volume (figure 1) is significantly affected by the magnitude of the applied pressure ($p = 0.009$) with the lowest stroke volumes seen with negative pressure application, especially in the whole, lower-body negative pressure chamber. There is also a significant interaction effect between pressure exposure area and magnitude on heart rate ($p = 0.034$, figure 2), with the largest deviation in heart rate from baseline control (ambient or zero pressure) seen at -40 mmHg applied external pressure in the LBNP (large area of pressure application). Mean arterial pressure (MAP) increases with increasing area of pressure exposure, but this trend was not significant. For this preliminary data set, total peripheral resistance (TPR) and MAP do not appear to be significantly affected by the magnitude or total area of pressure exposure.

DISCUSSION

Preliminary results suggest that both area and magnitude of external pressure application significantly affect the cardiovascular system. Completion of the entire study population (n = 20) is expected to confirm these initial findings. These results are important in establishing the ideal external pressure chamber size and applied pressure magnitude for safe, effective, and practical microgravity countermeasure development, as well as adverse effect avoidance in chronic wound patients, who have many cardiovascular comorbidities. Interpretation of these results with the bone, skin and muscle microvascular flow data will further support the development of effective therapies with minimal adverse events for astronauts and chronic wound patients.

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Keywords: stroke volume, cardiovascular, dose - response, Lower Body Negative Pressure (LBNP), space flight countermeasure

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Possible mechanisms of diverse spaceflight effects on endothelial function in different vascular beds

Olga L. Vinogradova[1,2]*, Oxana O. Kiryukhina[1,3,4], Dina K. Gaynullina[1,3], Olga S. Tarasova[1,3,4]

[1]*Laboratory of Exercise Physiology, Institute of Biomedical Problems (RAS), Moscow, Russia*
[2]*Faculty of Basic Medicine, Lomonosov Moscow State University, Moscow, Russia*
[3]*Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia*
[4]*Laboratory of Information Processes at the Cellular and Molecular Levels, Institute for Information Transmission Problems (RAS), Moscow, Russia*
**ovin@imbp.ru*

OBJECTIVES

The release of vasoactive substances from the endothelium is an important mechanism influencing arterial structure and function, whereas endothelium disfunction is typical for various cardiovascular disturbances (Triggle et al., 2012; Vanhoutte et al., 2016). Importantly, recent studies have demonstrated increasing risk for cardiovascular disease resulting from long-lasting space travel (Delp et al., 2016). This fact can be associated with the weightlessness-induced hemodynamic shifts and vascular remodeling (Prisby et al., 2006; Zhang, 2013; Zhang and Hargens, 2018). Another established endothelium-damaging factor of the space flight is space radiation which induces a sustained vascular endothelial cell dysfunction (Delp et al., 2016; Ghosh et al., 2016).

According to our recent observations, basilar artery (cerebral) and saphenous artery (hindlimb, mainly cutaneous) of C57BL/6 mice demonstrated qualitatively different alterations after 30-day-long spaceflight on the Bion-M No 1 biosatellite. The endothelium-dependent relaxation to acetylcholine was suppressed in basilar artery but somewhat augmented in saphenous artery (Sofronova et al., 2015; Tarasova et al., 2016). Beside different location in the mouse body (rostral vs. caudal), such diverse post-flight alterations can be associated with the inherent differences in the contribution of endothelium-derived mechanisms in these arteries. Of note, the contributions of key mediators of endothelium-derived relaxation (NO, prostacyclin and endothelium-derived hyperpolarizing factor, EDHF) can differ significantly in vessels of different branching order, as well as in vasculature of different organs (Hill et al., 2000; Kostyunina et al., 2016). Therefore, this study tested the hypothesis that murine basilar and saphenous arteries differ in the contribution of endothelium-derived mechanisms to the regulation of their smooth muscle tone.

METHODS

All experimental procedures were approved by the by the Biomedical Ethics Committee of the Institute for Information Transmission Problems, Russian Academy of Sciences (protocol 12-051), conforming to the U.S. National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (Eighth edition, 2011).

C57Bl/6 male mice aged 2 to 3 month were housed in the animal vivarium under controlled environmental conditions (12:12 hour light-dark cycle, 22-25°C) and provided food and water ad libitum. For isolated vessel studies mice were euthanized by cervical dislocation. Basilar and saphenous arteries were isolated and arterial segments (1 to 2 mm long) were mounted in wire myograph (620M, DMT, Denmark) to study their responses under isometric conditions. The preparations were kept at 37°C in the solution containing (mM): 120 NaCl, 26 NaHCO3, 4.5 KCl, 1.2 NaH2PO4, 1.0 MgSO4, 1.6 CaCl2, 5.5 D-glucose, 0.025 EDTA, 5 HEPES. The solution was continuously bubbled with gas mixture 5% CO2 + 95% O2 to maintain pH 7.4. Transducer readings were sampled at 10 Hz using E14-140 ADC (L-CARD, Russia) and PowerGraph 3.3 software (DISoft, Russia). All preparations were stretched to an internal circumference at which they developed maximal active tension (Mulvany and Halpern, 1977) and activated with two contraction/relaxation cycles (5 min/10 min). Saphenous arteries were contracted by the maximum concentration of α1-adrenoceptor agonist phenylephrine (10-5 M, Sigma). Basilar arteries were contracted by the maximum concentration of U46619 (10-6 M, Cayman Chemicals), the agonist of thromboxane A2 receptors, which are important regulators of cerebrovascular tone (Toth et al., 2011).

Relaxation responses to acetylcholine (in the concentration range from 10-8 M to 10-5 M) were studied cumulatively after precontraction with the respective agonist to 70 to 80% of the maximum active force (Figure 1). After washout, the segments were incubated with blockers of one or several endothelium-derived pathways and the concentration-response to acetylcholine was repeated. We used L-NNA (10-4 M, Alexis Biochemicals) to block NO-synthase, indomethacin (10-5 M, Sigma) to block cyclooxygenases and the combination of SKCa and IKCa channel blockers

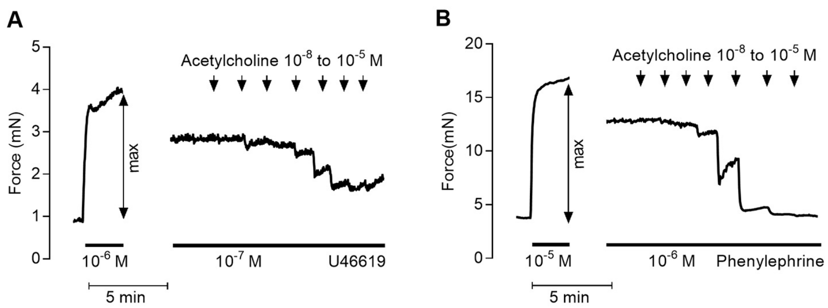

FIGURE 1: Representative recordings of basilar **(A)** and saphenous **(B)** arteries responses to graded concentrations of acetylcholine in a C57BL/6 male mouse.

(TRAM-34, 10-6 M, and UCL-1684, 10-7 M, both from Sigma) to eliminate the contribution of EDHF. Relaxation responses to NO-donor DEA/NO (in the concentration range from 10-9 M to 10-6 M) were studied using similar protocol. Before DEA/NO application the preparations were incubated with L-NNA for 15 min, to eliminate the interference of exogenous NO and endogenous NO effects.

The responses to each acetylcholine or DEA/NO concentration were calculated as percentage of the precontraction level. Statistical analysis was performed in GraphPad Prism 7.0 using Repeated Measures ANOVA. Statistical significance was reached at $P < 0.05$. All data are given as mean $\pm$ S.E.M.; n represents the number of animals.

RESULTS

Application of acetylcholine induced relaxation of basilar and saphenous arteries which reached the maximum at the concentration of 3*10-6 M (Figures 1 and 2). In the absence of pharmacological interventions, the response to acetylcholine was much larger in saphenous artery than in basilar artery. Combined blockade of NO, prostacyclin and EDHF pathways almost eliminated the response to acetylcholine in both arteries (Figure 2).

L-NNA given alone halved the response to acetylcholine in basilar and saphenous arteries (Figure 2 A,B). The additional inhibition of cyclooxygenase by indomethacin was not accompanied by a further decrease in arterial

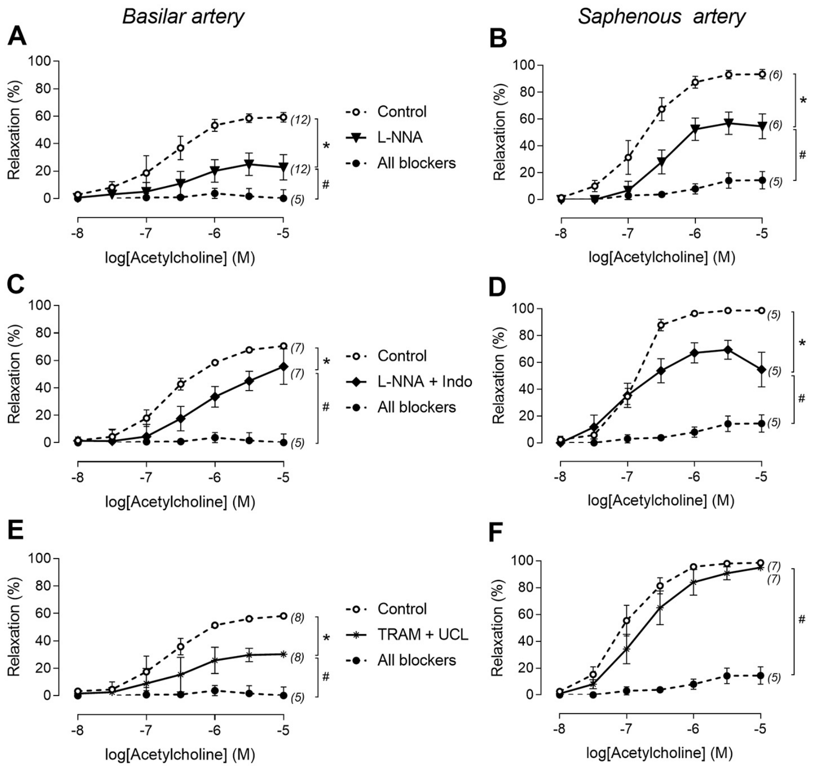

FIGURE 2: Pharmacological analysis of acetylcholine-induced relaxation of basilar (**A, C, E**) and saphenous (**B, D, F**) arteries in C57BL/6 male mice. **A** and **B** - the effects of L-NNA (10^{-4} M). **C** and **D** - the effects of L-NNA in combination with indomethacin (10^{-5} M), **E** and **F** - the effects of combined SK_{Ca}/IK_{Ca} blockade by TRAM-34 (10^{-6} M) and UCL-1684 (10^{-7} M). In each figure Control group corresponds to untreated arteries and All blockers group corresponds to arteries treated by the combination of L-NNA, indomethacin, TRAM-34 and UCL-1684 in respective concentrations. *p < 0.05 compared to Control; # p < 0.05 compared to All blockers (Repeated) measures ANOVA). Number in brackets indicates the number of animals.

relaxation response compared with the response in the presence of L-NNA alone (Figure 2 C,D). On the contrary, the response to acetylcholine was somewhat increased by indomethacin, which suggests rather constrictor than dilator influence of cyclooxygenase products in both studied arteries. Thus, in both arteries NO demonstrates a significant contribution to the relaxation in response to acetylcholine, this component of endothelium-dependent relaxation is comparable in basilar and saphenous arteries. Of note, relaxation responses to DEA/NO did not differ in basilar and saphenous arteries (data not shown).

The blockade of EHDF pathway halved the response to acetylcholine in basilar artery (Figure 1E). However, EDHF pathway blockade did not decreased the response of saphenous artery (Figure 1F), even though a pronounced EDHF contribution was observed in this artery after combined blockade of NO synthase and cyclooxygenase (Figure 1D). This observation suggests NO to inhibit the activity of EDHF pathway in saphenous artery. Such effect of NO may be attributed by the decrease of Ca2+ concentration in the cytoplasm of endothelial cells and thereby a weaker activation of SKCa/IKCa channels, which are essential the realization of EDHF-mechanism (Bauersachs et al., 1996).

CONCLUSIONS

The basilar and saphenous arteries of the mouse differ in the mechanisms of endothelium-dependent regulation of their smooth muscle tone. Endothelium-dependent relaxation response of basilar artery is caused by the additive effects of NO and EDHF pathways, each of which provides about half of the total relaxation response. However, saphenous artery demonstrates the redistribution of endothelium-derived pathways: reduced EDHF activity can be compensated by the increased contribution of NO and vice versa. Such redistribution of endothelium-dependent relaxation components in saphenous artery may underlie the unchanged or even elevated relaxation response after spaceflight in cutaneous vascular bed. On the other hand, cerebral arteries which lack the possibility of such redistribution can be more vulnerable to the influence of space flight factors than peripheral arteries. Violation of the endothelium-dependent regulation of cerebrovascular tone can be the reason for narrowing the range of cerebral blood flow control, the development of intracranial hypertension and visual impairment after long-lasting space flights (Delp et al., 2016; Zhang and Hargens, 2018).

ACKNOWLEDGEMENT

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Keywords: brain, skin, space flight, mouse (Black Swiss, C57BL/6), small arteries endothelium dysfunction

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GRAIN V2.0 (Influence of altered gravity on immune responses demonstrated with neutrophil migration performance) migration and activation of immune cells in altered gravity

Dominique Moser[1], Shujin Sun[2], Ning Li[2], Katharina Biere[1], Marion Hoerl[1], Sandra Matzel[1], Matthias Feuerecker[1], Cora Thiel[3], Yuxin Gao[2], Oliver Ullrich[3], Mian Long[2]*, Alexander Chouker[1]*

[1]Department of Anaesthesiology, Laboratory of Translational Research "Stress and Immunity", Klinikum der Universität München, Munich, Germany
[2]Key Laboratory of Microgravity (National Microgravity Laboratory), Center of Biomechanics and Bioengineering, and Beijing Key Laboratory of Engineered Construction and Mechanobiology, Institute of Mechanics, Chinese Academy of Sciences, Beijing, China
[3]Institute of Anatomy, Faculty of Medicine, University of Zurich, Zurich, Switzerland
**mlong@imech.ac.cn; *achouker@med.uni-muenchen.de*

INTRODUCTION

Astronauts returned from space often show immune dysfunctions related to an increased risk of infection. Because gravitational stress is assumed to be one of the major factors responsible for impaired immune function, several studies have been initiated to elucidate it´s impact on the immune system. Parabolic flight (PF) is a standardized model to induce repeated short-term periods of micro(μG)- and hypergravity (hyperG), resulting in acute gravitational stress. In the GRAIN V2.0 study which was carried out during the 30th DLR PF campaign, effects of gravitational stress on immune cells were investigated in vitro.

METHODS

Within the first part of the study, rolling speed and adhesion properties of peripheral blood mononuclear cells (PBMCs) were analysed during PF. PBMCs were injected in a flow chamber coated with the adhesion molecules PSGL-1 and ICAM-1 mimicking endothelial vessel walls. Cells passing the flow chambers were recorded by a microscope device connected to a camera (Fig. 1, representative picture).

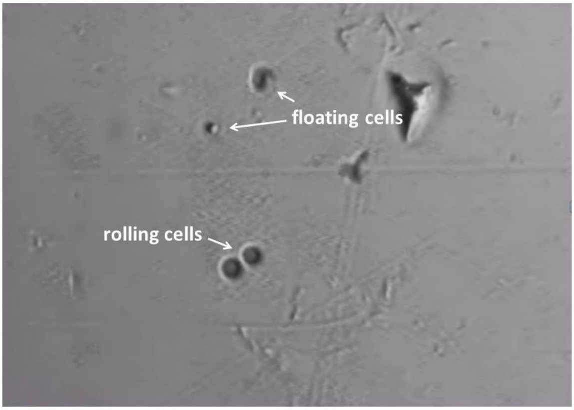

FIGURE 1: Exemplary excerpt from a video taken during one parabola. Arrows mark two rolling cells and two floating cells each.

In the second part, "Whole-Blood-Immune-Assays" were performed to determine changes in immune activation in response to a combination of different immunogenic stimuli and gravitational stress. After 6/24 hours incubation, plasmatic cytokine concentration and the activation state of monocytes in whole blood was determined.

RESULTS

Gravitational stress induced by PF leads to altered attachment and rolling properties of PBMCs on the adhesion molecule substrate. In µG, the rolling rate of cells on PSGL-1/ICAM-1 substrate decreased (µG: 0.018; hyperG: 0.179 & 0.237 normalG: 0.217). Furthermore, rolling cells in µG displayed an increased average speed (µG: 185 µm/sec.; hyperG: 142 & 151µm/sec.; 1G: 138µm/sec.).

Simultaneous exposure of whole blood to antigens and gravitational stress leads to a reinforced immune response in comparison to single stressors. However, both increased pro- and anti-inflammatory responses were detected. Expression of activation marker on monocytes and concentration of pro-inflammatory cytokines were remarkably increased after 6 hours. Also a reduced expression of pathogen recognition receptors and antigen-presenting molecules was recognized.

The observed immune enhancing effects were less pronounced or absent respectively after 24 hours incubation.

CONCLUSION AND OUTLOOK

In µG, rolling rate of PBMCs on adhesion molecule substrate is strongly reduced which is accompanied by an increased rolling speed. This effect is most probably due to lift forces during µG which mediate centralization of the cells and therefore increase the distance between cells and substrate. Under hyperG and 1G conditions, cells sediment to substrate again, which enables binding and may have a decisive impact on cytoskeleton architecture and immune cell activation.

Exposition of whole blood with immunogenic stimuli and gravitational stress results in increased but timely limited pro- and anti-inflammatory responses, which suggests an immunologic counterbalance to compensate inappropriate immune responses. In future experiments, immune enhancement after every single gravitational alteration that occurs during PF will be investigated. The results may give first hints to develop countermeasures for immune impairments both in space and on Earth.

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Keywords: hypergravity, immune cell migration, microgravity, parabolic flight, immue cell activation

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Gravitational stress during parabolic flights induce changes in the human leukocyte subsets

Ulrik Stervbo[1,2], Toralf Roch[1], Francis Labombe[3], Kaoutar Allou[3], Markus Kaymer[4], Antoine Pacheco[5], Jacques Vigne[5], Timm Westhoff[2], Felix Seibert[2], Nina Babel[1,2]*

[1]Berlin-Brandenburgisches Zentrum für Regenerative Therapien, Charité Universitätsmedizin, Berlin, Germany
[2]Centrum für Translationale Medizin, Marien Hospital Herne, Universitätsklinikum der Ruhr-Universität Bochum, Herne, Germany
[3]Laboratoire d'hématologie, Hôpital Haut-Lévêque, Pessac, France
[4]Beckman Coulter (Germany), Krefeld, Germany
[5]Beckman Coulter (France), Villepinte, France
**Nina.Babel@elisabethgruppe.de*

The human immune system is made up of several different cell types with distinct functions. The intricate interactions of these immune cells are required for an efficient function of the immune system leading to an appropriate response to foreign pathogens. Dysfunctions of the immune system might lead to a hyper-activity resulting in auto-immune or allergic diseases or to a hypo-activity allowing aberrant cells to form tumors (Iranzo and Villoslada, 2014).

Defense mechanisms against pathogens have evolved in all species of all taxonomic domains: from the restriction enzymes and loci of clustered, regularly interspaced, short palindromic repeat (CRISPR) protects bacteria and archaea from phage invasion to the germline encoded pattern recognition receptors of all invertebrates and vertebrates (Iwasaki and Medzhitov, 2010; Marraffini and Sontheimer, 2010). Vertebrates possess in addition a non-germline encoded adaptive subsystem, characterized by stochastic processes in generation of pathogen recognizing receptors (Boehm and Swann, 2014).

The gravitational field of earth has accompanied and shaped the evolution of all species and especially terrestrial organisms (Dubinin and Vaulina, 1976). It is, in fact, not until recently that mankind has produced means of altering the gravitational force subjected to a body. Centrifuges can increase the forces on a body, while the free falling international space station orbiting Earth creates a zero g environment. In addition, changes to the gravitational field might be actively sought after through various thrills such as amusement rides, skydiving, or bungee jumping (Gomez and Rao, 2016; Liu et al., 2016). Here we evaluate the effects of repeated changes in the gravitational field on circulating blood cells.

The data were collected from 20 volunteers (average age: 25.65 years, range: 19–43) during the 27th and the 28th DLR parabolic flight campaigns. The Airbus A310 performed one test parabola followed by 30 regular parabolas. The regular parabolas were performed in 6 sets of 5 parabolas, where each set was separated by steady flight for 5-8 minutes. Each parabola consisted of a single phase with µg, flanked by two phases of 1.8 g. Each phase lasted about 20 seconds. A steady flight phase of 1 g for about 2 minutes separated each parabola.

Blood samples were drawn just before the test parable (P0) and immediately after the last parabola of the last set (P30). To control for circadian effects, blood was taken on the ground the day before (C0 and C30). Whole blood was processed within an hour after arrival of the plane, and the immune status was evaluated by multi-parametric flow cytometry using the DuraClone IM Phenotyping Basic Panel (Beckman Coulter) per manufacturer's instructions. Briefly, all incubation steps were performed at room temperature for 15 minutes in the dark, and all centrifugation steps were performed at 200 g, for 5 minutes at room temperature. Erythrocytes were lysed using 2 ml VersaLyse (Beckman Coulter) per reaction tube, and the samples were collected on a Navios Flow Cytometer (Beckman Coulter).

We observed an increase in the frequency of Granulocytes (Figure 1A) during the parabolic flights, which was driven by an increase in Neutrophils (Figure 1B), as a decrease in the Eosinophils was observed (Figure 1C). These observations were also reflected in the cell count (Figure 2A-C), indicating an influx of Neutrophils during the cause of the parabolic flight.

The population of NK cells was found to be decreased after the parabolic flights (Figure 1D). This decrease could be explained by a decrease in the mature NK cells, as the frequency of the immature NK cells remained constant (Figure 1E-F). Similar observations were made for the cell count of the NK cell populations (Figure 2D-F).

Taken together, the data presented here show that repeated alterations of the gravitational field can cause changes to blood circulating immune cells.

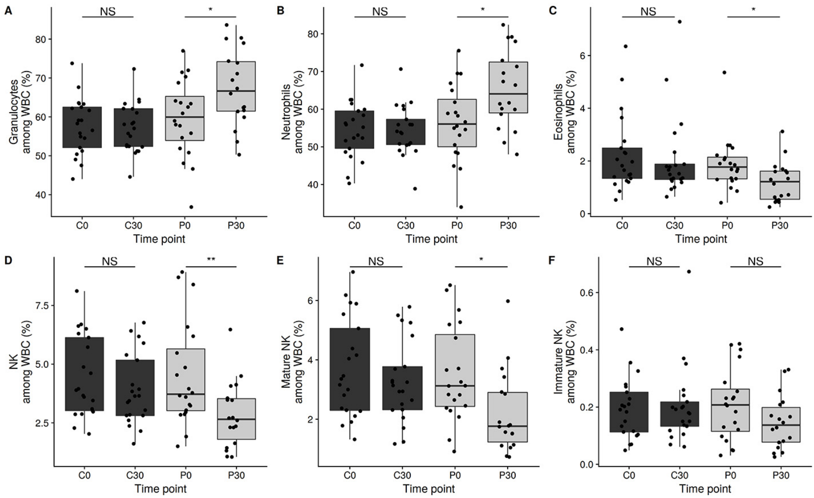

FIGURE 1: Gravitational stress disrupts the composition of circulating blood cells. The frequency of the indicated cell populations among all white blood cells were identified by flow cytometry. The boxes represent the 25th, 50th, and 75th percentile and the whiskers represent the range excluding outliers. Each point represents a single donor. Asterisks indicate p-values (** $p < 0.01$; * $p < 0.05$) for a Wilcoxon test.

We observed in particular changes related to the innate immune cells, which acts as a first line of defense against invading pathogens. This could indicate an impaired functional response to pathogens, which is in line with the observations from space flights (Cervantes and Hong, 2016). However, the true functional consequences remain to be confirmed.

We have shown here, that gravitational stress as experienced on a parabolic flight, can lead to alterations of the composition of blood circulating immune cells. This could have consequences for the efficacy of the immune system and potentially leading to an inappropriate response self- and non-self-antigen.

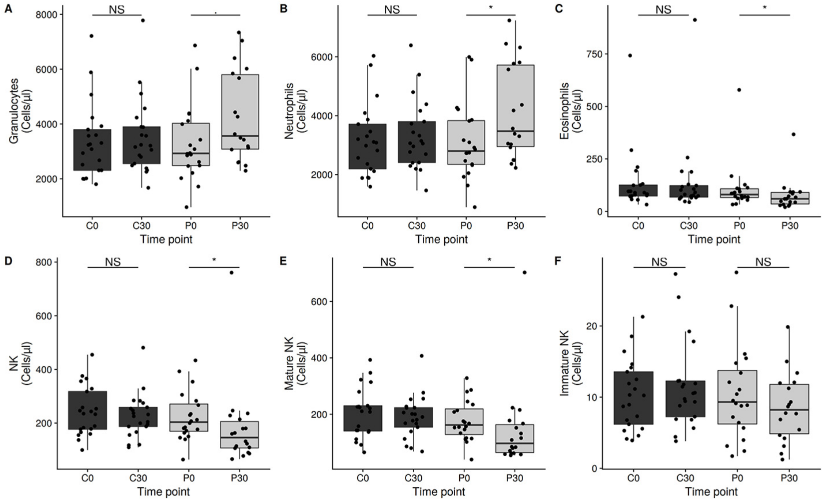

FIGURE 2: Gravitational stress disrupts the cell numbers of circulating blood cells. Total numbers of the indicated cell populations among all white blood cells were identified by flow cytometry. The boxes represent the 25th, 50th, and 75th percentile and the whiskers represent the range excluding outliers. Each point represents a single donor. Asterisks indicate p-values (* $p < 0.05$) of a Wilcoxon test.

Keywords: innate immunity, flow cyometry, parabolic flight, white blood cell, micro gravity

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Influence of factors of short-term suborbital flight on functional activity of human lymphocytes

Irina Alchinova[1]*, Margarita Polyakova[1,2], Mikhail Karganov[1,2], Mikhail Baranov[1], Nikolay Mullin[3], Sergey Kalinkin[4], Kirill Morozov[4], Nikolay Balugin[5], Vladimir Yushkov[5]

[1]Laboratory of Space Pathophysiology, Research Institute for Space Medicine, Federal Biomedical Agency of Russia, Moscow, Russia
[2]Laboratory of Physico-Chemical and Environmental Pathophysiology, Institute of General Pathology and Pathophysiology, Moscow, Russia
[3]Skolkovo Institute of Science and Technology, Moscow, Russia
[4]Moscow Polytechnic University, Moscow, Russia
[5]Central Aerological Observatory, Federal State Institution, Dolgoprudny, Moscow region, Russia
**alchinovairina@yandex.ru*

Many different technologies and methods are used for the evaluation of the influence of spaceflight factors on the body. For instance, the effects of microgravity are studied in vitro on a clinostat (for cells) and in vivo using the tail suspension test (for mice). The effects of radiation can be easily modeled under in ground conditions. Exposure to spaceflight factors, even during short-term missions, may affect the number and functions of studied cells. Any extreme exposure triggers activation of general adaptation systems, but the rate and degree of these processes and, most importantly, the degree of exhaustion and the development of compensatory responses can vary. These factors ultimately determine viability of the organism.

Despite of the fact that the main trend in international astronautics is the transition from near-Earth orbital flights to long-distance spaceflights to the Moon and Mars, the initial phase of space tourism will involve primarily low-earth suborbital trajectories. Suborbital crew and passengers will be exposed to various potentially dangerous factors during flights. The main problem in evaluation of biological risk of anthropogenic and other influences in suborbital flights is the necessity to analyze the effect of doses not surpassing or slightly surpassing the threshold doses. In this range the response to the studied factors is maximally individual. Biological consequences of these influences can be revealed only in multilevel studies. High radiation level in combination with other stress factors can induce damage to cell structures, changes in their functioning, which, in turn, can lead to activation of defense systems. Taking into account the fact that increased radiation level is related to solar activity and depends on flight altitude, even-short-term exposure to extreme factors may lead to exhaustion of the adaptive resources and under these conditions hypersensitivity of the organism can develop even after subthreshold exposures.

An established space tourism industry will involve longer orbital flights and possibly permanent facilities including hotel and resort facilities. These flights and facilities present an additional risk because they involve longer durations of potential exposure to elevated radiation levels [1].

The main goal of this experiment was to investigate the cellular and molecular responses of human blood cells (lymphocytes) to gravity changes.

METHODS

On July, 27 2017 meteorological rocket MMR-06 was launched. It carried a special thermostat (370C) module with two 75-ml cultural flacks. Start acceleration was ~40 g. The measurement phase covered the altitude range of 64431 m. Ballistic flight to the apogee took 116 sec, microgravity part of epy flight lasted 60 sec. The horizontal distance of the flown part was about 30 km.

Whole blood lymphocytes from a healthy female donor were routinely isolated by centrifugation in Diacoll-1077 density gradient. The medium was centrifuged (100,000g, 2 h) and filtered (0.2-μm Millipore filter) before the experiment to avoid extra particles during dynamic light scattering analysis. After washout, the cells in RPMI-1640 medium were transferred to 2 flight and 2 ground flasks in a concentration 0.44x106 cells/ml. Flasks temperature during the flight was 38+100C, the ground flasks were kept at the same temperature.

After landing the flight and ground cell suspensions were centrifuged (3500 rpm, 15 min). The cells were used for comet assay, culture fluids were frozen.

Electromagnetic radiation is among flight factors producing maximum damaging effect on cell DNA. Control irradiation of samples was performed at the Central Aerological Observatory. The electromagnetic radiation from the equipment inside the rocket head (“upper” sample), the maximum theoretical irradiation from the equipment inside the rocket head (“side” sample), and the effect from the tracking radar ("radar" sample) were simulated. The exposure for the first two samples was 2 h and for radar sample - 30 min.

Subfractional composition of the culture fluid was determined using laser correlation spectrometer for dynamic light scattering. The method is based on a change in spectral characteristics of monochromatic coherent radiation of a helium-neon laser. Measurement of the culture fluid (0.2 mL) and processing of the spectra were performed in accordance with the conventional technique [2,3]. The particle size distribution presented after mathematical processing in the form of a histogram allows characterization of dispersion composition of a certain biological fluid and classification of the distribution according to chosen informative zones of the spectrum.

The Comet Assay or single cell gel electrophoresis (SCGE) assay is a quick, sensitive, and relatively simple method for detecting DNA damage at the level of individual cells. Analysis of DNA damage degree was performed in accordance with the protocol [4].

RESULTS

Percent of live cells tested with eosin was almost the same in both flight and ground samples – 91 and 92%, respectively. Control ground experiments were performed to reveal separate effects of some flight factors – temperature, acceleration, electromagnetic field, etc. Temperature variations and overload 40 g produced no changes in the genetic material. Comet test revealed difference in degree of cell DNA damage. Cells after flight demonstrated mainly pronounced degree of DNA damage (Fig.1).

Taking into account the equal quantity of normal cells in both samples, we suppose that the influence of flight factors consists in accelerated destruction of cells with preexisting defects.

DNA comet analysis showed the maximum percentage of cells with damaged DNA in the radar sample.

The presence of a large number of type 1 DNA comets in this point indicates massive de novo injuries caused by irradiation.

Dynamic light scattering of cultural fluids showed shifts in their composition. Flight samples contained more 67-123-nm particles than control ones with

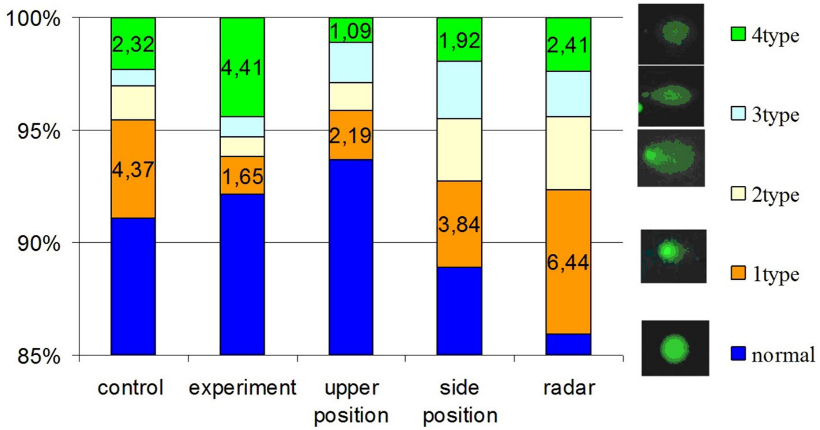

FIGURE 1: The percentage of comets of different types in the samples.

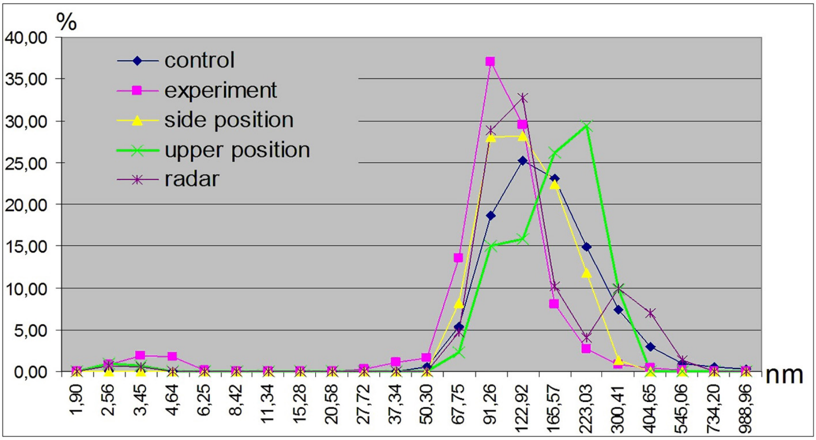

FIGURE 2: LC histograms of culture fluid samples.

a maximum at 91 nm. This may indicate that DNA fragments are released from cells to the culture fluid (Fig.2).

The difference between the LC histograms of the test sample and ones characterizing the medium after different irradiation regimens shows that electromagnetic radiation induced changes in the “radar” and “side” samples similar to the changes observed during the experiment (namely, increase in the percentage contribution of 67-123-nm particles into the light scattering).

CONCLUSIONS

Factors of suborbital flight did not cause additional cell death, but aggravated destruction processes in the damaged cells.

Electromagnetic irradiation in points “radar” and “side” is considered by us as the theoretically maximum possible exposure, however, similar shifts in LC histograms showed considerable contribution of the effect of electromagnetic radiation into complex effect of all flight factors.

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Keywords: cell death, electromagnetic radiation, lymphocyte, gravity changes, suborbital flight

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Altered anandamide metabolism in microgravity: The "RESLEM" experiment

Natalia Battista[1], Monia Di Tommaso[1], Aleandro Norfini[2], Marco Passerai[2], Valerio Chiurchiù[3], Mauro Maccarrone[3]*, Monica Bari[4]*

[1]*Facoltà di Bioscienze e tecnologie agro-alimentari e ambientali, Università degli studi di Teramo, Teramo, Italy*
[2]*Kayser (Italy), Livorno, Italy*
[3]*Facoltà di Medicina e Chirurgia, Università Campus Bio-Medico di Roma, Rome, Italy*
[4]*Dipartimento di Medicina Sperimentale e Chirurgia, Università degli Studi di Roma Tor Vergata, Rome, Italy*
**m.maccarrone@unicampus.it; *Bari@med.uniroma2.it*

To date it is widely accepted that human immune response is impaired during Space flight conditions, thus prognostic, diagnostic and therapeutic markers of microgravity-induced immunodepression are highly desirable (Hughes-Fulford, 20011). Our previous studies, performed in the course of the 28th parabolic flight campaign of the European Space Agency (ESA) (Maccarrone et al., 2001), under simulated microgravity (Maccarrone et al., 2003), as well as in the frame of our previous "ROALD" (Role of Apoptosis in Lymphocyte Depression) project (Maccarrone et al., 2008), have highlighted the key-role of 5-lipoxygenase (5-LOX) as a gravity responder that triggers apoptosis of human peripheral blood mononuclear cells (PBMCs), and hence immunodepression, under authentic microgravity conditions (Battista et al., 2012). In this context, we should recall that anandamide (AEA), the first endocannabinoid discovered more than 25 years ago, is considered a peripheral marker for immune disorders, and that during neuroinflammatory processes the expression of the main AEA-synthesizing enzyme (N-acylphosphatidyl-ethanolamine specific phospholipase D, NAPE-PLD) and AEA-degrading enzyme (Fatty Acid Amide Hydrolase, FAAH) are altered in immune cells (Maccarrone, 2017). The results obtained during the ROALD experiment have demonstrated that microgravity induces in PBMCs an early increase in 5-LOX activity and, subsequently, in leukotrienes B4 (LTB4) production, speaking in favour of an early engagement of 5-LOX along the signaling pathway that leads to apoptosis (Battista et al., 2012). It is worth reminding that AEA, unlike other endocannabinoids, inhibits FAAH activity by promoting the release of arachidonate, to be converted by LOXs into hydroperoxides, which act as competitive inhibitors of FAAH. The observation that all LOX inhibitors (MK886, caffeic acid), as well as the hydro(pero)xides of AEA generated by LOX (Maccarrone et al., 1998), yield the same effects on FAAH seems to stress the hypothesis that LOX pathway might regulate FAAH activity (Maccarrone et al., 2000). Additionally, previous findings showed a significant increase of circulating AEA levels in humans exposed to brief or prolonged periods of weightlessness (Chouker et al., 2010; Strewe et al., 2012).

On this basis, we speculated that under real microgravity conditions AEA immunosuppressive effects might be associated with inhibition of lymphocyte proliferation throughout the involvement of 5-LOX (Maccarrone and Finazzi Agrò, 2003) and that this regulatory mechanism might be involved in controlling AEA levels, due to an unbalanced expression of the main enzymes that regulate its metabolism. Therefore, we proposed the "RESLEM" (Role of the Endocannabinoid System in human Lymphocytes Exposed to Microgravity) project, that has been selected by ESA following the 2009 International Life Science Research Announcement (ILSRA-2009) (Battista et al., 2011). Our proposal was aimed at studying the gene and protein expression of the enzymes that regulate the endogenous tone of AEA, in order to provide new insights in the immune response of lymphocytes in Space, and to identify its role as modulator of cell survival and death. The experiment was part of the International Space Station (ISS) Increment 29-30 (PromISSe) mission organized by the European Space Agency (ESA) in 2011. RESLEM was launched on December 21, 2011 from the Baikonur Cosmodrome (Kazakhstan) onboard Soyuz rocket mission 29S, operated by the Russian Space Agency. After 126 days onboard the ISS, the RESLEM samples were recovered from the returning Soyuz 28S landed in Kazakhstan on April 27, 2012, kept at 25 °C in a yellow box and transported to the University of Teramo, where they were received on April 29, 2012 by science team for postflight analysis.

Human PBMCs were isolated from whole blood, taken from two human healthy donors, and were purified by gradient concentration, using the density separation medium Histopaque-1077. Cells were resuspended in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) containing L-glutamine (2 mM), gentamicin sulfate (50 μg/ml), HEPES (40 mM), and 10% fetal bovine serum, all purchased from Sigma-Aldrich (St. Louis, MO, USA). Cells were loaded into a specific hardware developed by Kayser Italia composed of 8 experimental units (EUs), each fitting into a KIC-SL container. After arrival on the ISS, RESLEM experiment containers were transferred into KUBIK 3 facility, pre-conditioned at 37°C, in the Columbus module. One set of samples was in a static position (0g) and another was put in the centrifuge (1g). During inflight operations, human PBMCs were activated automatically for different times (0, 3, 24, and 48 hrs), following a well-established procedure to investigate the effect of microgravity on immune function by using Concanavalin A

(15 μg/ml solution in each culture chamber; Invitrogen) (Walther et al., 1998). Then, cell suspensions were fixed with RNAlater (900 μl/culture chamber; Ambion, Austin, TX, USA), and were immediately moved by the astronauts into the minus 80°C laboratory freezer for ISS (MELFI) facility, where they were stored until download with Soyuz 28S. The experimental design and timeline are reported in Supplementary Figure S1.

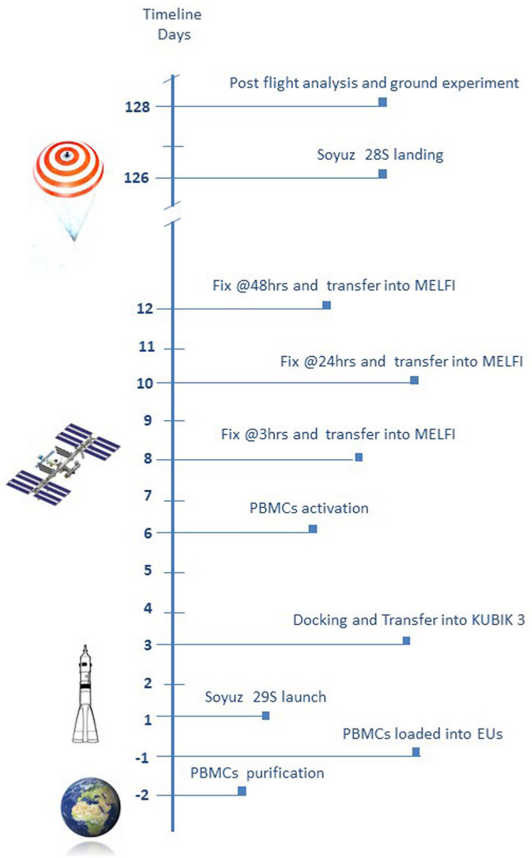

FIGURE S1: Timeline and experimental procedures of RESLEM project during pre-flight, on orbit and post-flight operations.

Briefly, RNA was extracted from human PBMCs using the RNeasy extraction kit (Qiagen, Crawley, UK) and quantitative real-time RT-PCR (qRT-PCR) reactions were performed using the RT-PCR SuperScript III Platinum Two-Step qRT-PCR Kit (Invitrogen, Carlsbad, CA, USA), as already described (Battista et al., 2012). NAPE-PLD and FAAH protein expression were evaluated by using both an ELISA assay and Western blot analysis (Battista et al., 2012).

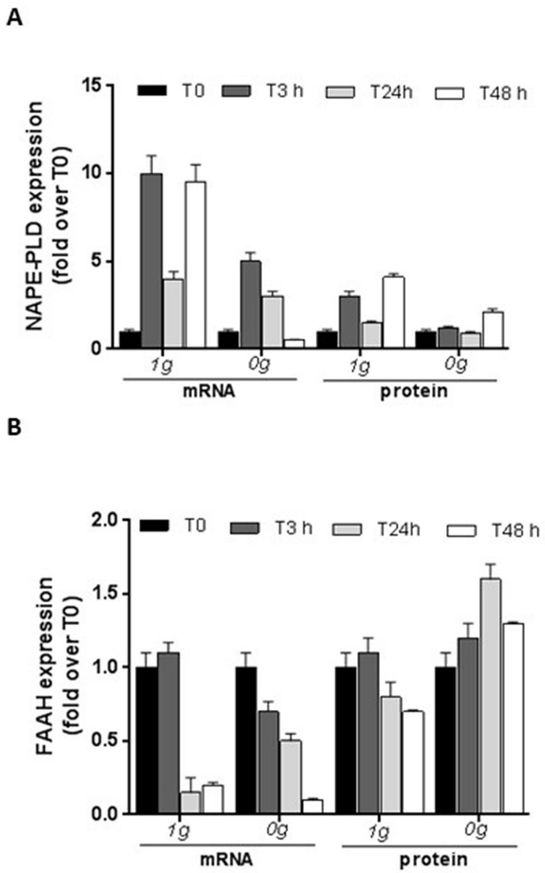

FIGURE 1: (**A**) NAPE-PLD and (**B**) FAAH mRNA and protein expression in microgravity (0g) and inflight control (1g) PBMCs samples onboard the ISS.

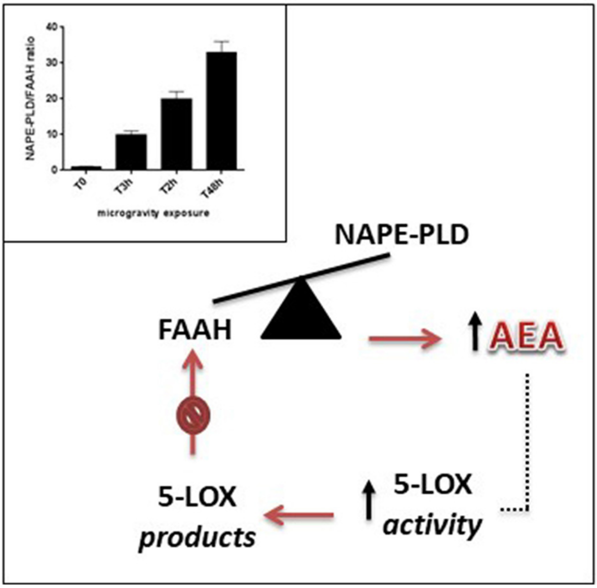

FIGURE 2: Overall scheme of hypothetical effect of microgravity conditions on AEA metabolism in lymphocytes. Inset graph shows NAPE-PLD:FAAH ratio.

Our results demonstrate that microgravity enhances the expression, both at transcriptional and translational level, of the main enzymes responsible for AEA endogenous tone (Figure 1). Interestingly, our data highlight that human PBMCs are active in synthesizing AEA, showing a NAPE-PLD:FAAH ratio >1 within 48 hrs of exposure to microgravity (Figure 2). These findings provide a possible explanation for the increased levels of circulating endocannabinoids and are in line with the data obtained during parabolic flights as well as in long-duration space missions to the ISS. In the perspective of Space exploration and colonization, the RESLEM project has disclosed an unprecedented potential engagement of endocannabinoid signaling in lymphocyte apoptosis and immunodepression, two events that have been already documented in Space. Additionally, endocannabinoid-based drugs can serve as novel therapeutics to be exploited as countermeasures against weightlessness sickness.

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Keywords: immunosuppression, lipoxygenase, lymphocytes, anandamide, microgravity

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NASA's use of ground and flight analogs in reducing human risks for exploration

Barbara J Corbin*, Leticia M Vega

Human Research Program, Johnson Space Center (NASA), Houston, United States
**barbara.j.corbin@nasa.gov*

Human exploration in the Lunar or Mars vicinity poses multiple risks to crew health and performance. In order to enable safe, reliable, and productive human space exploration within and beyond low Earth orbit, NASA's Human Research Program (HRP) performs research to help reduce the risks to human health & performance during exploration missions. HRP research leads to the development and delivery of: human health, performance, and habitability standards or requirements; countermeasures and other risk mitigation solutions; and advanced habitability and medical support technologies. Figure 1 lists the 26 risks to human health and performance that NASA

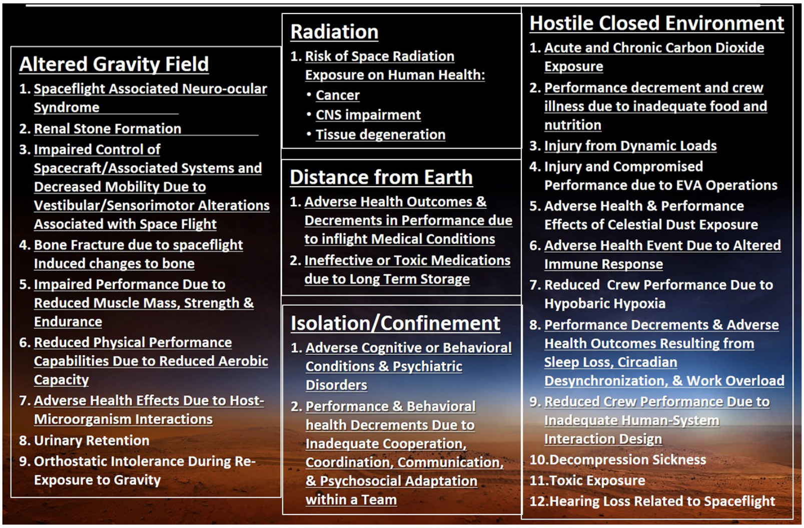

Figure 1: Human Health and Performance risks maintained by the NASA Human System Risk Board. Risks are grouped into five main stressors or hazards: radiation, altered gravity fields, isolation and confinement, hostile closed environments, and distance from Earth.

grouped into five main stressors or hazards: radiation, altered gravity fields, isolation and confinement, hostile closed environments, and distance from Earth. HRP utilizes multiple research venues to understand and mitigate these human exploration risks.

Although the International Space Station (ISS) provides an excellent platform for much of HRP's biomedical, behavioral and human factors research, it has its limitations as an analog for long duration exploration applications. The ISS is close to Earth, protected from deep space radiation. Near real-time communication to ground control or to family members is possible. It also has evacuation and re-supply capabilities, is relatively large, is limited to 6-month increments, and its utilization is highly constrained. In contrast, exploration vehicles will be far more autonomous and austere. To that end, NASA is investigating new ways to utilize the ISS to mimic portions of the target exploration missions. The HRP is proposing year-long ISS missions to further characterize the impact of spaceflight and effectiveness of counter-measures extending beyond 6 months. Also under investigation are ways to limit interactions with the ground, developing hardware and procedures the reduce reliance on ground control, reducing the number of visiting vehicles and delaying communications, and increasing autonomous operations. In addition, HRP utilizes multiple ground analogs to mimic portions of the target exploration missions that are difficult to replicate on ISS. Ground analogs can mimic portions of a target mission and provide a pathway to more effectively address human health and performance questions quicker and in a more controlled manner than ISS allows.

NASA's HRP currently leverages national and international partnerships to conduct research in six different ground analogs, covering five of the six primary spaceflight hazards. The NASA Space Radiation Laboratory (NSRL) simulates space radiation. The Deutsches Zentrum Für Luft- Und Raumfahrt E. V. (DLR) :envihab facility coupled with parabolic flight simulate the altered gravity fields. NASA's Human Exploration Research Analog (HERA) and the Institute of Biomedical Problems (IBMP) Science-Experimental Complex (NEK) facility simulate isolation and confinement. Finally, Antarctic stations simulate a hostile environment.

Radiation research utilizes the NASA Space Radiation Laboratory (NSRL) located at the Brookhaven National Laboratory. The NSRL provides multiple heavy ion and proton irradiations, including a Galactic Cosmic Ray Simulation, with energies ranging from 50- 1500 MeV, in a 20x20 cm beam profile.

In addition, support laboratories, specimen housing and control rooms allow deep space radiation exposures to cells, tissues and rodents. HRP sponsors three exposure runs annually focusing on translation of tissue and animal research to humans, countermeasure testing, single ion to mixed field translation and low dose-rate studies more closely aligned with space exposure rates.

HRP utilizes bed rest and parabolic flight to simulate the microgravity environment. NASA completed a 30-day 6-degree head down tilt bed rest study with altered atmospheric conditions (0.5% CO_2) in collaboration with DLR. During this VIIP and Psychological :envihab Research (VaPER) study, 5 of the 11 subjects developed Spaceflight Associated Neuro-ocular Syndrome (SANS) symptoms, indicating this bed rest paradigm may be a valuable ground analog for the SANS (formally known as VIIP) risk. NASA, the European Space Agency (ESA) and DLR are planning a 60-day bed rest campaign with Artificial Gravity in the DLR :envihab facility. This campaign, identified as AGBRESA, implements three NASA and Seven ESA proposals that cover the full complement of altered gravity risks. NASA, ESA, DLR and the Centre national d'études spatiales (CNES) also recently collaborated on an International parabolic flight campaign using the Novespace aircraft. Completed May 28- June 8, 2018, eight studies (4 ESA, 1 CNES, 2 DLR, 1 NASA/DLR) were run during 31 Parabolas at 0.25 g, 0.5 g, and 0.75 g.

For isolation and confinement research, HRP sponsors annual campaigns of four 45-day isolation missions with a crew of four in the Human Exploration Research Analog (HERA). The HERA is 3-story, 3 module habitat at the NASA Johnson Space Center in Houston, Texas. It supports four crewmembers per mission, has a mission control, adjustable lighting, flight vehicle and virtual reality simulators. Four campaigns representing 66 research studies from NASA and DLR have been completed to date, with the fifth campaign of 14 studies planned to begin in January 2019. Longer isolation studies are planned in collaboration with the IBMP in Moscow utilizing the Russian NEK facility for isolation studies ranging from four months to one year, identified as SIRIUS missions. The NEK facility supports six crew in a single story, 4 module habitat that includes a mission control and a surface simulator and is environmentally controlled. The four month mission is scheduled to begin in February 2019.

Antarctic stations (both US and non-US) have become an invaluable analog for multiple studies, with studies in McMurdo, South Pole, Neumayer and Concordia stations.

Analog environments mimic portions of a target mission and provide a pathway to effectively address exploration human health and performance questions. Collaborations provide unique opportunities to address critical research questions requiring long duration and controlled simulation or isolation. Through the established working groups and multilateral panels, NASA is negotiating expedited releases of research opportunities with international participation to encourage multilateral approaches to future analog research campaigns. NASA's collaborative use of global analog facilities through multilateral campaigns or missions leverage the global scientific community, allow NASA to mimic four of the five major space flight stressors, focus high quality research on exploration issues and provide sufficient power to accelerate the development of countermeasures to drive sound recommendations for exploration missions.

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Keywords: radiation, risk, NASA, microgravity, analogs, analogue, NEK, :envihab

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Cardiospace French Chinese cooperation in gravitational physiology

Jean-Christophe LLORET[1]*, Laurent Arnaud[1], Guillemette Gauquelin[1], Yuan Ming[2], XueMin YIN[2], Yinghui LI[2]

[1] Science Orbital Projects, Centre National d'Etudes Spatiales, Paris & Toulouse, France
[2]China Astronaut Research and Training Center, Life Sciences, Beijing China
*jean-christophe.lloret@cnes.fr

COOPERATION FRAMEWORK AND SCIENTIFIC OBJECTIVES

France and China have signed a cooperation framework agreement on the peaceful uses of outer space in 1997. In a few years, a long-term cooperation in space physiology has been developed between French Space Agency (CNES) and the Astronaut Center of China (ACC): following a bedrest organized by ACC in 2007, it was decided in 2012 to fly a medical equipment provided by CNES, during a Chinese human space flight. It became the project Cardiospace, designed to study how the cardiovascular system adapts during human spaceflight.

Cardiospace scientific cooperation focusses on cutaneous micro-circulation and macro-circulation. Cutaneous micro-circulation is interesting by itself because of its extent and its role in thermoregulation and its way to reflect the general micro-circulation. Macro-circulation is studied along with its morphological aspects.

CARDIOSPACE MEDICAL EQUIPMENT

CNES coordinated the development of Cardiospace equipment, a unique system integrating and synchronizing different medical instruments that provide standard cardio-vascular parameters (continuous blood pressure, respiratory rate, and electrocardiogram) together with more specific measurements: cutaneous microcirculation with a laser-dopler device and macro-circulation with a sonographer (Fig. 1). It has to be emphasized that the development of a laser-doppler for use in space had never been done before.

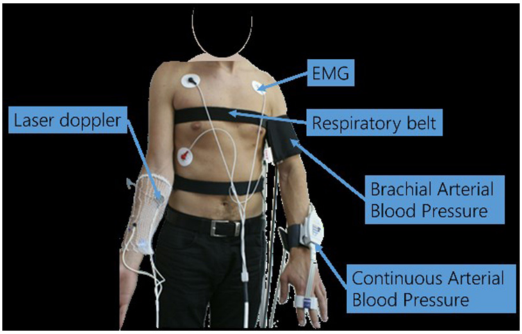


FIGURE 1: Cardiospace medical probes.

SCIENTIFIC EXPERIMENTS AND RESULTS

Mid-September 2016, the Chinese Space Laboratory, TianGong-2, was launched, carrying a broad range of payloads including Cardiospace equipment that was used successfully by the two Taikonauts of the Shenzhou 11 mission (figure 2).

Thanks to Cardiospace, for the first time during a space flight, endothelial vascular impairments were observed at the cutaneous microcirculation level (see micro-circulation protocol, Fig. 3).

Complementary to the space mission, a second model of Cardiospace was used for ground trials as part of a 180-day confinement experiment organized by ACC in Shenzhen (Fig. 4). The objective was to isolate the confinement factor in space flight and thus complement the measurements carried out in orbit. Five French scientific teams participated to this experiment to study cardio-vascular system but also back pain, psychological and ethological factors.

FUTURE COOPERATION

The success of Cardiospace development and use on-board Tiangong-2 offers the prospect of a more challenging project, Cardiopace-2.

FIGURE 2: Cardiospace and Shenzhou 11 taïkonautes on-board Tiangong-2.

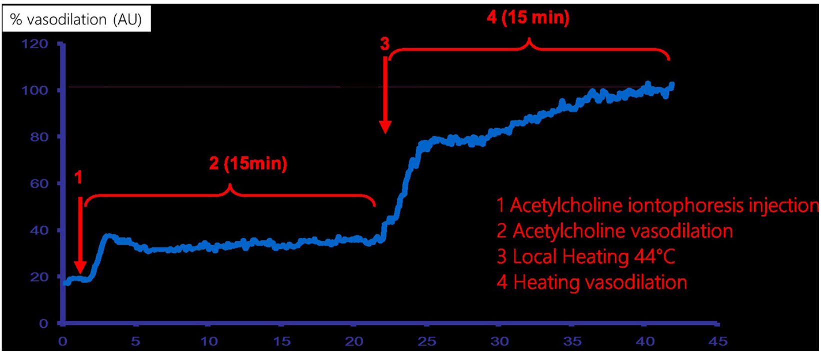
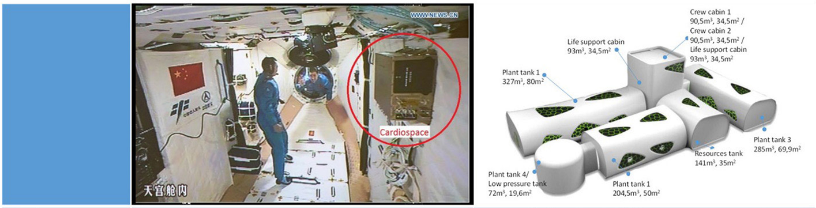


FIGURE 3: Micro-circulation protocol, typical curve.



Mission	Onboard experimentation	Controlled ecological life support system (CELSS)
Location	**Tiangong-2**	**Shenzhen**
Dates	1 month (2016, 15 Oct. – 17 Nov)	6 month (2016 ,15 June – 15 Dec.)
Subjects	2 taikonautes (Shenzhou 11 expedition)	4 subjects in cabin 4 subjects outside (control)
Measurements	6 recording sessions (12h)	48 recording sessions (96h)
Achievements	Laser-Doppler used for the first time in space	Cardiovascular data + back-pain + psychology + ethological data

FIGURE 4: Cardiospace on-board and on-ground experimentations in 2016.

The development of this new equipment in cooperation between ACC and CNES is planned from 2018 to 2021 for utilization onboard the future Chinese Space Station. Such a project would allow to consolidate the scientific results obtained on Tiangong-2 by acquiring a more representative base of scientific data as operations on board of the Chinese station are planned at least for 10 years. Instruments that have never been used in space are also foreseen to explore new fields of research.

Keywords: instrumentation, microcirculation (skin), gravitational physiology, cardiospace, French-Chinese cooperation

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Estimation of gait characteristics during walking in lower gravity environment using a wearable device

Léo Lamassoure*[1], Keisuke Araki[1], Akihito Ito[1,3], Kiyotaka Kamibayashi[2,3], Yoshinobu Ohira[2,3], Nobutaka Tsujiuchi[1,3]

[1]*Graduate School of Engineering, Mechanical Engineering Department, Doshisha University, Kyoto, Japan*
[2]*Graduate School of Engineering, Health and Sports Science Department, Doshisha University, Kyoto, Japan*
[3]*Research Center for Space and Medical Sciences, Doshisha University, Kyoto, Japan*
**lolamassoure@gmail.com*

Responses of walking patterns at 3.5 km/h and 0% inclination in 8 male subjects to reduction of body weight were estimated using a lower body positive pressure (LBPP) treadmill. Gait patterns and ground reaction forces (GRF) were estimated using a wearable device for motion analysis. In response to the reduction of body weight, the GRFs lowered accordingly. The gait patterns shifted from rear-foot strike to fore-foot strike, especially when the body weight was reduced from 60 to 40% vs. the 1-G condition. Further, the angle of ankle joints increased and that of knee joints and the movement ranges of hip joint were decreased, on the contrary.

INTRODUCTION

New space missions to The Moon and Mars are planned. In this context, a better understanding of the influence of low-gravity environments on gait characteristics is required (3/8-G) [1-3]. In a previous experiment, electromyogram activities and joint angles were measured during walking and/or running on a LBPP treadmill [4, 5]. It clearly indicated that the mobilization of the ankle plantar-flexor, slow-twitch, and soleus muscles was reduced in response to the gravitational unloading. However the close relationship between these parameters and the ground reaction forces was still unclear. Therefore, the current study was performed to investigate the walking patterns, including ground reaction force and gait patterns during walking on a LBPP treadmill using a wearable device [5, 6].

METHODS

Eight healthy male students volunteered as subjects (height: 170 $\pm$ 5 cm, weight: 70.9 $\pm$ 5.4 kg). The Human Use Committees at Doshisha University approved the study. All experimental procedures were conducted in accordance with World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

The wearable devices were composed of sandal-type shoes each equipped with two forceplates in their soles, and of sensors for joint angles (acceleration, gyroscopes, and geomagnetic). The subjects were equipped with the wearable devices (sampling frequency 100 Hz) and walked for 60 sec at 3.5 km/h and 0% inclination with 100, 80, 60, 40, or 20% body weight support on a LBPP treadmill (AlterG Inc., USA). The levels of loading were assigned randomly for each subject.

RESULTS

The normalized GRFs reduced gradually with the body weight (BW) (Fig. 1). The level of the GRF at toe-off was slightly greater than that at touch down, when the body-weight support was 100-60%. But it was reversed following further decrease below 40%.

Joint angles were affected differently by the reduction of the BW. For the ankle, the absolute value of the plantar flexion peak increased when the BW was reduced, especially between 60 and 40% of BW. For the knee, the peak value of the flexion angle reduced, especially at the 60-40% and 40-20% transitions. For the hip joint, the movement range decreased, most significantly below 60% of BW.

An increase in the total duration of the average gait cycle also appeared with the reduction of the BW. Figure 1 shows clearly that the duration of the stance phase remained the same for all weight rates, but the swing phase got longer as the weight rate decreased.

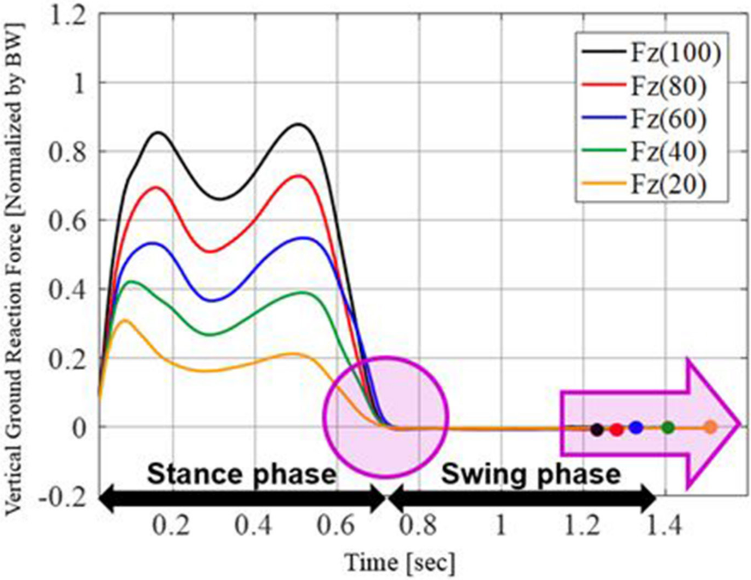

FIGURE 1: Fz (vertical GRF), during walking, for each weight rate.

DISCUSSION

These results explain more precisely the evolution of the gait pattern observed on subjects walking at low weight rates. For the ankle, the increase of the plantar flexion peak could explain the shift in foot-strike pattern that happens at low weight rates, while the lengthening of the swing phase observed is in accord with previous research [6, 7]. The ANOVA results indicate that the changes in joint angles are mostly significant when changing from 60% to 40% of BW, suggesting it is between these two weight rates that the gait transition happened.

CONCLUSION

In response to the reduction of body weight, the GRFs were lowered accordingly. The gait patterns were shifted from rear-foot to fore-foot strike, especially when the body weight was reduced from 60 to 40% vs. the 1-G condition. Further, the angle of ankle joints was increased and that of knee joints and the movement ranges of hip joint were decreased, on the contrary.

ACKNOWLEDGEMENT

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Keywords: gait, measurement, low-gravity environment, wearable device, AlterG

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Motor-cognitive functions in Parkinson's disease patients across the program of "Dry" immersion: A pilot study

Alexander Meigal[1]*, Liudmila Gerasimova-Meigal[1], Olesya Tretiakova[1], Kirill Prokhorov[1], Nataliya Subbotina[1], Nina Popadeikina[1], Irina Saenko[2]

[1]Department of Human and Animal Physiology, Pathophysiology, Petrozavodsk State University, Petrozavodsk, Russia

[2]Center of Aviation and Space Medicine and Technologies, Institute of Biomedical Probkems (RAS), Moscow, Russia

**meigal@petrsu.ru*

Motor and cognitive signs of Parkinson's disease (PD) have specific progression speed (Lawton et al., 2018). Some motor-cognitive instrumented tasks, e.g. choice reaction time (CRT) appear as cognitively demanding, while others, e.g. simple reaction time (SRT) or tapping test (TT) - as ones with lower need for cognitive effort (Müller et al., 2002). In PD patients, the program of "dry" immersion (DI) sessions provoked decrease of UPDRS-III and rigidity scores by 16%, while that of Hamilton's depression rate scale - by 40% (Meigal et al., 2018). Therefore, we hypothesize that the cognitively demanding tasks might have been more affected by a program of DI in comparison to merely motor tasks. To proceed, we compared the effect of DI on a set of reaction time tasks with different share of mental operation - TT, SRT, SRT at distraction of attention (SRT/DA), CRT, and prediction motion task (PMT).

A total of 10 PD patients (9 m, 1 f, Hoehn and Yahr staged 1-3) participated in the program of DI. They stood immersed in a bathtub (MEDSIM, IBMP, Moscow, Russia, Twater=31-32°C), wrapped in a thin waterproof material, head-out-of-water, 7 times (45 min each session) within 35 days. The data was collected at 4 study points: before the 1st (preDI), after the 7th immersion (DI7), 2 weeks (2W) and 2 months post-DI (2M). Six PD patients (all males) without application of DI formed the reference group. The study was performed with a help of PC-based tester (NS-Psychotest, Neirosoft Ltd., Ivanovo, Russia). To execute the TT task, the subjects performed tapping as quickly as possible on a contact board (55x60 mm) with a contact pencil, within 30 s. The count of taps was measured. The visual SRT task was evaluated as the time between light (red flash) stimulus (n=30) on

PC-based portable control panel and pressing the button with the thumb of the dominant hand on the same panel. The SRT/DA paradigm appeared as a SRT task (red circle, 20 mm in diameter, emerging on the center of PC screen, random in time, n=30) supplemented by distracting visual stimuli (circles of varied color and size, appearing irregularly in time and space of PC screen). In the CRT paradigm subjects had to press the appropriate panel button (red button on red light stimulus, and green button - on the green one, n=20). In all these tests reaction time (ms) was measured. To perform PMT, subjects pressed the button on control panel (n=30) when a dynamic object (growing red segment, 135°/s) on PC screen was colliding with a static object (green radius, 45 mm). The accuracy (% of precise hits) was estimated. The SPSS 21.0 (IBM Corp., USA) was used for statistics (multiply comparisons between 4 study points by Friedman non-parametric test with further post-hoc corrections).

In the reference group, none of the tests has significantly modified along the investigation period. In the study group, before the program of DI, SRT centered around 290 ms (249-335 ms, 25-75%), SRT/DA was 343 ms (300-358.5 ms), and CRT - 486 ms (407-724 ms). The SRT has not changed across the study, while the SRT/DA has decreased by some 8% (315.5, 308-353, p=0.042), and the CRT - by 20% (417, 355-457 ms, p=0.022) at 2W study point. At the point 2M all parameters tended to restore their values. Normalized values of SRT, SRT/DA and CRT along the study are presented on figure 1. The count of hand taps during TT task centered around 185 per 30 s (6.05 Hz), and it stood unchanged by DI in both groups (see Figure 1). In the PMT, precise hits were 51% (40-62) and did not change over the study.

Before the DI program, the three RT tasks clearly ranged by their duration from the shortest (SRT) through SRT/DA to the longest (CRT) thus reflecting gradually increasing cognitive complexity of the task. The reaction to DI program has also ranged from a prominent (20% decrease) effect on CRT, through a modest effect (8% decrease) on SRT/DA to a negligible effect (<2% decrease) on SRT. Such finding prompts that the more the task was cognitively demanding, the more it was affected by the DI program. Thus, the motor component of the reaction time tasks was not modified by DI. That conclusion is supported by the fact that the tapping count in TT task and proportion of accurate hits in PMT have not changed through the DI program. That result is in line with earlier finding that performance of complex, but not simple motor tasks is improved by anti-PD therapy (Müller et al., 2003), what supports the original hypothesis of that study. As the reaction

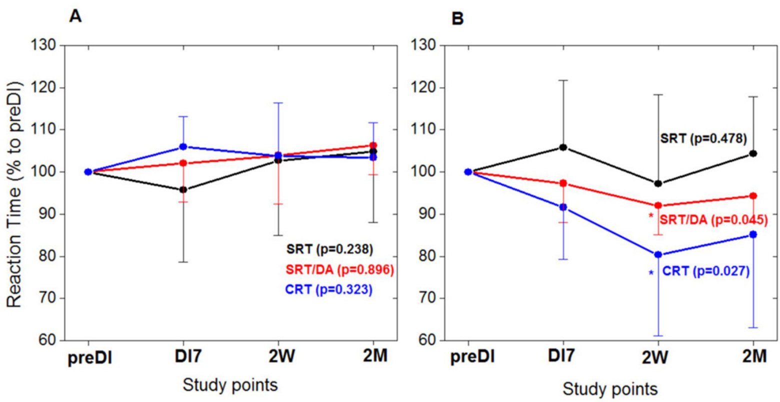

FIGURE 1: Reaction time in psychophysiological test in Parkinson's disease patients with (right panel) and without (left panel) program of dry immersion.
SRT - simple reaction time, CRT -choice reaction time, SRT-DA - SRT with distraction of attention, preDI - before the program of dry immersion, DI7 - after the 7th immersion, 2W - two weeks after the program, 2M - two months after the program of dry immersion.

time tasks have well-established neurophysiologic basis, they can be used to evaluate the mechanisms of DI therapy. In further studies, the battery of motor-cognitive tasks could be expanded to the more complex ones, e.g. recognition/discrimination tasks.

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Keywords: choice reaction time, Parkinson's disease (PD), dry immersion, tapping test, simple reaction time (SRT)

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Plantar mechanical stimulation prevents neurochemical alterations in the hippocampus induced by stimulated microgravity

Anna Berezovskaya[1], Sergey Tyganov[2], Boris Shenkman[2], Margarita Glazova[1]*

[1]Laboratory of Comparative biochemistry of cellular functions, Institute of Evolutionary Physiology and Biochemistry (RAS), Saint Petersburg, Russia
[2]Myology Laboratory, Institute of Biomedical Problems (RAS), Moscow, Russia
**MGlazova@iephb.ru*

INTRODUCTION

Fifty seven years have passed since the first manned flight into space, but our knowledge about alterations in the CNS is still limited. MRI studies demonstrated the long-term space flight significantly changed the brain morphology in astronauts resulting in decreased of gray matter and increased volume of the ventricles (Roberts et al., 2017). Moreover cognitive impairments were documented in astronauts after long-terms space missions (Casler and Cook, 1999). Obviously all these changings are mediated by neurochemical/molecular disturbances in the CNS and particular in the hippocampus, which controls memory and navigation by coordination of many inputs from forebrain and lower brain structures. In the hippocampus of rodents (in space and using hind-limb suspension model) it was demonstrated the alterations in the expression of glutamate receptors, neurotrophins and apoptotic proteins (Yasuhara et al., 2007; Santucci et al., 2012; Naumenko et al., 2015; Shang et al., 2017; Wang et al., 2017). In all these experiments biological materials were collected after 7 and more days in space or tail suspension. The initial stage of the brain alterations during exposure to unloading is still unexplored. Among the main causes of the brain function impairment during gravitational unloading the sensory deprivation is considered as the most important one. And withdrawal of the support afferentation under conditions of real or simulated microgravity is believed to trigger on the brain remodeling (Kozlovskaya et al., 1988). The roles of support afferentation are usually analyzed by means of the plantar mechanical stimulation during unloading (Layne et al., 1998; Kyparos et al., 2005). However the effects of plantar mechanical stimulation on functional activity of neurons and/or cognitive function have not been studied yet. In our study we analyzed effect of short-term stimulated microgravity (SM) and checked whether the repeated plantar mechanical stimulation (PMS) affects functional status of the hippocampal neurons.

MATERIALS AND METHODS

36 adult Wistar rats were recruited in the experiments. The rats were housed in individual cages at 12/12 light-dark cycle with free water and food access. All procedures were approved by the Biomedical Ethics Committee of the Institute of Biomedical Problems.

THE RATS WERE DIVIDED FOR 3 GROUPS

C – vivarium control, SM group - 3 days hind-limb suspension, PMS group – 4 hours repeated plantar mechanical stimulation each day during hind-limb suspension. The rats were anaesthetized and perfused with 4% formalin for immunohistochemical study (n=4 for each groups); or the hippocampi were dissected and homogenized for Western blot assay (n=8 for each groups).

USED ANTIBODIES FOR IMMUNOSTAINING AND WESTERN BLOT

GAD67 (Millipore), VGLUT2 (Millipore), NR2B (Abcam), pCREB (Ser133; Millipore), Akt (Cell Signaling), pAkt (Ser473; Cell Signaling), GSK3b (Cell Signaling), pGSK3b (Ser9; Cell Signaling), GAPDH (Abcam).

Statistical analysis was done by a nonparametric Kruskal-Wallis test.

RESULTS

Our data demonstrated that SM significantly reduced VGLUT2 immunostating in the hippocampus that was confirmed by immunoblotting. These data proposed a decrease in glutamate release, since it was shown that the expression of VGLUT2 correlates with glutamate secretion (Liguz-Lecznar and Skangiel-Kramska, 2007). In opposite PMS restored VGLU2 expression (Fig.1). Analysis of NMDA receptor subunit NR2b did not reveal any difference between all groups. Immunostaining for glutamate decarboxylase GAD67, which synthesize GABA,

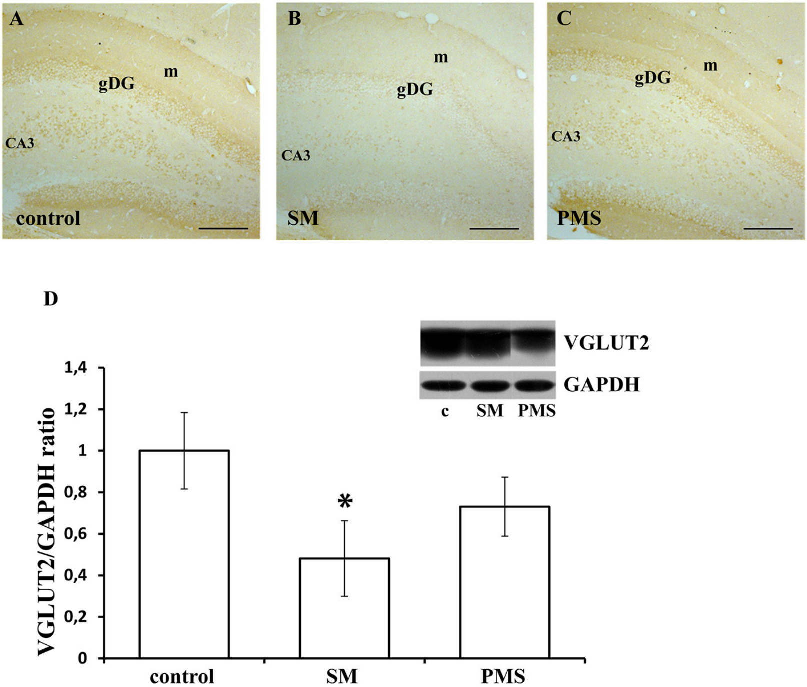

FIGURE 1: Plantar mechanical stimulation protects glutamatergic system from negative effects of stimulated microgravity. A-C - VGLUT2 immunostaining revealed a decreased VGLUT2 expression in the Dentate Gyrus of rats after 3 days hind-limb suspension (**B**) in comparison with control (**A**); in rats exposed to plantar mechanical stimulation (**C**) the VGLUT2 immunostaining intensity was the same with control. gDG - granular layer of the Dentate Gyrus; m - molecular layer of the Dentate Gyrus. Scale bar 200 mm. (**D**) - Western blot analysis of VGLUT2 expression in the hippocampus. Data are shown as mean +/– SEM. * - $p<0.05$.

also did not show the differences between the groups. Analysis of Akt signaling demonstrated that in SM group phosphorylation of both Akt and GSK3b was significantly increased in the hippocampus (Fig. 2). We also observed increased number of pCREB immunopositive nuclei in the dentate gyrus and CA3 region of the hippocampus that revealed the activation of this transcription factor. Akt and CREB are the main factors that regulate the neuronal survival. It was demonstrated that short-term hindlimb suspension (1-3 days uploading) can cause a systemic stress (Morey-Holton and Globus, 2002). Exhausting of

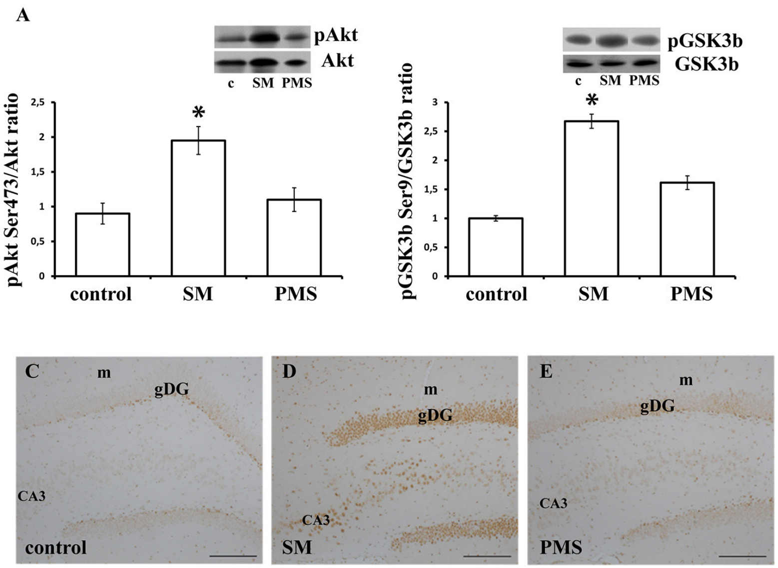

FIGURE 2: Plantar mechanical stimulation restores activity of Akt signaling pathway and CREB A, B - analysis of Akt (**A**) and GSK3b (**B**) activity were estimated by phosphorylation levels of Akt at Ser473 and GSK3b at Ser9. Data are shown as mean +/- SEM. * - $p<0.05$. C-D - pCREB immunostaining demonstrated an increased number of pCREB immunopositive nuclei in granular layer of the Dentate Gyrus and CA3 of rats after 3 days hind-limb suspension (**D**) in comparison with control (**C**); in rats exposed to plantar mechanical stimulation (**E**) the pCREB immunostaining intensity did not differ significant from the control. gDG - granular layer of the Dentate Gyrus; m - molecular layer of the Dentate Gyrus. Scale bar 200 mm.

glutamatergic system in the hippocampus, probably caused by stress, may be a risk factor for cell damage, and the activation of Akt and CREB supposes the activation of neuroprotective mechanisms. On the other hand PMS during SM prevented damage of glutamatergic hippocampal system (Fig.1) and restored normal activity the members of Akt pathway and CREB (Fig.2).

CONCLUSION

Repeated PMS during 3 days' hindlimb uploading prevented damage of glutamatergic hippocampal system and restored normal activity of

Akt/CREB signaling. Thus our data revealed that plantar mechanical stimulation protects hippocampus from the negative effects of SM.

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Keywords: dentate gyrus, hippocampus, Akt, CREB, GAD67, VGLUT2, hind-limb suspension, Gsk3 beta

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Changes in intrinsic functional brain connectivity after first-time exposure to parabolic flight

Angelique Van Ombergen[1]*, Floris L Wuyts[1], Ben Jeurissen[1], Jan Sijbers[1], Floris Vanhevel[2], Steven Douglas Jillings[1], Paul M Parizel[1], Stefan Sunaert[3], Paul H Van de Heyning[1], Vincent Dousset[4], Steven Laureys[5], Athena Demertzi[5]

[1]*University of Antwerp, Antwerp, Belgium*
[2]*Department of Radiology, Antwerp University Hospital, Antwerp, Belgium*
[3]*Translational MRI, KU Leuven, Leuven, Belgium*
[4]*Department of Radiology, Centre Hospitalier Universitaire (CHU) de Bordeaux, Bordeaux, France*
[5]*COMA Science Group, University de Liege, Liege, Belgium*
**angelique.vanombergen@uantwerpen.be*

Space is a hostile environment for humans and spaceflight induces several physiological changes in the human body, such as fluid shifts, neurovestibular disturbances, bone loss and muscle atrophy1. Space crew are known to adapt fairly well to some of these detrimental effects, depending on the site of action and the applied countermeasures1. The central nervous system also seems capable of adaptation to microgravity by the process of neuroplasticity, as previously shown in animals2–4. Yet, little is known about the effects of micro-gravity and gravity transitions on the human brain5. Recently, in a functional MRI study with a single cosmonaut, we showed that long-duration spaceflight induced functional changes in the right insula and in sensorimotor-cerebellar connectivity6. In addition, head-down bed rest studies, a spaceflight analog, have also shown alterations in brain functional connectivity, particularly in sensorimotor areas7.

In parallel to spaceflight research, ground-based models have been developed to overcome some of the logistic challenges related to human spaceflight research. Such a "ground-based" alternative is parabolic flight (PF), during which a specific parabolic trajectory is carried out, wherein the acceleration of the aircraft cancels the gravity acceleration. A hypergravity phase, characterized by 1.5-1.8g, precedes and follows the microgravity phase. Microgravity resembles zero g and lasts around 20–25s. In between parabolas, the aircraft flies in normal 1g conditions8. PF consists of gravity transitions, (microgravity, hypergravity and normal gravity phases), generated during 31 parabolas. An entire flight lasts around 3 to 3.5h (Figure 1).

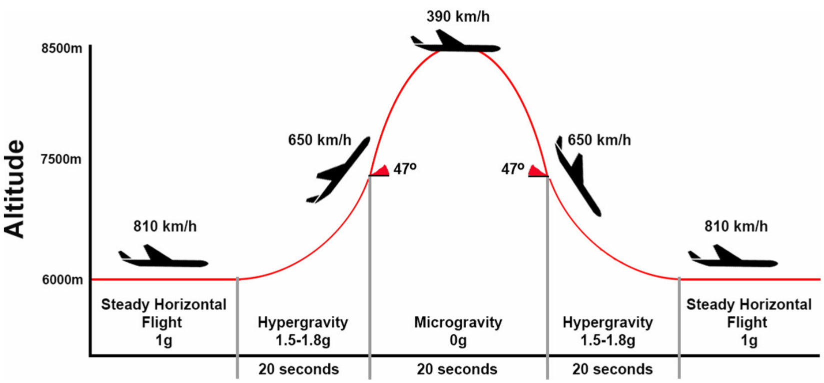

FIGURE 1: Parabolic flight trajectory.

The present study aimed to measure the effects of acute gravitational transitions, as induced by PF, on the brain of naïve human subjects. During a PF, and especially during the microgravity phase, the vestibular input is largely disturbed and therefore might cause an incongruity with the normal terrestrial expectations regarding verticality and spatial orientation9. As no previous neuroimaging investigations have been performed under these conditions 5,10,11, a data-driven approach was implemented to investigate changes in fMRI functional connectivity during resting state.

To this aim, we included 28 healthy participants (11 female; mean(SD) age of 31 (7) years). Each volunteer participated in one parabolic flight of an ESA parabolic flight campaign in Bordeaux, France (flown by Novespace), over the course of 2014-2015. Prior to the PF, all selected participants received scopolamine (0.25mg/1mL; 0.7mL for males and 0.5mL for females), a muscarinic receptor antagonist known to alleviate motion sickness12. To account for the effects of the drug, an independent control group of 12 adults (4 female; mean (SD) age 24 (3) years) who received scopolamine was also included. These participants had no previous experience with PFs.

Each participant received two 3T resting-state functional MRI scans. For the PF group, pre- and post-flight data were acquired on a 3T GE MR 750 W (GE Healthcare, Milwaukee, Wisconsin, USA) MRI scanner at the University of Bordeaux and University Hospital of Bordeaux (France), using a 32-channel head coil.

For the scopolamine control group (non-PF): two scanning sessions took place, a baseline medication-free session and 3 hours after the administration of scopolamine (Antwerp University Hospital, Belgium). Pre- and post-scopolamine data were acquired on a 3T Siemens MAGNETOM Prisma scanner (Siemens, Erlangen, Germany), using a 32-channel head coil. During the resting state scanning period, an identical MRI sequence was used as for the PF group. For a full overview of sequence parameters, readers are referred to the full publication13.

Data preprocessing was performed with Statistical Parametric Mapping 12 (SPM12; www.fil.ion.ucl.ac.uk/spm) and statistical analysis with the CONN v.16 functional connectivity toolbox (www.nitrc.org/projects/conn). Statistical analysis adopted a hypothesis-free (voxel-to-voxel) approach and made use of the intrinsic connectivity contrast (ICC)14, which characterizes the strength of the global connectivity pattern between each voxel and the rest of the brain. For a full overview of data preprocessing and analysis, readers are referred to the full publication13.

For the ICC analysis, the main effects of each group at pre- and post-scan were investigated. For the PF group, between-condition differences were identified in posterior cingulate cortex and right parietal gyrus. For the non-PF group, no post-pre scan differences in ICC connectivity were found. The interaction analysis revealed that the modification of the connectivity pattern was observed in the right temporo-parietal junction (rTPJ)/the angular gyrus (rAG) in the PF group in comparison to the scopolamine group, at post-scan as compared to pre-scan assessment ($T(38)= -3.32$, $p<.001$ FWE cluster-level, permutation testing; cluster size: 260 voxels, peak coordinate x,y,z=[58 -64 18]) (Figure 2).

With no a priori assumptions, we found a decrease of the ICC scores in the rTPJ/rAG after the PF. These results suggest the rAG/TPG has reduced participation in whole-brain connectivity after short-term exposure to altered gravity, most possibly related to vestibular function alterations. Previous investigations also suggest that the rAG is involved in the processing and integration of vestibular, visual and proprioceptive input15. For example, inhibition of the right TPJ caused difficulties with the perception of the upright and maintaining an internal representation of verticality16,17.

These results are relevant for long-duration spaceflight, as well as for space tourism, where less-trained humans will be exposed to similar and

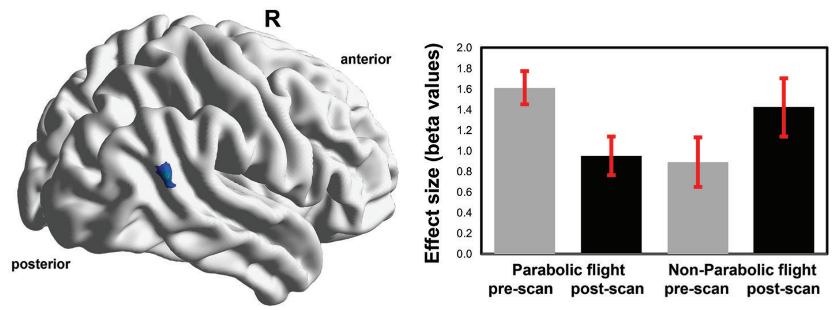

FIGURE 2: The hypothesis-free exploration of connectivity changes points to the right angular gyrus/temporoparietal junction as the region with lower scores on the intrinsic connectivity contract, implying that this region has decreased participation in whole-brain connectivity at post-flight scan. Bars indicate effect sizes (beta values) and error bars 90% CI in the same cluster.

even more extreme gravitational transitions. Taken together, our findings shed light on the understanding of how the brain is affected by short-term alteration of gravitational input and the internalization of gravity in the human brain.

FULL PUBLICATION

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Keywords: brain connectivity, microgravity, parabolic flight, aerospace, rsfMRI = resting state fMRI, fMRI — functional magnetic resonance imaging

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Postural stability of cosmonauts after long space flights

Nikita Valerievich Shishkin*, Vladimir V. Kitov*, Tatiana A. Shigueva, Elena S. Tomilovskaya*, Inessa B. Kozlovskaya

Sensory-motor physiology and countermeasures department, SSC RF – Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia
**chachaturan@yandex.ru; *finegold@yandex.ru; *arctg@yandex.ru*

The studies of the characteristics of postural stability has revealed deep disorders of equilibrium function after space flight of different duration (Kozlovskaya et al., 1981-1990; Paloski et al., 1992; Wood, 2012-2015).

Computer dynamic stabilometry method (CDP) was invented and first used for reflex function assessing by Lewis Nashner (Nashner, 1976). The results of works of American researchers, who were using the CDP, have shown that the restoration of the vertical balance function after short 4-10-day space missions occured in the first hours after landing, covering up to 50% of the post-flight decrease in the comprehensive assesment of EquiScore equilibrium during the first 3 hours (Black, Paloski, 1998).

The method of computer dynamic stabilometry included 6 Sensory Organization Tests (SOT), namely – the posture with eyes open or closed in stable or moving visual environment or support surface, during which the motion of the center of pressure of the subject is recorded. Later modified SOT2 tests (posture with eyes closed on a fixed surface) and SOT5 tests (eyes closed, movable support surface) were added to the series of tests. Modification consisted in addition to the tasks the dynamic head movements in the sagittal plane with a frequency of 0.33 Hz and an amplitude of 40o (Jain et al., 2010).

More recent studies of the postural stability after long-term space flights (Wood et al. 2012, 2015) showed that the basic recovery of EqScore rate in the original SOT2 and SOT5 tests can be clearly detected within the first 3 days after the flight, decelerating over the next 7 days, while the restoration of stability in the modified tests is ongoing throughout the ten-day study period with reduce of this improving’s dynamic. The authors assumed that the greater complexity of the tests, including dynamic head movements, is associated with the readaptation of the vestibular system, expressed in the reassessment of otolith signals and complete blocking of slow otolith signals. Postural stability studies, included in the Russian scientific-reseach program of post-flight studies, do not capture the early recovery period after the flight, focusing on the features of restoration of posture regulation system in the period from 3 to 10 days after the SF.

26 Russian cosmonauts - members of ISS crews took part in the research. The flight duration ranged from 115 to 199 days. All participants signed an informed consent to participate in the experiment. The examination was performed using the method of computerized dynamic posturography developed by L. Nashner in the 1970s with modifications introduced by NASA researchers (Black, Paloski, 1982-1993). During the examination, the cosmonaut was in a vertical position on the tensometric platform of the NeuroCom «SMART EquiTest» installation equipped with a controlled screen closing the field of vision of the subject from three sides – from the front, right and left.

The support surface and visual environment rotates in direct proportion to anterior-posterior (AP) body sway (sway referencing). By disrupting somatosensory feedback and removing vision, this condition is sensitive to adaptive changes in how vestibular feedback is used for postural control

The study was carried out twice before flight and on the 3rd, 7th (8) and 10th (11) days after landing. The 1-way ANOVA with Bonferroni's multiple comparisons test has been used for statistical analysis of the results.

The analyzed parameter of the tests is the value of Equilibrium Score calculated by the formula EqScore = [1-(P-Psway)/12,5°]*100, where (P-Psway) – the angle between the maximum deviations of the center of gravity in the sagittal plane during the test; 12.5° - maximum (P-Psway), observed in the normal population. Before flight, this parameter ranged from 91 to 93 during vertical stance with eyes open, naturally decreasing along the growing complexity of the task: for example, in the SOT2 test (eyes closed) it varied in a range from 88 to 90 points, in the SOT5 test (eyes closed, the movable surface) – from 55 to 63 points. The lowest values of the indicator were recorded in the SOT5 test with dynamic head movements - from 28 to 41 points.

The analysis of the obtained data showed that the gradual decrease in the rate of recovery of the EqScore index after 3 days after landing is observed only in standard tests, while in the tests with head movements this did not happen.

Thus, at a vertical posture on a fixed surface with eyes closed (SOT2), the values of the studied indicator on the 3rd, 7th and 10th days did not differ significantly, averaging 87.14+0.86 points, and when performing a similar test with head movements (SOT2m) the difference between the preflight data (82+2.03) and 10th day (78.21+2.48) becomes insignificant. 3rd and 7th days data (74.56+2.44 and 74.73+2.69) have the significant difference with the preflight.

In the test of vertical stance with closed eyes on the movable support (SOT5), EqScore values increased significantly from 3 to 10 days after the SF completion (52.75+2.85 and 61.25+2.58. When performing the complicated test with dynamic head movements (SOT5m), the values of the studied indicator were 20.19+4.54 on the 3rd day after the SF, progressively increasing in the course of further studies-up to 26.96+3.58 on the 7th day and up to 35.78 + 3.24 on the 10th day after the flight in opposition to reduce of improving's dynamic in similar American study (Wood, 2015).

Our study has shown that recovery of EqScore to the initial level in standard tests SOT1-5 was observed during on the 7th day after landing. In tests with addition of dynamic head tilts(SOT2m and SOT5m) progressive recovery of the studied index continued up to day 10 after landing. Unlike in american studies (Wood et al., 2015), there was no decrease in the dynamics of SOT2m (Fig. 1)

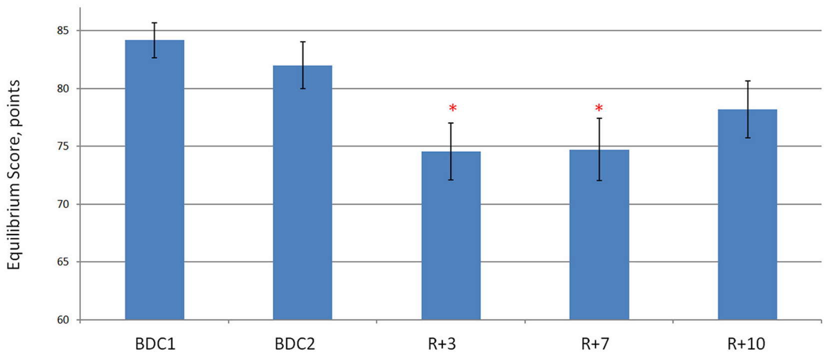

FIGURE 1: SOT2m - EqScore alteration for test with eyes closed, stable surface and sagittal dynamic head tilts (0,3 Hz); BDC1, BDC2 - baseline studies, R+3, R+7, R+10 - 3rd, 7th, 10th day after landing; * - significant difference compared to baseline; SOT2m is used for assessing of regulation effectiveness of closed loop feedback in the absence of visual control. Dynamic head tilts are used for increasing of diagnostic accuracy (Jain, 2010).

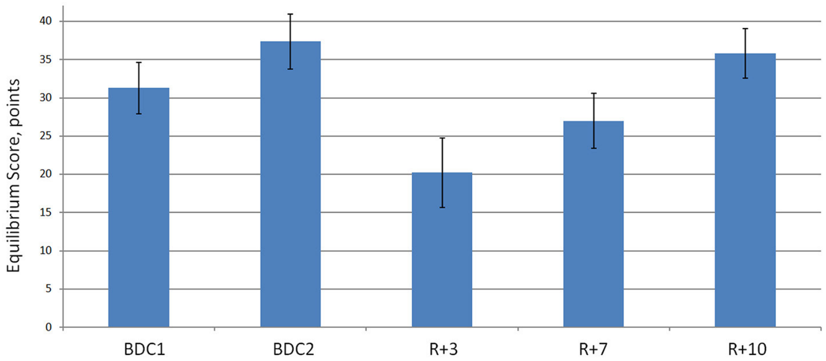

FIGURE 2: SOT5m - EqScore alteration for test with eyes closed, movable surface and sagittal dynamic head tilts (0,3 Hz); BDC1, BDC2 - baseline studies, R+3, R+7, R+10 - 3rd, 7th, 10th day after landing; SOT5m is used for assessing of posture vestibular regulation. Dynamic head tilts are used for increasing of diagnostic accuracy (Jain, 2010).

and SOT5m (Fig. 2) EqScores. The period from 7 to 10 days after landing coincides with the period of recovery of the otolith function (Kornilova et al. 2012), which suggests that the restoration of the stability index in the SOT2m test on the 10th day could be associated with the restoration of the ability of the Central nervous system to quickly track the orientation of the head in space, associated with the function of the otolith apparatus.

FUNDING

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Keywords: space physiology, dynamic posturography (CDP), cosmonaut, vestibular postural reactions, human postural stability

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Effects of microgravity on characteristics of the accuracy control of movements

Tatiana A. Shigueva[1]*, Vladimir V. Kitov[1], Lyubove E. Amirova[1], Roman N. Chuprov-Netochin[2], Elena S. Tomilovskaya[1], Inessa B. Kozlovskaya[1]

[1]Sensory-motor physiology and countermeasures department, SSC RF – Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia

[2]School of Biological and Medical Physics, Moscow Institute of Physics and Technology, Dolgoprudny, Russia

**t.shigueva@gmail.com*

INTRODUCTION

Results of previous studies have shown that hypogravitational motor syndrome is characterized by alterations in all components of the motor system (Kozlovskaya et al., 1987; Reschke et al., 1998). Motor control disturbances, such as kinematics changes of locomotion and the decline of postural stability are constantly registered after exposure to weightlessness or simulated microgravity (Gurfinkel et al., 1969; Kozlovskaya et al., 1981). These changes are caused by the development of a number of negative motor disturbances such as decrease of muscle tone and maximal voluntary contraction force (Kakurin et al., 1971; Berry, 1973; Grigoriev et al, 2004), decline of accuracy of muscle contraction forces control, increase of motor task execution time and others (Chkhaidze, 1968; Chekirda et al., 1974; Kozlovskaya et al., 2003). These alterations form a hypogravitational ataxia syndrome (Grigoriev et al, 2004). Support withdrawal is one of important factors of weightlessness. According to the results obtained in IBMP RAS researches most of motor effects of microgravity are fully reproduced on Earth under conditions of Dry Immersion (DI), which seems to be one of the most adequate ground simulation model of weightlessness (Shulzhenko and Vil-Villiams, 1975; Kozlovskaya et al., 1988; Tomilovskaya et al, 2013).

The goal of the present work was to study effects of support withdrawal on precision characteristics of programmed movements.

METHODS

The studies were carried out with participation of 20 healthy male volunteers that were exposed to Dry Immersion (DI). Twelve of them experienced DI for 5 days, eight others – for 7 days.

To obtain precise voluntary movements characteristics a force gradation task with single-joint isometric plantar flexions has been used. During the task a subject performed a number of efforts - from the minimal to the maximal one with the minimal difference between neighboring movements. The initial minimal effort that is considered as an absolute threshold of the precision control system and the mean difference between neighboring efforts – considered as a differential threshold - were analyzed. The cases in which the subsequent effort didn't exceed the previous one were defined as an error. The number of properly executed efforts and errors were also analyzed.

The aim of the second part of the study was to analyze the recruitment order of spinal motor neurons participating in execution of the task.

While executing the second task a subject maintained a small muscle effort (up to 7% from maximal voluntary contraction). During the task the motor units (MU) activities of the leg extensors (m. soleus and m. gastrocnemius lateral) were recorded using sterile needle concentric electrodes. The number of MU displayed on the screen under these conditions didn't exceed 4 or 5. Peak amplitude and duration of interspike intervals (ISI) were analysed.

Experiments were performed before DI, twice in the course of DI and on the next day after its completion. The Wilcoxon nonparametric criteria was used for statistical data analysis.

RESULTS AND DISCUSSION

Under conditions of simulated microgravity the motor task was executed as usual correctly. However more precise analysis of the data has revealed a decrease of leg movement control system accuracy expressed by a decrease of the number of distinguished efforts and an increase of the differential thresholds.

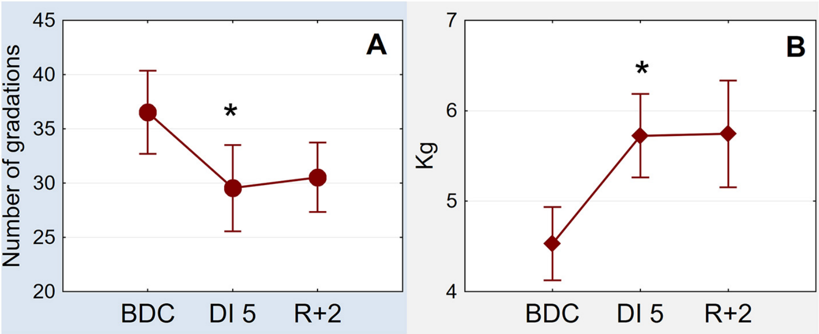

FIGURE 1: (**A**) The number of gradations. (**B**) The values of the differential threshold of effort. DI5 – 5th day of DI, BDC – base data collection. * – significant changes in comparison to BDC, p<0,05.

The number of gradations decreased significantly (p<0,05) to 29,5 ±14,3 on day 5 of immersion (versus 36,5±13,8 in base data collection) (Figure 1A). The value of differential threshold of effort increased significantly (p<0,05) up to 4,5 ±1,5 (versus 5,7±1,7 in base data collection) on day 5 of immersion (Figure 1B).

Motor unit recruitment order in the task of small isometric effort was assessed by the frequency characteristics of MU in the course of 7-day dry immersion experiment.

In full accordance with previous data (Sugajima et al., 1996; Kozlovskaya and Kirenskaya, 1986, 2004) small plantar flexion effort (0-5,5% of maximal voluntary contraction of about 140 kg) before exposure to DI was provided by the activity of small motoneurons. The number of MU involved in the task execution averaged 68,6% (of total number) in m. soleus (Figure 2A) and 77,3% – in m. gastrocnemius lat. (Figure 2B); the average interspike intervals (ISI) were in the range of 100-160 ms.

The order of MU involvement in both muscles under DI conditions changed significantly. MUs with ISIs of 100-130 ms were not registered at all during the task execution on the 3d day of DI. The most of MUs involved had ISIs of 160-200 ms. Only 59.3% of MU with ISI in the range of 130-160 ms were involved in the task execution in m. soleus (Figure 2A), and 28% – in m. gastrocnemius

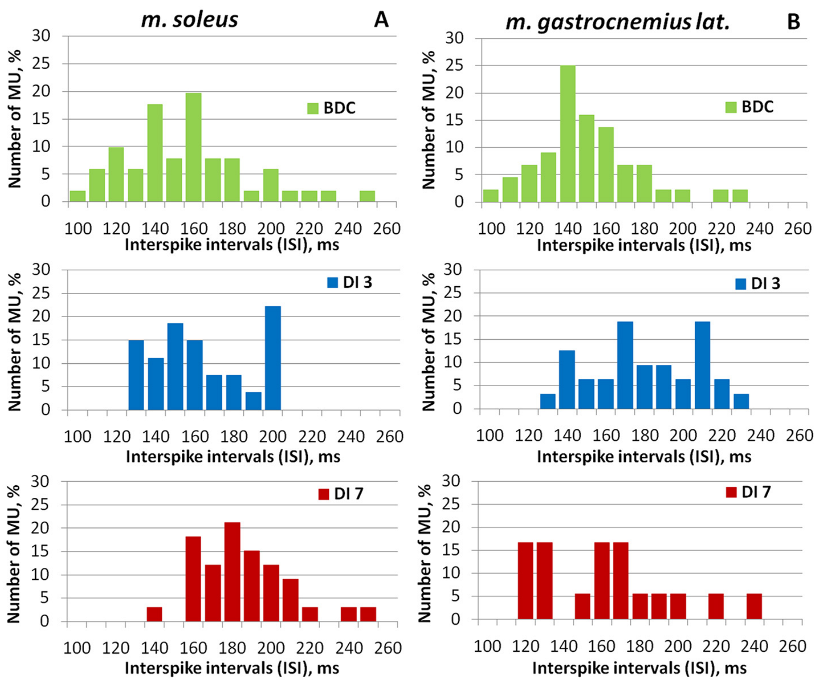

FIGURE 2: Histogram of the distribution of MU by ISI in m. soleus and m. gastrocnemius lat. (**A**) before DI; (**B**) in the course of DI; BDC – base data collection, DI3 – 3d day of DI, DI7 – 7th day of DI.

lat. (Figure 2B). Increase of ISI values in case of constant low-level isometric effort maintenance shows that the small effort under microgravity conditions is provided by the larger MUs. The altered order of MUs involvement remained also on the 7th day of DI and even after its completion.

Thus, our studies have shown a decrease of precision in programmed movements under conditions of immersion which is caused not only by changes in cortical programming mechanisms (Kozlovskaya and Kirenskaya, 2004), but also by recruitment order alterations in motor neuron pool (violation of Henneman law) (Henneman et al., 1965) which decreases its ability to control precisely movements of low contraction level due to inactivity of small (postural) motor units.

CONCLUSIONS

Exposure to support withdrawal conditions is followed by a decrease of precision in muscle force control.

Results of motor neuron recruitment order study showed that disturbances in precise movement control is caused not only by changes in programming mechanisms but also by changes in recruitment order of small motor neurons responsible for execution of low-level contractions.

ACKNOWLEDGEMENT

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Keywords: motor control, microgravity, dry immersion, motor unit (MU), hypogravitational motor syndrome, support unloading

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Changes in the characteristics of voluntary movements after long duration spaceflight

Nikolay Osetskiy[1]*, Vladimir Kitov[1], Ilya Rukavishnikov[1], Igor Kofman[2], Inna Sosnina[1], Nataliya Lysova[1], Liubov Amirova[1], Marissa Rosenberg[2], Elena Fomina[1], Elena Tomilovskaya[1]*, Millard Freeman Reschke[2], Inessa Kozlovskaya[1]

[1] The department of sensory-motor physiology and countermeasures, Institute of Biomedical Problems (RAS), Moscow, Russia
[2] Neuroscience laboratory, Johnson Space Center (NASA), Houston, United States
**n.osetskiy@gmail.com; *finegold@yandex.ru*

Exposure to weightlessness in the course of spaceflight (SF) is connected tightly to physiological alterations of vestibular functions, the support deprivation, and a significant decline of the proprioceptive information density (Grigoriev et al., 2004), which affects negatively the ability to control a precision of voluntary movements. These dicturbances are resulted in the appearance of the alterations of complicated and complex movements performance, like voluntary walking, vertical posture standing, and hand-precision movements (Kozlovskaya et al., 1981; 1990; Paloski et al., 1992; Grigoriev et al., 1993; Black et al., 1998; Wood et al., 2015).

The characteristics of voluntary movements representing motor reaction tasks have been studied before and after long duration SF. Eleven Russian cosmonauts that were included in the “Field Test” Russian-American experiment performed a series of 4 following tasks: recovery from fall, voluntary walking, a series of tandem (heel-to-toe) walks, and a force discrimination task. Additionally, the severity of motion sickness (MS) was assessed as well, before and after each task by answering a questionary about the rate of their MS symptoms using a 1 to 20 scales from 1 (no symptoms) up to 20 (vomiting). To assess the biomechanical and kinematic parameters of voluntary movements, Emerald inertial measurement unit sensors were placed on the head, torso, trunk, wrists, ankles, and toes (APDM, Inc., Portland, OR).

In the recovery from fall task, subjects begin in the prone postion. After a verbal command “go”, subjects come to a stable standing position. The performance metric for this task is the time of the transition from prone to a stable vertical posture. The voluntary walking task included standing up from a chair, walking in a straight line and stepping over an obstacle. The obstacle consisted of a thin metal rod balanced on top of two foam blocks. The walks were performed 3 times, with the obstacle height changed from the 5 to 10 cm, and after that - finally 15 cm. Performance was assessed using the height and length of the step and the time of walk. The tandem walk consisted of 3 trials of heel-to-toe walking for approximately 10 steps, with arms crossed over the chest and eyes closed. For a fourth time this was repeated with the eyes open, with gaze directed ahead. Tandem walk was scored by percent correct steps, assessed from a video recording made during each test session. A step was considered incorrect if there was a large gap between heel and toe, if there was a long pause, if the subject unfolded arms or opened his eyes, if the subject took an intermediate step or crossed over the stable foot with the stepping foot. For the force discrimination task, subjects held a hand dynamometer that measured the force of their squeezes. They were instructed to start with a squeeze with the minimal amount of force possible and continue to squeeze and release with just noticeably higher amount of force until they hit a maximum strength. In this task of grading efforts the number of gradations performed, the number of errors (when the subsequent effort did not exceed or was equal to the strength of the previous one), the minimal force and the average amplitude of the effort increase were analyzed.

Baseline data were collected twice prior to the SF: 60 and 30 days before launch (L-60 and L-30, respectively). Post-flight test sessions were conducted in the medical tent at the landing site approximately 1 hour after flight, and then - on the 3-4th, 6-8th and 12th days after the SF completion (R+0, R+4, R+7, R+12 respectively).

Statistical data analysis was conducted using ordinary one-way ANOVA with post hoc test Bonferroni, $p<0.05$ was chosen as a significance criteria.

Analysis of the results showed that the most profound changes in characteristics of the voluntary movements were observed at the day of the landing (R+0). The baseline recovery from fall time to stand lasted, on average, 7.9 ± 1.7 s, which was an average over subjects and over the two baseline sessions (L-60 and L-30). The time to stand increased significantly on R+0 up to 14.8 ± 3.0 s ($n=9$, $p<0.0001$, $t=8.046$). On the test day R+4 we still observed a significant time increase to 10.5 ± 2.3 s ($n=10$, $p=0.0148$, $t=3.125$). On the R+7 - R+12 the time to stand did not last longer than the baseline one.

The step length during the voluntary walking task for the baseline pre-flight data collections was 1.32 ± 0.1 m ($n=4$) on average. This step length significantly decreased on R+0 to 0.73±0.08 m (n=2) ($p=0.002$, $t=6.594$). In subsequent sessions, the mean length of the step did not vary from the baseline values. Similar changes were observed in the height of the steps: 0.15±0.02 m (n=4) pre-flight as compared to 0.085±0.006 m (n=2) ($p=0.0255$, $t=3.485$) on the landing day (R+0).

Subjects performed the tandem walk task with 12.10±0.74 (n=10) correct steps, that was significantly lower for this task on the landing day to 3.60±1.34 (n=5) ($p<0.0001$, $t=13.23$). Recovery back to baseline performance was not as quick as for the other tasks. We found a score of 5.60±1.71 (n=10) ($p<0.0001$, $t=12.39$) on R+4 and 8±1 (n=8) ($p<0.0001$, $t=8.045$) - on the seventh day after the landing. On R+12, the score did not differ from the baseline data (Figure 1).

The number of gradations performed on the landing day was 23.0±5.3 (n=5), whereas the baseline values were 25.0±8.9 (n=5). No significant difference was observed. The re-adaptation period values were 27.5±8.4 (n=4) on R+4, 30.5±14.0 (n=4) on R+7 and 25.2+6.4 (n=5) on R+12, with no significant difference as well.

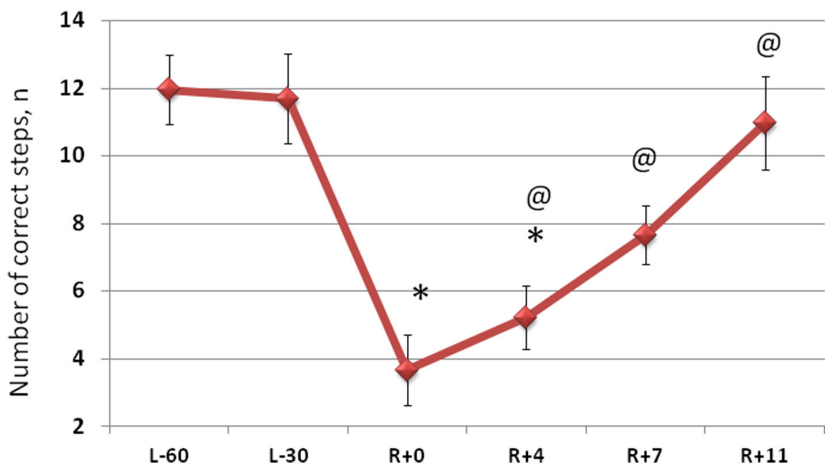

FIGURE 1: The number of correct tandem walking steps (heel-to-toe) performed by the cosmonauts before and after long term spaceflight, n=11; * - significant difference compared to BDC values, p < 0,05; @ - significant difference compared to R+0 difference, p < 0,05.

Table 1: Motion sickness score (0-20 scale) in 11 cosmonauts during the Field Test experiment performance at the landing day (during the 1st hour after landing). Primary evaluation – evaluation before the beginning of the test, before sts – evaluation before "sit-to-stand" task, before fr – evaluation before "fall recovery" task, after fr – evaluation after "fall recovery" task, before first walk (obstacle) – evaluation before the first "walking with obstacle avoidance" task, after first walk (obstacle) – evaluation after the first "walking with obstacle avoidance" task, before first tandem walk – evaluation before the first "tandem walk" task, after first tandem walk – evaluation after the first "tandem walk" task. n/d – the absence of data due to the termination of the experiment.

	Primary evaluation	**Before sts**	**Before fr**	**After fr**	**Before first walk (obstacle)**	**After first walk (obstacle)**	**Before first tandem walk**	**After first tandem walk**
subject No1	7	7	5	6	7	7	n/d	n/d
subject No2	11	7	7	6	5	7	n/d	n/d
subject No3	15	15	15	17	17	18	n/d	n/d
subject No4	5	7	7	10	n/d	n/d	n/d	n/d
subject No5	8	10	8	7	7	8	8	10
subject No6	18	n/d	n/d	n/d	n/d	n/d	n/d	n/d
subject No7	10	17	15	18	n/d	n/d	n/d	n/d
subject No8	10	10	6	8	10	15	15	15
subject No9	10	10	13	16	n/d	n/d	n/d	n/d
subject No10	14	14	8	10	10	12	n/d	n/d
subject No11	8	8	6	7	10	10	n/d	n/d

Subjects motion sickness scores varied greatly within subjects and throughout the test session (Table 1). However, there was some tendencies common for all the participants. After 2 minutes of prone position the symptoms severely decreased in all of the subjects whereas after recovery from fall the symptoms were almost as pronounced as at the beginning of the test. It has to be noted that during the task some of the subjects quit to fully perform the test complaining of the dizziness and nausea which were produced after the fall.

CONCLUSIONS

The results of the study demonstrated a visible decrease of the functional capacities and the significant deterioration of the characteristics of voluntary movements after the accomplishment of the long duration SF as well as extremely low functional performance level immediately after return from space. All the mentioned changes were most pronounced in the acute period of re-adaptation to gravity conditions, that means days R+0 - R+3. These data are crucially important due to the plans for interplanetary missions and future work on Lunar and Martian surface.

Further investigations will broaden our knowledge on the subject and collect sensory-motor system recovery dynamic data in post-flight period.

ACKNOWLEDGEMENT

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Keywords: physiology, posture, motion sickness, voluntary movement, microgravity, spaceflight, dynamometer, fall recovery, afferentation

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Isolation, sleep, cognition and neurophysiological responses – An investiogation in the human exploration research analog (HERA)

Timo Klein[1,2]*, Andrea Rossiter[3], Jan Weber[1], Tina Foitschik[1], Brian Crucian[4], Stefan Schneider[1,2], Vera Abeln[1]

[1]Institute of Movement and Neuroscience, German Sport University Cologne, Germany
[2]VasoActive Research Group, School of Health and Sport Science, University of the Sunshine Coast, Australia
[3]Centre of Human & Applied Physiological Sciences, King´s College London, United Kindom
[4]Johnson Space Center (NASA), Houston, United States of America
**t.klein@dshs-koeln.de*

INTRODUCTION

Space and isolation missions are known to be stressful for the human organism and negatively impact on the quality of sleep and mood of crewmembers. The maintenance of cognitive performance is of outmost importance for astronauts' safety and for mission success during space travels. While short bouts of microgravity have been associated with greater cognitive performance than during gravitational conditions on earth (Wollseiffen et al., 2016), long-term isolation analogues on earth were linked with impaired cognitive performance (Basner et al., 2014; Gemignani et al., 2014). However, the underlying neurophysiological mechanisms remain unclear and efficient countermeasures are missing. Furthermore, high levels of stress are associated with a reduction of neurotropic factors like BDNF and IGF-1. This study aimed to investigate the effect of isolation on sleep, mood, cognition, neurotrophic factors and brain cortical activity during 30 days of isolation under space flight analogue conditions.

METHODS

Sixteen participants (aged: 37±7 years) were isolated in four missions, during each mission 4 participants stayed inside the HERA habitat at NASA for the duration of 30 days. Seventeen participants (aged: 32±9 years) were tested as a non-isolated control group simultaneously at the German Sport University Cologne. Both groups were asked to exercise on a daily basis during the

30 days of the interventions. Throughout the interventions participants wore wrist belt actigraphs to assess total light exposure during sleep, sleep efficiency and wake time after sleep onset (WASO). On mission days (MD) -5, 7, 28 and +5 participants completed a sleep diary and the Self-assessment questionnaire for Sleep and Awakening quality (SSA), the Positive Affect and Negative Affect Scale (PANAS-X) and cognitive tasks (speed match, chalkboard challenge, memory matrix by lumosity.com). Resting state brain activity as a marker of stress was assessed by a five-minutes resting electroencephalography in relaxed sitting position with eyes-closed (Brain Products, Munich). Also, intravenous morning cortisol, melatonin, BDNF and IGF-1 were assessed. Effects of the intervention (isolation vs. non-isolation) and time were determined using repeated measures ANOVA.

RESULTS

Cortisol level was significantly increased during isolation in comparison to non-isolated participants ($p<0.01$). Melatonin ($p=0.37$), BDNF ($p=0.92$) and IGF-1 ($p=0.09$) values showed no difference between the groups and remained unchanged during the mission. There were no group effects for total light exposure during sleep ($p=0.61$), sleep efficiency ($p=0.54$), WASO ($p=0.73$) and subjective sleep quality (SSA, $p=0.10$). Mood was also similar between the groups as there were no group by time interaction on the positive ($p=0.38$) and negative affect scale ($p=0.20$). Cognitive performance was not affected by isolation as no differences were shown between the groups (speed match, $p=0.22$; chalkboard challenge, $p=0.75$; memory matrix, $p=0.29$). Frontal cortical current density did not show any differences between the isolated and the non-isolated groups ($p=0.40$) and remained unchanged throughout the intervention as there was no group*time interaction ($p=0.72$).

CONCLUSION

During 30 days of isolation sleep, mood and cognition were not impaired although high levels of cortisol suggest increased levels of stress. The maintenance of sleep quality through a daily exercise routine during isolation might have positively affected CNS function and structural markers, as brain cortical activation and neurotropic factors were not impaired during isolation. Further results from HERA campagne 4 in 2017/2018 might allow further analyses and insights into possible effects of isolation on sleep, mood, cognition and its underlying neurophysiological mechanisms as well as the effect of physical activity as participants exercise less during isolation.

ACKNOWLEDGEMENT

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Keywords: exercise, BDNF, EEG, brain cortical activity, space flight analog

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The definition of the cellular and molecular mechanisms of plants gravisensitive

Olga Artemenko

Department of Cell Biology and Anatomy, Institute of Botany (NAN Ukraine), Kiev, Ukraine
oartemenko66@gmail.com

Future long-term expeditions require a huge amount of metabolic resources - water, food, oxygen, requiring special conditions and equipment, and they are too heavy for existing missiles. In addition, such life support systems cannot exist without a high level of circulation. A comparison of the results of protein study and gene's transcription in plants showed a positive, some limited interaction between the regulation of protein synthesis and gene expression in general plant reactions under space flight conditions (Kordyum, Chapman, 2017). The leading areas of modern biology space are cell biology under conditions of altered gravity and definition the cellular and molecular mechanisms plants gravisensitive. The mechanisms of gravitropism and graviresistance are complementary, the first being mostly sensitive to the direction of the gravity vector, and the second to its magnitude (Kordyum, 2014). At a global molecular level, the consequence of gravity alteration is that the genome should be finely tuned to counteract a type of stress that plants have never encountered before throughout evolution. An increase in the transcription level of certain cell cycle's genes delays the transition of cells from the G1- phase to S- phase in the first cell cycle that leads to a proliferative pool decrease. But an increase in the proliferative activity occurs at the subsequent stages of growth, that may testify the work of adaptation mechanisms and the renewal of normal cell functioning. These adaptation mechanisms are likely involved multigene families. Multigene families and redundant genes present an advantage in that they can experience changes without the risk of being deleterious and, for this reason, they should play a key role in the response to gravitational stress.

The coordinated regulation of the interaction of plant cells is carried out by the synthesis of special hormones (cytokinin and auxin) which cause the signaling cascade in the cells (Herranz, 2014; Tank, 2014). In the event that the signal leads to changes in the level of gene's expression, the transcription factor (TF) is often the end point of the cascade. TF - is one of the groups of proteins that provides for the reading and interpretation of genetic information. They bind DNA and promote the initiation of a program to increase or decrease transcription of the gene. So, they are need for the normal functioning of the body at all levels. One of the most numerous types of TF plants is MYB-proteins (MYB-myeloblastosis). This family of TF contains proteins that controling such processes as root development, patterning of the leaf, formation of trichomes, cell cycle, circadian rhythms, transmission of a photochromic signal, etc. The MYB protein DNA-binding domain contains one to three repeats and approximately 50 amino acids. This type of TF interacts specifically with genes containing (C / T) AAS (G / T) G-nucleotide sequences.

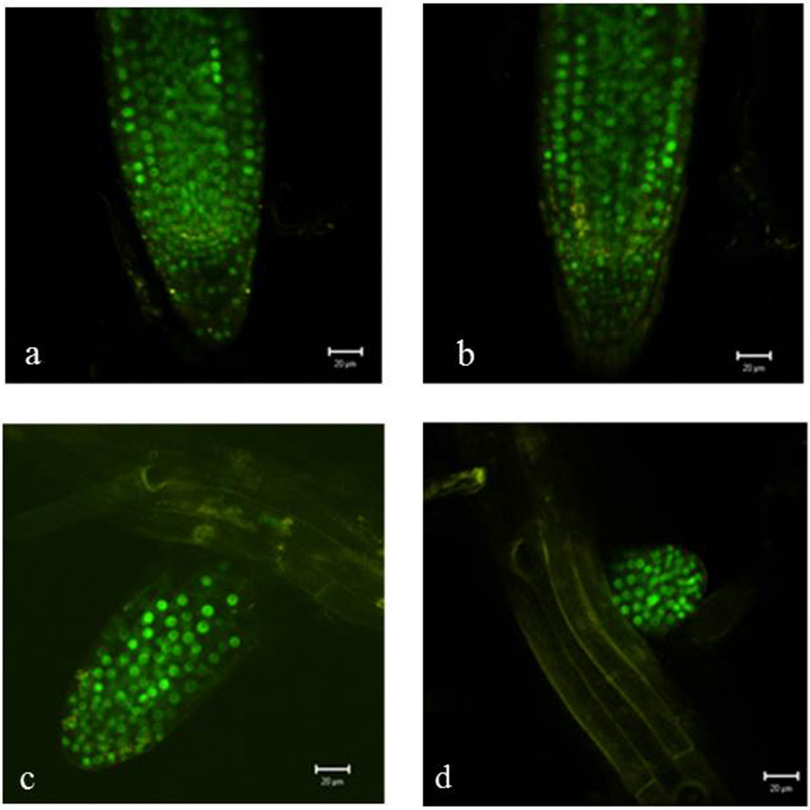

FIGURE 1: Expression of MYB3R3 in the roots of 4 daily seedlings and in the *Arabidopsis thaliana* lateral roots rudiments (**a**, **c**-control, **b**, **d**-clinorotation).

We analyzed the expression of TF MYB3R3 in Arabidopsis thaliana root cells in a stationary meristem of four-day seedlings and in the rudiments of the lateral roots (Fig. 1). In conditions of clinorotation observed a loss of TF expression in comparison with control, which may be due to a decrease in the activity of the cell cycle and growth of the root. At the same time, in the rudiments of the lateral roots, when there is a peak of proliferative activity in the places of the formation of lateral roots, the expression of TF is also at a high level in both control and clinorotation. The difference in the size of the lateral roots of control and experiment is due to a later onset of growth of the lateral roots in the clinorotation conditions, which is confirmed by the earlier known data on the reduction of growth parameters in the conditions of the changed gravity (Artemenko, 2015). The obtained data on the influence of clinorotation on the process and duration of the cell cycle phases, as well as subsequent changes in the growth and development of plants in these conditions, and are key in the development of onboard greenhouses and bioregenerative life support systems. Investigating the control of cell cycle events and the interaction between the molecular and cellular mechanisms of these processes is important for understanding undesirable changes in plant growth and development, since the quality and quantity of products that cosmonauts will use depend on it.

Keywords: cell cycle, plant, MYB, microgravity, transcription factor

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Clinorotation impacts the plasmalemma lipid bilayer and its functional domains—rafts in plant cells

Elizabeth Kordyum*, Iliya Bulavin, Tamara Vorobyova

Department of Cell Biology and Anatomy, Institute of Botany (NAN Ukraine), Kiev, Ukraine
**cellbiol@ukr.net*

Currently the presence of functional microdomains, which became known as "lipid rafts", in the plasmalemma (Pl) of plant cells has been shown. It is supposed that rafts enriched on cholesterol and sphingolipids modulate the protein interrelation and in this way they include in many vitally important cell processes. In this work, we firstly analyzed the composition of fatty acids and sterols both in the Pl isolated from the roots of Pisum sativum seedlings grown during 6 days under slow horizontal clinorotation and in lipid rafts isolated from it. Rafts obtained from the root plasmalemma in the stationary conditions and under clinorotation were similar to those in other plant species. Clinorotation induced changes in the percentage of saturated and unsaturated fatty acids in the Pl and raft fractions. The percentage of cholesterol increased in Pl and especially in rafts under clinorotation. Nevertheless, the plasmalemma unsaturation index was similar to that in control for maintenance of the membrane fluidity necessary for normal cell viability. At the same time, a high level of cholesterol in lipid rafts clearly shows their increased rigidity in simulated microgravity, that can impact membrane activities. This work presents new information for future spaceflight and ground-based experimental designs.

INTRODUCTION

Properties and functions of biological membranes, especially plasmalemma (Pl), may consider as the most sensitive indicators of the influence of gravity or altered gravity on a cell. The Pl is one of the most dynamic supramolecular structures in a cell and it is the intermediate link between the cytoplasm and extracellular environment and involved in numerous processes such as transport of metabolites and ions, endocytosis, cell proliferation and differentiation, defence from pathogens. Currently it has been revealed the presence of functional microdomains with the specific localization and content of lipids and proteins in the Pl of plant cells, that became known as "lipid rafts". It is supposed that rafts enriched on cholesterol and sphingolipids take part in many vitally important cell processes (Brown and London, 1998; Cacas et al., 2012). The investigations of lipid rafts help to explain the biochemical processes which occur in cell membrane in the normal conditions and in responses to stress and can't be explained by using the other models.

It has been experimentally shown that Pl is gravisensitive: changes in the percentage of phospholipids and fatty acids in Pl under clinorotation (Polulyakh, 1988), as well as an impact of gravity on ion channels and fluidity of artificial and cellular membranes (Sieber et al., 2004). At the same time there is no information about the influence of real or simulated microgravity on lipid rafts in plant cells. The purpose of our work was to examine the composition of fatty acids and sterols in Pl and lipid rafts isolated from roots of Pisum sativum seedlings grown during 6 days under slow horizontal clinorotation.

MATERIAL AND METHODS

Roots of pea seedlings grown in 6 days in the stationary conditions and under clinorotation (2 rpm) were used. The Pl fraction was obtained using the two-phase water-polymer system optimized for pea seedling roots. Rafts were separated in the sucrose gradient under height speed centrifugation (31 000 rpm, 16 hours). The fractions of Pl and rafts were obtained with an ultracentrifuge "Optima L-90K" (Beckman Coulter, Germany) and controlled by the electron-microscopic method with a transmission electron microscope JEM 1230 (Jeol, Japan). The composition of saturated and unsaturated fatty acids, and sterols was determined by gas chromatography with a chromatograph HRGC 5300 (Carlo Erba Instruments, Italy).

RESULTS AND DISCUSSION

It was shown that lipid rafts from the root PL of pea seedlings grown in the stationary conditions and under clinorotation have the appearance of thin tapes of 80–100 nm in length and 6–13 nm in width (Figure 1), they were similar to those in other plant species on the structure and size, and also enriched with cholesterol and saturated fatty acids. In the stationary conditions, it was found that saturated fatty acids prevailed in the fraction of rafts (64.5%), especially palmitic acid. The percentage of unsaturated fatty acids was 35.5 %, especially linoleic acid.

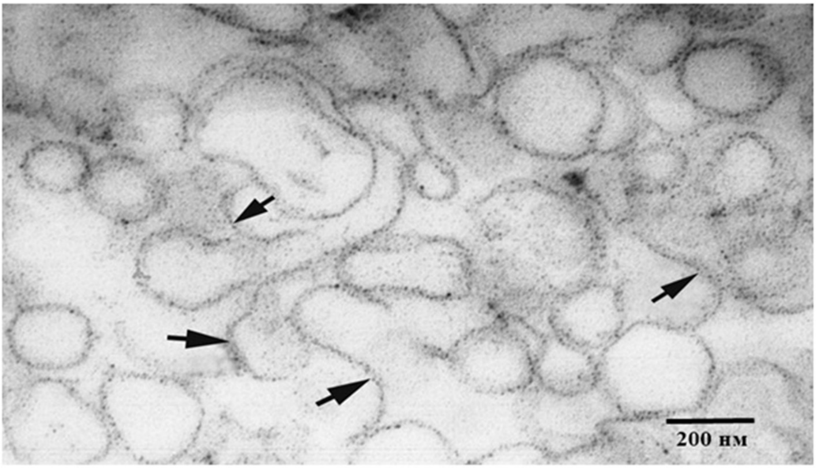

FIGURE 1: Rafts obtained from roots of pea 6-day old seedlings (electron transmission microscopy). Arrows indicate rafts.

Under clinorotation, saturated fatty acids also prevailed in the fraction of rafts, and their percentage slightly increased up to 66.67%. Arachidonic acid prevailed among unsaturated fatty acids while the percentage of linoleic acid significantly decreased. The percentage of cholesterol increased 7 times in lipid rafts in comparison with control. Changes in the percentage of individual saturated and unsaturated fatty acids in Pl occurred under clinorotation but the unsaturation index was similar to that in control for maintenance of the membrane fluidity necessary for normal cell viability.

At the same time, the obtained data clearly show the increased rigidity of the lipid domains in the Pl over the stability of the membrane fluidity under simulated microgravity. Since the rafts contain protein complexes necessary for the perception and transduction of external signals, vesicular transport etc. (Lingwood and Simons, 2010), a significant increase in sterols may cause the changes in membrane permeability and functions of respective proteins, that will affect cell vital activity in microgravity.

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Keywords: sterols, rafts, pisum sativum, plasmalemma, clinorotation

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“Rhizogenesis in vitro” from leaf explants as a model for studying root cell differentiation under real and simulated microgravity

Iliya Bulavin

Department of Cell Biology and Anatomy, Institute of Botany (NAN Ukraine), Kiev, Ukraine
iliyavbulavin@ukr.net

A new model of “Rhizogenesis in vitro” from A. thaliana leaf explant petioles was investigated under the simulated microgravity conditions (linorotation). Some ultrastructural changes were revealed in the cells of the roots formed de novo under clinorotation. Auxin distribution as the physiological parameter showed that in vitro roots did not perceive the gravity stimulus under clinorotation, but they reacted to gravity after clinorotation. Based on the experimental data, we consider that in vitro roots are gravisensitive and propose the model of “Rhizogenesis in vitro” for using in spaceflight experiments.

INTRODUCTION

Numerous spaceflight biological experiments have shown the essential changes in cell behavior of multicellular and unicellular organisms in comparison with that on Earth (Halstead and Dutcher, 1987; Kordyum, 2014; Kittang et al., 2015). In our investigations, we used the model “Rhizogenesis in vitro” to study cell differentiation in the root cap and growth zones under clinorotation. An advantage of this model is the possibility to study the influence of clinorotation at the beginning of root initiation de novo and next morphogenetic processes unlike experiments in vivo with embryonal roots formed in seeds in the stationary conditions.

MATERIAL AND METHODS

Arabidopsis thaliana wild type (Columbia), GFP-MAP4 and DR5rev::GFP transgenic plants were selected. Rhizogenesis in vitro from leaf explants of the plants growing in the sterile condition in vivo was reached by using the modified Murashige-Skoog medium contained 1/10 of MS mineral salts, without vitamins and hormones. The structure of roots formed in vitro was investigated by using the methods of light microscopy (Axioscope, Carl Zeiss, Germany), electron transmission microscopy (JEM 1230, Jeol, Japan) and confocal microscopy (LSM 5 Pascal, Carl Zeiss, Germany).

RESULTS AND DISCUSSION

Anatomical investigations of A. thaliana wild type roots formed de novo in the stationary conditions showed that they consisted of a root cap with differentiated statocytes and growth zones (meristem, distal elongation zone (DEZ), central elongation zone (CEZ), and mature zone). Under clinorotation, roots formed in vitro preserved their anatomical structure. A cell number in the meristem and DEZ, as well as a length and a cell size of these growth zones did not also differ from control samples.

Electron-microscopic investigation showed that graviperceptive cells - statocytes were fully differentiated and preserved their polarity in the stationary conditions. Under clinorotation, amyloplasts revealed mainly a tendency to group in the cell center, rarely – in the cytoplasm whole volume. Under clinorotation, the ultrastructural organization of cells in protodermis and epidermis of DEZ was in common similar to control. At the same time, a size of mitochondria changed in protodermis cells. In the epidermis of DEZ, changes were observed in a size of mitochondria, vacuoles and ER-bodies.

For vital visualization of the tubulin cytoskeleton orientation in roots formed in vitro, we firstly obtained and used roots from leaf explants of A.thaliana transgenic plants GFP-MAP4. Cortical microtubules were found in root cells in control and under clinorotation. In the meristem and DEZ cells in control, cortical microtubules oriented more or less perpendicularly and shifted to oblique orientation in the CEZ. Under clinorotation, cortical microtubules preserved their orientation in the meristem and CEZ. In cells of the DEZ, the disorientation of microtubules was observed that may indicate gravisensitivity of the tubulin cytoskeleton in this zone. This process is probably connected with specific physiological properties of the DEZ (Ishikawa and Evans, 1995; Kalinina, 2006).

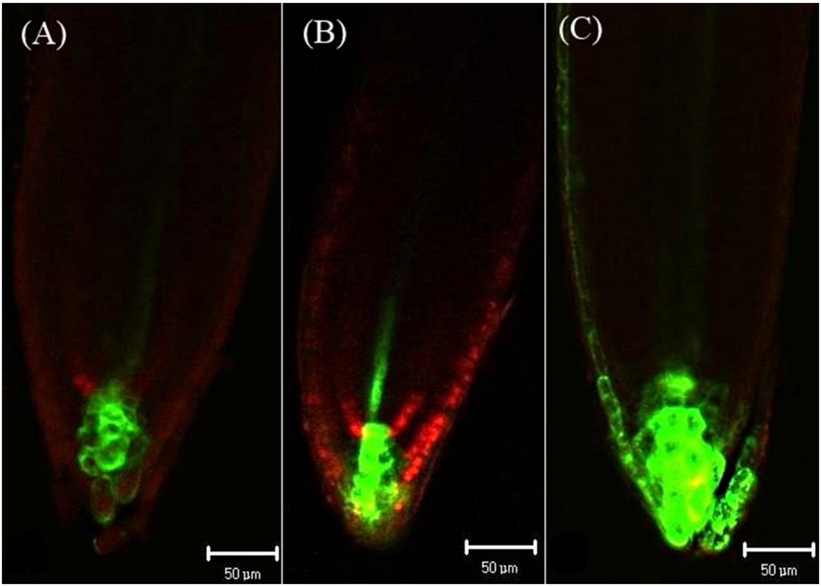

FIGURE 1: Auxin distribution in roots formed de novo in vitro: (**A**) – control, (**B**) – clinorotation, (**C**) – gravistimulation after clinorotation.

Auxin (IAA) plays an important regulatory role in the plant life cycle and takes part in a root gravitropic reaction. We firstly regenerated roots in vitro from leaf explants of A. thaliana DR5rev::GFP transgenic plants, in which DR5rev green fluorescent protein marks auxin localization, and checked auxin distribution in them. In roots growing vertically (Figure 1A), auxin-dependent reporter DR5rev green fluorescent protein was revealed in the root central cylinder and in the cap columella. Under clinorotation (Figure 1B), DR5rev::GFP signal was only noted in cap cells of roots which had not a visible bending. Under gravistimulation of clinorotated roots (Figure 1C), the DR5rev::GFP localization was revealed in the columella and epidermis. Thus, our data demonstrate that roots formed de novo in vitro under clinorotation preserve their sensitivity to gravity.

On the whole, the results of performed experiments clearly showed the similarity of the anatomical structure and differentiation of cells, specialized and not specialized to gravity perception, in roots of A. thaliana wild type formed in vitro to those of embryonal roots. Structural and functional reactions of roots formed de nono demonstrated their gravisensitivity. Thus, a model of rhizogenesis from leaf explants in vitro is proposed for using in plant space biology, especially in spaceflight experiments.

Keywords: structure, leaf explants, clinorotation, A.thaliana, rhizogenesis in vitro

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