PROGRAM & ABSTRACT BOOK

40th Annual Meeting of the International Society for Gravitational Physiology (ISGP) and Space Life Science and Medicine Meeting

May 26-31, 2019, Nagoya, Japan, at Noyori Conference Hall, Nagoya University

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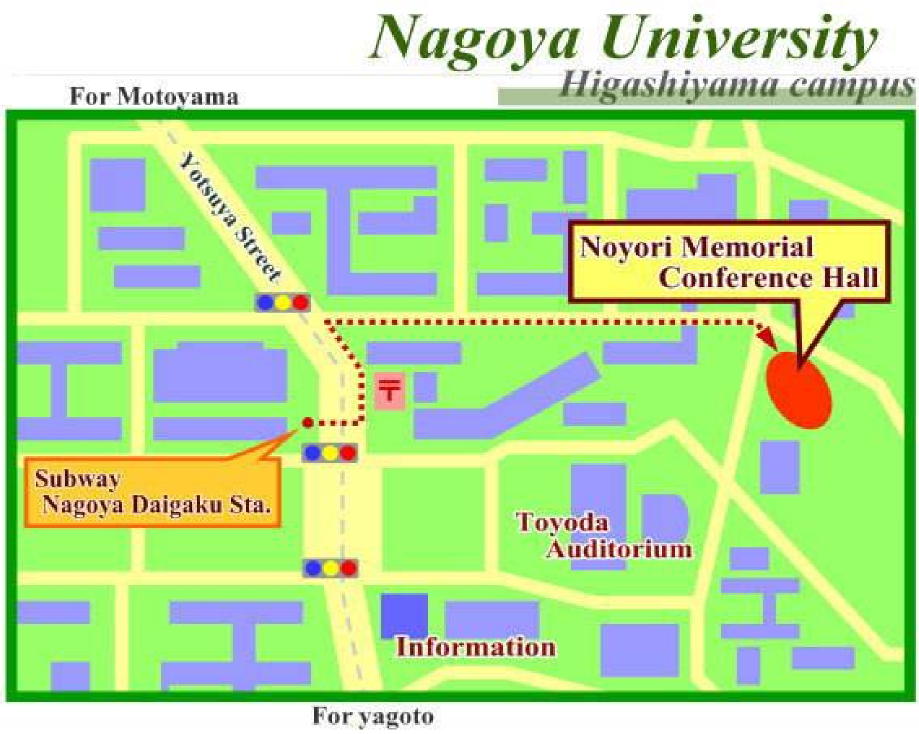
Access guidance to the conference venue from Nagoya Daigaku Station (Nagoya University): You can reach Noyori Memorial Conference Hall by 5 - 8 minutes walk from Nagoya Daigaku Station Exit2.



Committees

Local Organizer

Convener:
Satoshi Iwase, Aichi Medical University
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Keisho Katayama, Nagoya University
Naoki Nishimura, Nihon Fukushi University
Secretariat:
Maki Sato, Aichi Medical University

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Hironobu Morita
Masao Yamasaki
Yutaka Hirata
Katsumasa Goto
Sachiko Yano
Jun Hidema
Akihisa Takahashi
Masahiro Terada
Hiroshi Akima
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Alexandre Chouker (Germany)
Charles Fuller (USA)
Peter Norsk (USA)
Jeffrey Alberts (USA)
Alan Hagens (USA)
Michael Delp (USA)
Olga Vinogradova (Russia)
Inessa Kozlovskaya (Russia)
Yoshinobu Ohira (Japan)
Tadaaki Mano (Japan)
Dag Linnarson (Sweden)
Marc-Antoine Custaud (France)
Alain Maillet (France)
Richard Hughson (Canada)

The Symposium is jointly organized by

International Society of Gravitational Physiology

And

Space Life Science and Medicine Program of "Leadership Development Program for Space Exploration and Research, Nagoya University"
Co-sponsored by Research Center for Physical Fitness and Sports, Nagoya University

Under the Auspices of
The physiological Society of Japan
Japanese Society for Biological Sciences in Space
Japanese society of Aerospace and Environmental Medicine

2019 ISGP Annual Gravitational Physiology Meeting Proceedings

Frontiers in Physiology section
Environmental, Aviation and Space Physiology

The Proceeding submissions will be online with a text limitation of 1500 words maximum with up to 2 figures. While these submissions will provide an open access documentation of our meeting contents, all authors are encouraged to submit a summary of their work, taking care not to prejudice the full publication of their studies at a later time. The authors also need to obtain any necessary permission for republishing any previously published data or figures. All presenters are strongly encouraged to send their Proceedings. For submission of the proceedings please proceed to https://www.frontiersin.org/events/40th_ISGP_annual_meetingThe_International_Society_for_Gravitational_Physiology/6802

The submission deadline will be the end of August 2019.

The final online submission link will be identified on our meeting website, the ISGP website, as well as provided after the meeting to all registered participants.

As the ISGP moves forward with this new venture, feel free to communicate with us any questions, comments to suggestions. At this time, any such communications can be sent to MACustaud@chu-angers.fr.

We look forward to your contribution.

Practical Information

Venue of the Conference: Noyori Conference Hall, Nagoya University
At Nagoya University Higashiyama Campus
Five to 8 min walk from Subway Station: Nagoya Daigaku (Nagoya University) on Meijo Line
The map is shown on pages 9-10.

Morning Symposia Agenda (9:00 to 12:30)

Monday:	Current Concepts
Tuesday:	Identifying & Reducing Risks to Brain and Behavior during Extended Human Spaceflight
Wednesday:	Insights into the Immune Challenges facing the Space Exposome – from cells to man
Thursday:	Bone: Are we there yet?

Coffee, Tea, or Beverages
Will be served during the dedicated breaks in the Reception Hall.

Lunches
Lunches are not included in the registration fees. Lunch can be taken in the Restaurants or Buffets in Nagoya University Campus. Also, we will sell lunch box in reception desk on application (approx. 1,000 JPY).

Oral Sessions
The oral sessions will take place in the afternoon from 13:30 on Monday through Thursday. The time for presentation will be 15 min including Q & A. The chairpersons will take a role to manage the presentations.

Poster Sessions
The poster sessions will take place Tuesday and Thursday from 15:30-16:30 in the Reception Hall, along with the coffee break.
The size of the poster is to be within 900 wide× 1800 height mm.

WiFi
WiFi is available free of charge. WiFi names and passwords will be provided at the reception desk.

Social Events

Sunday May 26 – Welcome reception and Pre-registration

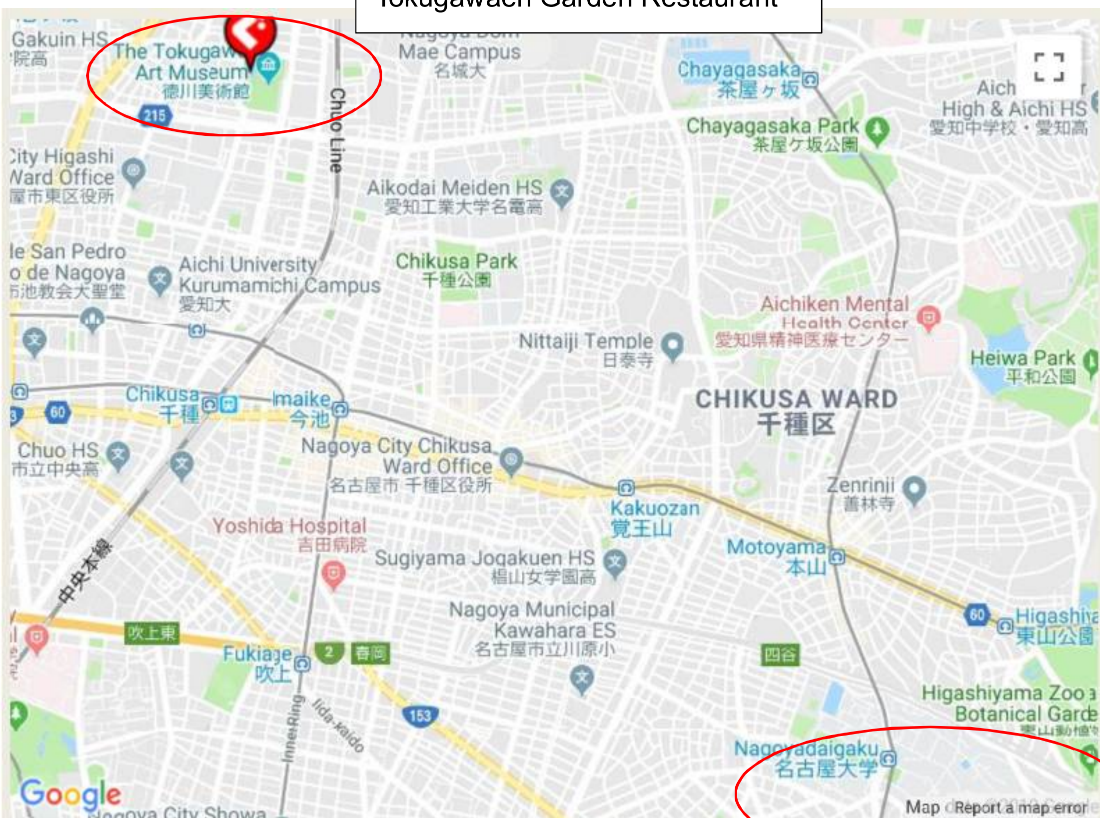
The Welcome Reception will take place at HUB, Nagoya Sakae-Nishiki Street.
Address: Ark Bldg 1F, Nishiki 3-22-7, Naka-ku, Nagoya 460-0003

After collecting your badge and conference kit, drinks and snacks are served during 19:00 – 21:00.

Wednesday May 29, Conference Dinner 19:00 – 21:00

The Conference Dinner will be organized at Tokugawaen Garden Restaurant.
Address: Tokugawa-cho 1001, Higashi-ku, Nagoya 461-0023.
The direct bus service from Toyota Auditorium Nagoya University to Tokugawa-en will start at 18:00 (We will inform on site).
https://www.aichi-now.jp/en/spots/detail/9/

Tokugawaen Garden Restaurant



Nagoya University Higashiyama Campus

Friday, May 30 – Conference tour to Gujo Hachiman

A full day tour to Gujo Hachiman will be organized. The tour will include a guided visit to the city area, visit to the Food Replica Workshop, visit to the towel making workshop, and lunch at Japanese Soba noodles. Leave: 09:00, back to Nagoya ~19:00.

The social events are included in the registration fee, however, for logistical purposes all participants will need to pre-register in order to attend the events.

Known as "Little Kyoto", the small castle town of Gujo Hachiman in Gifu Prefecture is easily accessible from Nagoya and offers an authentic Japanese small town experience with temples, a castle, old-fashioned narrow streets.

Exploring Gujo Hachiman

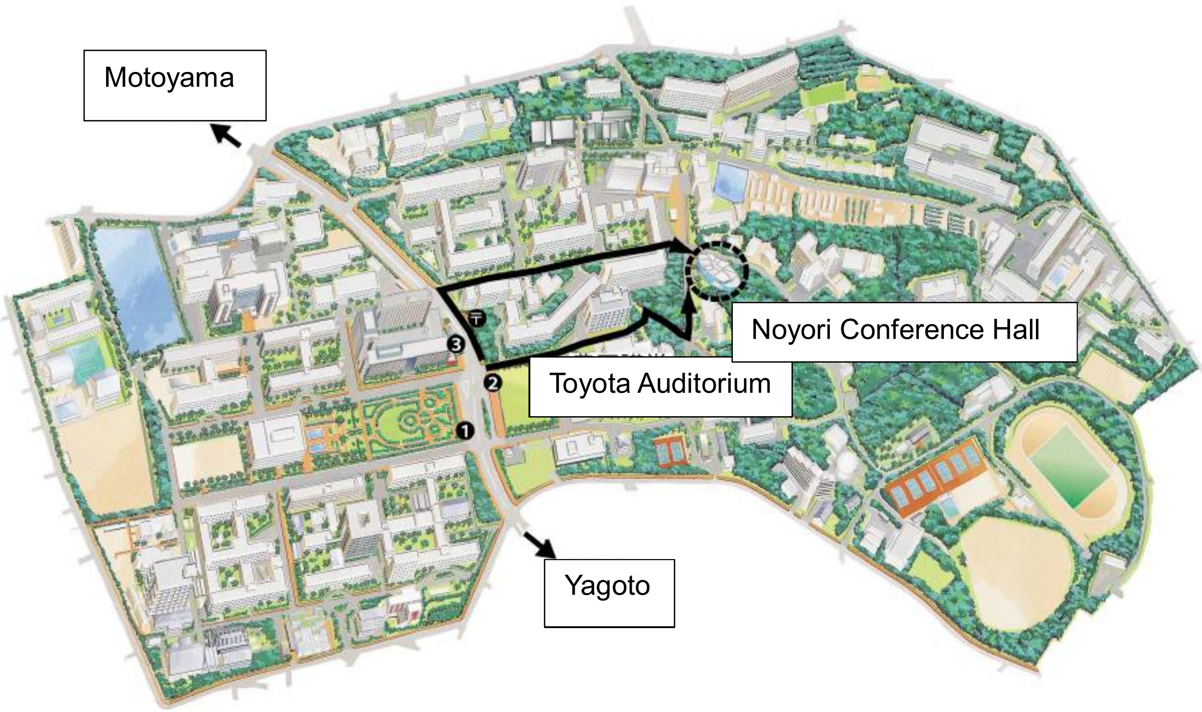
The town is best experienced on foot. The old town, temples, castle, the narrow traditional streets, and the beautiful Yoshida River are all within easy walking distance. So before you go, make sure you download a city map and the two self-guided walking tours from the official English website

wwwGujo Hachiman.com/kanko.

The Home of Replica Food

A great thing about going to a restaurant in Japan is that you know exactly what they serve and how big the portions are by looking at the very realistic-looking wax samples in the restaurant window. If you've recently been to a restaurant and chose your lunch based on the wax models there's a good chance that the models were made in Gujo Hachiman. Take a little bit of Japanese culture home with you by making your very own replica food sample.

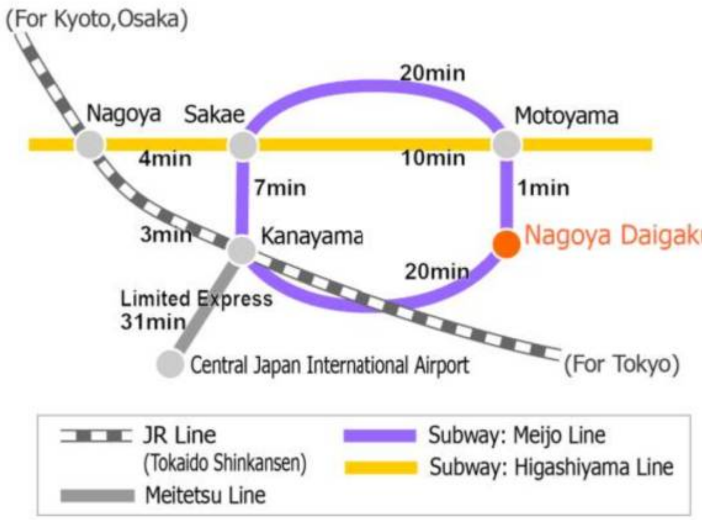
Campus Map of Nagoya University

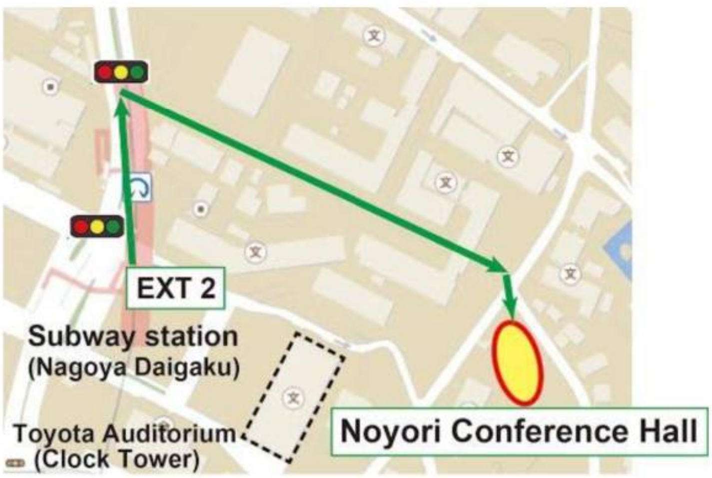


The restaurant list will be provided at the reception desk of Noyori Conference Hall.

Access Map

The conference site, Noyori Conference Hall in Higashiyama Campus of Nagoya University, can be easily accessed from the Central Japan International Airport, "Centrair." From Centrair, take the Meitetsu Line to Kanayama Station, and transfer to the Subway Meijyo Line to Nagoya Daigaku Station. Higashiyama Campus is just off the subway exit. For the campus map, refer to http://en.nagoya-u.ac.jp/map/index.html





Noyori Conference Hall in Nagoya University

Program Overview	
Sunday, May 26	
19:00 Welcome reception at HUB Sakae-Nishiki-dori	
Monday, May 27	
09:00	Welcome and Introduction
09:10	Current Concepts in Gravitational Physiology
10:30	Coffee Break
11:00	Current Concepts in Gravitational Physiology
12:30	Lunch Break
13:30	Oral 1: Cardiovascular/Respiratory
14:15	Oral 2: Environment
15:30	Coffee Break
16:00	Oral 3: Plant Physiology
16:30	Oral 4: Doshisha Session
Tuesday, May 28	
09:00	Identifying & Reducing Risks to Brain and Behavior during Extended Human Spaceflight
10:20	Coffee Break
10:50	Identifying & Reducing Risks to Brain and Behavior during Extended Human Spaceflight
12:30	Lunch Break
13:30	Oral 5: Neuroscience
14:45	Coffee Break and **Poster Session (P1-P9)**
15:45	Oral 6: Bone and Muscle
Wednesday, May 29	
09:00	Insights into the Immune Challenges facing the Space Exposome – from cells to man
10:30	Coffee Break
11:00	Insights into the Immune Challenges facing the Space Exposome – from cells to man
12:30	Lunch Break
13:30	Oral 7: Countermeasure
14:30	Oral 8: Metabolism/Psychology
15:15	Coffee Break
15:45	Oral 9: Neuroscience/Cardiovascular
16:45	Oral 10: Radiation
19:00	**Conference Dinner at Tokugawaen Garden Restaurant**
Thursday, May 30	
09:00	Bone: Are we there yet?
10:30	Coffee Break
11:00	Bone: Are we there yet?
12:30	Lunch Break
13:30	Oral 11: Molecular
14:00	Oral 12: Reproduction
14:45	Coffee Break and **Poster Session (P10-P17)**
15:45	Oral 13: Immunology & Hematology

Friday, May 31	
09:00	Full day excursion to Gujo Hachiman (19:00 arrive at hotel)

Program

Sunday, May 26, 2019

19:00 –21:00 Welcome Reception and Pre-registration

Monday, May 27, 2019

09:00 – Registration Desk Open
09:00 –09:10 Welcome – Martina Heer and Satoshi Iwase

Current Concepts in Gravitational Physiology

Chairs: *Martina Heer, Charles Fuller*

09:10 –09:45 **Historical view on researches of the autonomic nervous system in microgravity**
Mano T.:

09:45 –10:30 **Current Status of SANS Research for Future Deep Space Missions**
Norsk P.:

10:30 –11:00 Coffee Break

11:00 –11:45 **Astaxanthin - Nutrient for Spaceflight?**
Schnakenberg, J.

11:45 –12:30 **Essential role of anti-gravitational ankle plantarflexion to obtain beneficial effects from centrifuge study**
Ohira, Y.

12:30 – 13:30 Lunch Break

Afternoon oral session

Oral 1: Cardiovascular/Respiratory 13:30 – 14:15

Chair by Custaud MA, Fuller CA

13:30 – 13:45 **Endothelial Dysfunction Induced by Simulated Weightlessness**
M.A. Custaud[1,6], R. Murphy[2], I. Larina[3], C. Gharib[4], G. Gauquelin-Koch[5], N.Navasiolava[6]

1. Laboratoire Mitovasc, UMR CNRS 6015 - INSERM U1083, Angers University, France
2. Dublin City University, Ireland
3. Institute for Biomedical Problems, Moscow, Russia
4. Université Claude Bernard, Lyon, France
5. CNES, Paris, France
6. Clinical Research Center, Angers Hospital, France

13:45 – 14:00 **Body Fluid Changes, Cardiovascular Deconditioning and Metabolic Impairment induced by 5-Day Dry Immersion**
Marc-Antoine Custaud[1], Elena tomilovskaya[2], Irina larina[2] Claude Gharib[3], Guilmette Gauquelin-Koch[4], Nastassia Navasiolava[5]

1. Angers University, France
2. IBMP, Moscow, Russia
3. Université Lyon 1, France

4. CNES, Paris, France
5. Clinical Research Center, CHU Angers, France

14:00 – 14:15 **Head-down tilt as a model for intracranial pressure changes during spaceflight**

Charles A. Fuller, Tana M. Hoban-Higgins
Department of Neurobiology, Physiology & Behavior, University of California, Davis. Davis, CA, 95616 USA

Oral 2: Environment 14:15-15:30

Chair Tanaka K, Tomilovskaya ES

14:15 – 14:30 **Cooling effects of wearer-controlled vaporization for extravehicular activity**

Kunihiko Tanaka
Gifu University of Medical Science

14:30 – 14:45 **The German space life sciences program**

Christian Rogon, Markus Braun, Peter Gräf
DLR Aerospace Center, Space Administration, Department of Microgravity Research and Life Sciences, Koenigswinterer Str. 522-524, 53227 Bonn, Germany

14:45 – 15:00 **Efficacy of lower body compression garments during the first 24 hours after long-duration spaceflight**

Staurt Matthew Clark Lee[1], L. Christine Ribeiro[1], Steven S. Laurie[1], Brandon R. Macias[1], Marissa J.F. Rosenberg[1], Igor S. Kofman[1], Ajitkumar P. Mulavara[1], Jacob J. Bloomberg[2], Millard f. Reschke[2], Michael B. Stenger[2]
1. KBRwyle, Houston TX USA
2. National Aeronautics and Space Administration, Johnson Space Center, Houston, TX USA

15:00 – 15:15 **Functional capacity after long-term spaceflights experiment “FIELD TEST”**

Tomilovskaya E.S.[1], Rukavishnikov I.V[1], Kofman I.S.[2], Cherisano D.M.[4], Kitov V.V.[1], Lysova N.Yu.[1], Osetskiy N.Yu[1], Rosenberg M[2], Grishin A.P.[3], Fomina E.V.[1], Reschke M.F.[4], Kozlovskaya I.B.[1]
1. RF State Scientific Center – Institute of Biomedical Problems of the Russian Academy of Sciences, 123007, 76A Khoroshevskoe shosse, Moscow, Russia, +74991952253, info@imbp.ru.
2. KBRwyle Neurosciences Laboratory, Johnson Space Center, Houston, TX
3. GCTC by Yu.A. , Star City, Russia
4. NASA Neurosciences Laboratory, Johnson Space Center (code-SK3), Houston,TX

15:15 – 15:30 **Atmosphere selection of Lunar surface modules to minimise Decompression Sickness probability and improve crew performance during EVAs**

Davide Barbero, *Matteo Devecchi, Lorenzo Rabagliati, Guillaume Thirion*
2nd level specializing Master in SpacE Exploration and Development Systems (SEEDS) 2018/19

Coffee Break 15:30-16:00

Oral 3: Plant Physiology 16:00 – 16:30

Chair by Kitaya Y, Furuichi T

16:00 – 16:15 **Development of Space Pant Factories in Controlled Ecological Life Support Systems**

Yoshiaki Kitaya
Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8154, Japan

16:15 – 16:30 **Molecular mechanisms of plant growth and developments in ISS, a closed environment under microgravity**

Takuya Furuichi[1], *Masataka Nakano*[2], *Shiho Matsunami*[1], *Aya Kato*[1], *Erika Nakazawa*[1], *Yui Nagao*[1], *Hiroko Fujita*[1], *Hidetoshi Iida*[3] *and Hitoshi Tatsumi*[4]

1. Department of Human Life Sciences, Nagoya University of Economics, Japan,
2. Research Institute for Science & Technology, Tokyo University of Science, Japan
3. Department of Biology, Tokyo Gakugei University, Japan
4. Department of Applied Bioscience, Kanazawa Institute of Technology, Japan

Oral 4: Doshisha Session 16:30-17:55

Chair by Ohira Y, Sakurai Y.

16:30 – 16:45 **Effects of inhibition of reactive oxygen species on the properties of rat soleus muscle during hindlimb suspension**

Yoshinobu Ohira[1,2], *Yusaku Ozaki*[3], *Hisashi Kato*[1, 4], *Tetsuya Izawa*[1, 3, 4]
[1.] Research Center for Space and Medical Sciences[2]. Organization for Research Initiatives and Development, [3.] Graduate School, [4.] Faculty of Health and Sports Science, Doshisha University, Kyotanabe City, Kyoto 610-0394, Japan.

16:45 – 17:00 **Brain Freedom from Body: Enhancement of Neuronal Activity by Brain-Machine Interface (BMI) in the Rat**

Yoshio Sakurai
Graduate School of Brain Science, Doshisha University, Kyotanabe, Kyoto 610-0394, Japan

17:00 – 17:15 **Oxygen and silicon extraction from lunar regolith simulant**

Takuya Goto[1,2], *Yuta Suzuki*[1], *Yasuhiro Fukunaka*[2], *Takehiko Ishikawa*[3]

1. Department of Science of Environment and Mathematical Modeling, Research Center for Space and Medical Sciences, Doshisha University, Kyoto 610-0321, Japan,
2. Department of Interdisciplinary Space Science, ISAS, JAXA, 2-1-1 Sengen, Tsukuba, Ibaraki 305-8505, Japan

17:15 – 17:30 **Modulation of leg muscle activity during treadmill walking by varying body weight unloading**

Kiyotaka Kamibayashi[1,2,3], *Atsuhi Oshima*[2], *Keisuke Araki*[4], *Nobutaka Tsujiuchi*[3, 4], *and Yoshinobu Ohira*[3, 5]

[1]Faculty and [2]Graduate School of Health and Sports Science, [3]Research Center for Space and Medical Sciences, [4]Graduate School of Science and Engineering, and [5]Organization for Research Initiatives and Development, Doshisha University.

17:30 – 17:45 **Study of Human Gait Characteristics under Different Low-Gravity Conditions**

Léo Lamassoure[1], *Keisuke Kitano*[1], *Keisuke Araki*[1], *Akihito Ito*[1, 3], *Kiyotaka Kamibayashi*[2, 3], *Yoshinobu Ohira*[2, 3], *and Nobutaka Tsujiuchi*[1, 3]

Graduate School of [1]Engineering and [2]Health and Sports Science, and [3]Research Center for Space and Medical Sciences, Doshisha University, Kyotanabe City, Kyoto 610-0321, Japan.

17:45 – 17:50 **Effects of denervation-related inhibition of antigravity activity during growing period on the properties of hindlimb bones in rats**

Yuki Maeda[1], *Hisashi Kato*[2, 3], *Ai Sugiyama*[1], *Seita Osawa*[1], *Tetsuya Izawa*[1, 2, 3], *and Yoshinobu Ohira*[3, 4]

[1]Graduate School and [2]Faculty of Health and Sports Science, [3]Research Center for Space and Medical Sciences, and [4]Organization for Research Initiatives and Development, Doshisha University, Kyotanabe City, Kyoto 610-0394, Japan [short period presentation]

17:50 – 17:55 **Effect of 9-week exercise training regimen on expression of developmental genes in adipose-derived stem cells of rats**

Seita Osawa[1], *Hisashi Kato*[2, 3], *Yuki Maeda*[1], *Hisashi Takakura*[1, 2], *Yoshinobu Ohira*[3, 4] *and Tetsuya Izawa*[1, 2, 3]

[1]Graduate School and [2]Faculty of Health and Sports Science, [3]Research Center for Space and Medical Sciences, and [4]Organization for Research Initiatives and Development, Doshisha University, Kyotanabe City, Kyoto 610-0394, Japan. [short period presentation]

Tuesday, May 28 2019

Identifying & Reducing Risks to Brain and Behavior during Extended Human Spaceflight

Chairs: *Thomas J. Williams, Jeffrey R. Alberts*

09:00 –09:10 **Introduction and Overview**

09:10 –09:45 **NASA Human Research Integration: Potential Synergistic Risks to CNS Due to Altered Gravity and Stress from Isolation & Confinement**
Thomas J. Williams, Ajit Mulavara, Alexandra Whitmire

09:45 –10:20 **Effects and Implications of Spaceflight on Sensorimotor Performance**
Peter Norsk, Ajit Mulavara

10:20 –10:50 Coffee break

10:50 –11:25 **Structural and Functional Adaptation of the Vestibular Otoliths to Altered Gravity from Microgravity to Hypergravity**
Richard Boyle

11:25 –12:00 **Translating Behavioral Neuroscience from Animal Investigations to Human Applications for Safety and Success in Spaceflight**
Jeffrey R. Alberts

12:00 –12:30 **Integrated Discussion of Panelists' Perspectives.** *Audience Participation Encouraged*

12:30 – 13:30 Lunch Break

Afternoon session

Oral 5: Neuroscience 13:30 – 14:45

Chair by Hirata Y, Gollhofer A

13:30 – 13:45 **Tilt-Translation ambiguity problem in normal and cerebellectomized goldfish evaluated by the vestibulo-ocular reflex**
Yutaka Hirata[1,2], *Masanori Nakano*[1]
1. Dept. Computer Science, Chubu University Graduate School of Engineering
2. Dept. Robotic Science and Technology, Chubu University College of Engineering

13:45 – 14:00 **Sleep Homeostasis during Long Duration Cephalic Fluid Shifts**
Charles A. Fuller[1], *Tana M. Hoban-Higgins*[1], *and Patrick M. Fuller*[2]
Department of Neurobiology, Physiology, & Behavior, University of California, Davis. Davis, CA, 95616 USA
Department of Neurology, Beth Israel Deaconess Hospital, Harvard Medical School, Boston, MA

14:00 – 14:15 **The role of MAP-kinase p38 activation in the m.soleus slow-to-fast fiber type shift during gravitational unloading**

Kristina Andreevna Sharlo, Ekaterina Mochalova, Svetlana Belova, Tatiana nemirovskaya, Boris Shenkman
State Scientific Center of Russian Federation, Institute of Bio-medical Problems of the Russian Academy of Sciences.

14:15 – 14:30 **The effect of varying gravity levels on postural control - neuromechanics of compensatory responses during perturbation**

Albert Gollhofer[1], Ramona Ritzmann[1,2], Kathrin Freyler[1]
1. University of Freiburg,
2. Praxisklinik Rennbahn, Basel, Switzerland

14:30 – 14:45 **Locomotion task of stepping over the obstacle after long duration space flight**

Nataliya Yurevna Lysova[1], Elena Sergeevna Tomilovikaya[1], Ilya Vyacheslavovich Rukavishnikov[1], Igor Semenovich Kofman[2], Vladmir Valerevich Kitov[1], Nikolay Yurevich Osetskly[1], Marissa Rosenberg[2], Alexey Petrovich Grishin[3], Millard f. Reschke[4], Inessa Benediktovna Kozlovskaya[1], Elena Valentinovna Fomina[1, 5, 6]

1. State Scientific Center of Russian Federation, Institute of Bio-medical Problems of the Russian Academy of Sciences, Moscow, Russia
2. KBRwyle Neurosceince Laboratory, Johnson Space Center, Houston, TX
3. GCTC by Yu. A. Gagarin, Star City, Russia
4. NASA Neuroscience Laboratory, Johnson Space Center (code SK3), Houston, TX
5. Moscow Pedagogical Institute, Moscow, Russia.
6. RUDN University, Moscow, Russia

Coffee Break and Poster Session (P1-P9) 14:45 – 15:45

Oral 6: Bone and Muscle 15:45 – 17:30

Chair by Shenkman B, Stevens L.

15:45 – 16:00 **A gender comparison of the loss of muscle mass during a 10-day normoxic and hypoxic bed rest: the FemHab project**

Igor B. Mekjavic[1,2], Adam C. McDonnell[1], Tadej Debevec[1,3], Polona Jaki Mekjavic[4], Ola Eiken[5]

1. Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Ljubljana, Slovenia
2. Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6;
3. Faculty of Sport, University of Ljubljana, Ljubljana, Slovenia;
4. University Clinical Centre Ljubljana Eye Clinic, Ljubljana, Slovenia
5. Eye Clinic, University Medical Centre Ljubljana, SI-1000 Ljubljana, Slovenia
6. Department of Environmental Physiology, Swedish Aerospace Centre, Royal Institute of Technology, Stockholm, Sweden

16:00 – 16:15 **Using the video analysis of movements and analysis of EMG in the assessment of the functional state of the musculoskeletal system at gravitational unloading**

Alexey Shpakov[1], *Anton Artamonov*[1], *Alina Puchkova*[1], *Dmitry Orlov*[1], *Andrey Voronov*[2]

1. Research Institute for Space Medicine Federal Research Clinical Center of federal Biomedical Agency of Russia
2. Federal Research Center of Phyusical Education and sports

16:15 – 16:30 **Cartilage biology and morphology during long-duration space flight – first results of "CARTILAGE"**

Anna-Maria Liphardt[1, 2], *Gert-Peter Brueggemann*[1], *Frank Zaucke*[3], *Felix Eckstein*[4], *Wilhelm Block*[5], *Annegret Muendermann*[6], *Seungbum Koo*[7], *Jochen Mester*[8], *Anja Niehoff*[1, 9]

1. German Sport University Cologne, Institute of Biomechanics and Orthopaedics, Köln, Germany
2. Friedrich-Alexander University Erlangen-Nuremberg (FAU), Internal Medicine 3 – Rheumatology and Immunology, Universitätsklinkum Erlangen, Germany
3. Dr. Rolf M. Sch2iete Research Unit for Osteoarthritis, Orthopaedic University Hospital Friedrichsheim, Frnakfurt/Main, Germany
4. Paracelsus Medical University, Institute of Anatomy, Salzburg, Austria Chondrometrics GmbH, Ainring, Germany
5. German Sport University Cologne, Institute of Cardiovascular Research and Sport Medicine, Department of Molecular and Cellular Sport Medicine, Köln, Germany
6. University Hospital Basel, Department of Orthopaedics, Basel, Switzerland
7. School of Mechanical Engineering, Chung-Ang University, Seoul, Republic of Korea
8. German Sport University cologne, training Science and Sport Informatics, Köln, Germany
9. Cologne Center for Musculoskeletal Biomechanics, Medical Faculty, University of Cologne, Köln, Germany

16:30-16:45 **Metabolic signals and their sensors are involved in the control of slow myosin expression in unloaded rat soleus muscle**

Boris S. Shenkman[1], *Kristina A. Shario*[1], *Natalia A. Vilchinskaya*[1], *Inna I. Paramonova*[1], *Grigory R. Kalamkarov*[2]

1. State Scientific Center of Russian Federation, Institute of Bio-medical Problems of the Russian Academy of Sciences.
2. N.M. Emmanuel Institute of Biochemical Physics

16:45 – 17:00 **Effects of support withdrawal on the spine and trunk muscles size: Dry Immersion results**

Ilya V. Rukavishnikov, Elena S. Tomilovskaya, Inessa B. Kozlovskaya,
State Scientific Center of Russian Federation, Institute of Bio-medical

Problems of the Russian Academy of Sciences, Moscow, Russia

17:00 – 17:15 **Alteration in the biomechanical characteristics of arbitrary walking after long space flights**

Alina Alexandrovna Saveko[1], *Vladimir Valerievich Kitov*[1], *Ilya Vyacheslavovich Rukavishnikov*[1], *Nikolay Yuryevich Osetskiy*[1], *Igor S. Kovman*[2], *M. Rosenberg*[2], *Elena Sergeevna Tomilovskaya*[1], *Millard F. Reschke*[2], *Inessa Benediktovna Kozlovskaya*[1]

1. Russian Federation State Research Center Institute of Biomdical Problems, Russian Academy of Sciences
2. National Aeronautics and Space Administration

17:15 – 17:30 **Molecular markers and functional characteristics of human skeletal muscle deconditioning after short-term dry immersion.**

Stevens L, Montel V, Cochon L and Bastide B

UREPSSS, Physical Activity, Muscle and Health, Eurasport, University of Lille, France

Wednesday, May 29, 2019

Insights into the Immune Challenges facing the Space Exposome – from cells to man

Chairs: Alexander Chouker, TBD

09:00-09:30	*N.Li, M.Log:* The Immune-cell skeleton under µG -Stress – a biomechanical view
09:30-10:00	*J.P. Frippiat:* B lymphopoiesis under simulated microgravity, an example of accelerated aging?
10:00-10:30	*M. Murakami*: Neural pathways regulate immune cell-gateways in the CNS

10:30-11:00 Coffee break

11:00-11:45	*A. Choukèr*: Hypersensitivities in Space and in Space Analogues
11:45-12:30	*B.Crucian*: Interdisciplinary/Operational versus Specific Immunological Countermeasures for Deep Space Exploration

12:30 – 13:30 Lunch Break

Afternoon oral session

Oral 7: Countermeasure 13:30 – 14:15

Chair by Tomilovskaya E, Iwase S

13:30 – 13:45 **The effectiveness of plyometrics as an integrated countermeasure: evidence from bed rest, parabolic flights and artificial gravity studies**

Andreas Kramer

University of Konstanz

13:45 – 14:00 **Adaptation to changes in the gravitational field occurs more successfully at repeated spaceflight**

Elena Valentinovna Fomina[1, 2, 3], *Nataliya Yurevna Lysova*[1], *Tatyana Borisovna Kukoba*[1, 2, 3],

1. Institute of Bio-medical Problems of the Russian Academy of Sciences.
2. Moscow Pedagogical Institute, Moscow, Russia
3. RUDN University

14:00 – 14:15 **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 — as well as the deep space gateway.**

Satoshi Iwase[1], *Naoki Nishimura*[2], *Kunihiko Tanaka*[3], *Tadaaki Mano*[3], *Kazuhito Shimada*[4]

1. Department of Physiology, Aichi Medical University, Nagakute-480-1195, Aichi, Japan, 2. Nihon Fukushi University, 3. Gifu University of Medical Science, 4. Japan Aerospace Exploration Agency

Oral 8: Metabolism and Psychology 14:15 – 15:00

Chair by van Loon J, Tobita K.

14:15– 14:30 **Hypergravity and simulated microgravity affects adipocytes and fat metabolism**

Nathalie Reilly[1], Jessica Legradi[1], Mark Lammers[1], Peter Cenijn[1], Jack van Loon[2,3]

Affiliations: Vrije Universiteit Amsterdam[1], Academic Centre for Dentistry Amsterdam (ACTA)[2], European Space Agency – European Space Research and Technology Center (ESA-ESTEC)[3]

14:30 – 14:45 **Long-term space flight simulation studies provide a different view on the principles of salt and water metabolism in humans**

Jens Marc Titze

Duke-National University of Singapore

University Clinic Erlangen, Germany

Duke University, USA

14:45 – 15:00 **Individual variation of the psychological responses to hypoxic bedrest**

Kunihito Tobita[1,2], Adam C. McDonnell[1], Igor B. Mekjavic[1,3]

1. Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Ljubljana, Slovenia
2. Department of Sustainable System Sciences, Osaka Prefecture University, Osaka, Japan
3. Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Coffee Break 15:00 – 15:30

Oral 9: Neuroscience and cardiovascular 15:30-16:30

Chair by Macias B, Schneider S.

15:30 – 15:45 **Neurocognitive performance is enhanced during short periods of microgravity**

Stefan Schneider, Petra Wollseiffen, Timo Klein

German Sport University Cologne, Köln, Germany

15:45 – 16:00 **Spaceflight associated neuro-ocular syndrome: ISS vs analog**

Brandon Macias[1], Steven Laurie[1], Stuart MC Lee[1], Karina Marshall-Goebel[1], Robert Ploutz-Snyder[2], David Martin[1], Douglas Ebert[1], Alan R. Hagens[3], Scott Dulchavsky[4], Miohael B. Stenger[5]

1. KBRwyle/NASA JSC
2. University of Michigan, Ann Arbor, MI, USA
3. University of California, San Diego, CA, USA
4. Henry ford Hospital, Detroit, MI, USA
5. NASA Johnson space Center, Houston, TX, USA

16:00 – 16:15 **Jugular veins demonstrate enhanced constriction and structural remodeling following spaceflight in mice**

M.D. Delp[1], P. Ghosh[1], H. Park[1], A.E. Cullen[1], J. Goldsmith[1], J.J. Maraj[1], K. Evanson[1], A. Narayanan[1.2], X.W. Mao[3], J. Willey[4], D.C.

Zawieja[2], B.J. Behnke[5]
1. Department of Nutrition, Food, and Exercise Sciences, Florida State University, Tallahassee, FL, USA
2. Department of Medical Physiology, Texas A&M University Health Science Center, Temple, TX, USA
3. Department of Basic Sciences, Loma Linda University School of Medicine and Medical Center, Loma Linda, CA, USA
4. Department of Radiation Oncology, Wake Forest University School of Medicine, Winston-Salem, NC, USA
5. Department of Kinesiology, Kansas State University, Manhattan, KS, USA

16:15 – 16:30 **Transient cerebral blood flow responses during microgravity**

Timo Klein[1], Marit L. Sanders[2], Petra Wollseiffen[1], Heather Carnaham[3], Vera Abein[1], Christopher D. Askew[4], Jurgen A. H. R. Claassen[2], Stefan Schneider[1]
1. Institute of Movement and Neuroscience, German Sport University Cologne
2. Department of Geriatric Medicine, Radboud Alzheimer Center, Radboud University Medical Center, Donders Institute for Brain, Nijmegen, The Netherlands
3. Offshore Safety and Survival Centre, Marine Institute, Memorial University of Newfoundland, Canada
4. School of health and Sport Science, University of the Sunshine Coast, Maroochydore, Australia

Oral 10: Radiation 16:30 – 17:15

Chiar by Delp M, Takahashi A.

16:30 – 16:45 **Impact of space flight or simulated microgravity combined with space radiation exposure on retinal oxidative damage**

Xiao Wen Mao[1], Marjan Boerma[2], George Nelson[1], Dai Shiba[3], Masaki Shirakawa[3], Satoru Takahashi[4], Michael Delp[5]
1. Dept. of Basic Sciences, Division of Radiation Research, Loma Linda University School of Medicine, Loma Linda, CA, USA, 92354
2. Division of Radiation Health, Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A
3. JEM Utilization Center, Human Spaceflight Technology Directorate, JAXA, Japan
4. Department of Anatomy and Embryology, University of Tsukuba, Japan
5. Department of nutrition, Florida State University, Tallahassee, FL

16:45 – 17:00 **Space Experiments for "Cancer Progression" in the International Space Station**

Akihisa Takahashi[1*], *Masafumi Muratani*[2], *Asako Sawano*[3], *Atushi Miyawaki*[3]

1. Gunma University Heavy Ion Medical Center, Gunma 371-8511, Japan,
2. Tsukuba University, Ibaraki, Japan,
3. RIKEN, Saitama, Japan.

17:00 – 17:15 **Evident biological effects of space radiation in astronauts**

Honglu Wu[1] *and Maria Moreno-Villanueva*[2]

1. NASA Johnson Space Center, Houston, Texas, USA
2. University of Konstanz, Konstanz, Germany

Bus starts in front of Toyota Auditorium for Conference Dinner

19:00 – 21:00 Conference Dinner at Tokugawaen Garden Restaurant

Thursday, May 30, 2019

Bone: Are we there yet?

Chairs: Anna-Maria Liphardt, Kazuhito Shimada

09:00 –09:45 **Bone health during long-term space flight – do we know the risk?**
Liphardt, A.-M.

09:45 –10:30 **Influences of gravity change on the crosstalk between muscle and bone**
Kaji H.:

10:30-11:00 Coffee break

11:00 –11:45 **Effect of bisphosphonate on microgravity-induced bone loss**
Shimada K.

11:45 –12:30 **The beneficial effect of a Collagen Peptide supplementation on the maintenance and improvement of functional health parameters**
Gollhofer, A.

12:30 – 13:30 Lunch Break

Afternoon session

Oral 11: Molecular 13:30 – 14:00

Chair by Vilchinskaya N, Mochalova E

13:30 – 13:45 **The influence of the shifted balance of high-energy phosphates to AMPK dephosphorylation and expression of slow myosin at the early stage of gravitational unloading.**
Vilchinskaya N., *Paramonova I., Shenkman B.*
Cell Biophysics Laboratory, State Scientific Center of Russian Federation Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia.

13:45 – 14:00 **HDACI regulate Atrogin-1/MAFbx mRNA expression in unloaded rat soleus muscle.**
Ekaterina P. Mochalova, *Svetlana P. Belova, Boris S. Shenkman, Tatiana L. Nemirovskaya.*
Institute of Biomedical Problems, RAS, Khoroshevskoe sh. 76a, 123007, Moscow, Russia

Oral12: Reproduction 14:00 – 14:45

Chair by Shimizu T, Usik M.

14:00 – 14:15 **Morphology and motility of mice sperm after long-term modeling microgravity**
Maria A. Usik[1], *Irina V. Ogneva*[2]
1. Cell Biophysics Laboratory, State Scientific Center of the Russian Federation Institute of Biomedical Problems of the Russian Academy of Sciences, Khoroshevskoyoe shosse, 76a, Moscow, 123007, Russia
2. I. M. Sechenov First Moscow State Medical University, 8-2

Trubetskaya St., Moscow, 119991, Russia

14:15 – 14:30 **Cytoskeleton structure and according genes' expression in the testes and duct deference tissues of mice under space flight.**

Irina V. Ogneva[1, 2], *Maria A. Usik*[1], *Sergey S. Loktev*[1], *Yulia S. Zhdankina*[2], *Oleg I. Orlov*[1], *Vladimir N. Sychev*[1].

1. State Scientific Center of Russian Federation, Institute of Bio-medical Problems of the Russian Academy of Sciences.
2. I.M. Sechenov First Moscow State Medical University

14:30 – 14:45 **We Propose Again the Importance of Sexuality for Establishing a Happy and Peaceful Space Human Society.**

Tsuyoshi Shimizu[1,4], *Humihiko Yoshikawa*[2], *Kaori Kamijo*[3], *Yahiro Netsu*[3]

1. Shimizu Institute of Space Physiology, Suwa Maternity Clinic, Japan
2. Suwa Reproduction Center, Suwa Maternity Clinic, Japan
3. Suwa Maternity Clinic, Hospital for Obstetrics, Gynecology and Pediatrics, Japan
4. Fukushima Medical University (Professor Emeritus)

Coffee Break and Poster Session (P10-P17) 14:45 – 15:45

Oral 13: Immunology and Hematology 15:45 – 17:00

Chair by Frippiat J-P, Kim YH

15:45 – 16:00 **Analysis of femurs from mice embarked on board BION-M1 biosatellite reveals a decrease in immune cell development, including B cells, after one week of recovery on Earth**

Georg Tascher[1], *Maude Gerbaix*[2], *Stéphanie Ghislin*[3], *Evgenia Antropova*[4], *Galina Vassilieva*[4], *Laurence Vico*[2], *Jean-Pol Frippiat*[3] *and Fabrice Bertile*[1]

1. Université de Strasbourg, CNRS, IPHC UMR 7178, F-670000 Strasbourg, France
2. INSERM, U1059 Sainbiose, Université de Lyon-Université Jean Monnet, Faculté de Médecine, Campus Santé Innovation, Saint-Étienne, France.
3. EA 7300, Stress Immunity Pathogens Laboratory, Faculty of Medicine, Lorraine University, Vandoeuvre-lès-Nancy, France
4. Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia

16:00 – 16:15 **Socio-environmental stressors encountered during spaceflight partially affect the murine TCR repertoire and increase its self-reactivity**

Coralie Fonte[1], Sandra Kaminski[1], Anne Vanet[2,3], Stéphanie Ghislin[1] and Jean-Pol Frippiat[1].

1. EA 7300, Stress Immunity Pathogens Laboratory, Faculty of Medicine, Université de Lorraine, F-54500 Vandœuvre-lès-Nancy, France.
2. Paris Diderot University, University Sorbonne Paris Cité, F-75013 Paris, France.
3. Epôle de Génoinformatique, Institut Jacques Monod, UMR7592, CNRS, F-75013 Paris, France.

16:15 – 16:30 **Murine bone marrow progenitor cells from proximal and distal hindlimb bones after antiorthostatic suspension.**

Elena Markina, Polina Bobyleva, Olga Alekseeva, Irina Andrianova, Elena Andreeva, Ludmila Buravkova

State Scientific Center of Russian Federation, Institute of Bio-Medical Problems of Russian Academy of Science

16:30 – 16:45 **Anti-allergic effects of hypergravity are associated with restoration of Th1/Th2 balance and decrease in innate lymphoid type 2 cells.**

Young Hyo Kim[1, 2], Hyelim Park[1, 2], Ah-Yeoun Jung[1,2], Kyu-sung Kim[1, 2]

1. Department of Otorihnolaryngology, Head and Neck Surgery, Inha University, College of Medicine
2. Inha Institute of Aerospace medicine, Inha University College of Medicine

16:45 – 17:00 **Immune-cell responses under µG-Stress–a biomechanical view**

Mian Long[1,2], Ning Li[1,2], Chengzhi Wang[1,2], Shujin Sun, and Yuxin Gao

1. 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.
2. School of Engineering Science, University of Chinese Academy of Sciences, Beijing, China.

Friday May 31, 2019

Conference Tour to Gujo Hachiman

Poster Session

Tuesday Session

P1. Neuronal responses to the modulated gravity in vestibular nucleus

Gyutae Kim[1], Nguyen Nguyen[1,2], Kyu-Sung Kim[1,2]

1. Research Institute for Aerospace Medicine, Inha University, Incheon, Korea
2. Department of Otolaryngology Head & Neck Surg., Inha University Hospital, Incheon, Korea

P2. Comparison of the serotonin receptor expression in vestibular nuclei between short-term and long-term hypergravity stimulation

Hyun Ji Kim[1,2], Eun Hae Jeon[2], Yi Seul Kim[2], Kyu-Sung Kim[1,2]

1. Department of Otorhinolaryngology, Inha University, College of medicine, Incheon, Korea
2. Inha Institute of Aerospace Medicine, Incheon, Korea

P3. Tissue adhesive hydrogel glue for the bleeding control in space

Moonkang Heo[1,2], Daeyu Kim[2], Su-Geun Yang[1,2]

1. College of Medicine, Inha University
2. Inha Institute of Aerospace Medicine, Inha University

P4. Effects of centrifugation-induced hypergravity on the hypothalamic feeding-related neuropeptides gene expressions in mice via vestibular inputs.

Yoichi Ueta[1], Satomi Sonoda[1], Mitsuhiro Yoshimura[1], Takashi Maruyama[1], Chikara Abe[2], Hironobu Morita[2]

1. Department of Physiology I, University of Environmental and Occupational Health
2. Department of Physiology, Gifu University School of Medicine

P5. The effect of beta-GPA treatment on AMPK/mTORC1 signaling in rat soleus muscle at the onset of simulated microgravity

Vilchinskaya N.A., Mirzoev T.M., Paramonova I.I., Shenkman B.S.

Myology Lab, Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russian Federation

P6. Changes in the mice bone tissue elements content under hypergravitation

N.A. Lukicheva[1], O.E. Kabitskaya[1], G.Yu. Vassilieva[1], P.A. Khatyushin[2], L. Vico[3]

1. Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow;
2. Scientific and Production Association
3. IFRESIS, Saint-Étienne, France

P7. Effects of denervation-related inhibition of antigravity activity during growing period on the properties of hindlimb bones in rats

Yuki Maeda[1], Hisashi Kato[2, 3], Ai Sugiyama[1], Seita Osawa[1], Tetsuya Izawa[1, 2, 3], and Yoshinobu Ohira[3, 4]

[1] Graduate School and [2]Faculty of Health and Sports Science, [3]Research Center for Space and Medical Sciences, and [4]Organization for Research Initiatives and Development, Doshisha University, Kyotanabe City, Kyoto 610-0394, Japan

P8. Effect of 9-week exercise training regimen on expression of developmental genes in adipose-derived stem cells of rats

Seita Osawa[1], Hisashi Kato[2, 3], Yuki Maeda[1], Hisashi Takakura[1, 2], Yoshinobu Ohira[3, 4] and Tetsuya Izawa[1, 2, 3]

[1]Graduate School and [2]Faculty of Health and Sports Science, [3]Research Center for Space and Medical Sciences, and [4]Organization for Research Initiatives and Development, Doshisha University, Kyotanabe City, Kyoto 610-0394, Japan.

P9. Effects of low frequency electromyostimulation on characteristics of reflex excitability of calf extensor muscles

Tatiana A. Shigueva, Elena S. Tmilovskaya, Inessa B. Kozlovskaya

State Scientific Center, Russian Federation – Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia

Thursday Session

P10. The influence of a sustained 10 day bed rest with hypoxia on cartilage and subchondral bone in females: the FemHab study

Adam C. McDonnell[2], Matej Drobnič[1], Ola Eiken[3], Nik Žlak[1], Igor B. Mekjavič[2,4]

1. *Department of Orthopaedic Surgery, University Medical Centre Ljubljana, Zaloška 9, SI-1000 Ljubljana, Slovenia.*
2. *Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia.*
3. *Department of Environmental Physiology, Swedish Aerospace Physiology Centre, Royal Institute of Technology, Berzelius väg 13, S-171 65 Solna, Sweden.*
4. *Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6*

P11. Effect of simulated lunar gravity on function of respiratory system in humans

Alina Puchkova, Darya Stavrovskaya

Research Institute for Space Medicine of the Federal Research Clinical Center of Federal Biomedical Agency of Russia, Moscow, Russian Federation

P12. Sphingolipds are involved in disuse muscle atrophy: effects of inhibitor of acid sphingomyelinase clomipramine

Alexey A. Yakovlev, Vladimir a. Protopopov, Maria N. Shalagina, Alexey V. Sekunov, Nikita A. Ivanov, Irina G. Bryndina

Izhevsk State Medical Academy, Ulitsa Kommunarov, 281, Izhevsk, Udmurtskaja Respublika, Russia 426034

P13. Signaling consequences of p70S6K upregulation in rat soleus muscle at the early stage of mechanical unloading

Svetiana P. Belova, Ekaterina P. Mochalova, Timur M. Mirzoev, Tatiana L. Nemirovskaya, Boris S. Shenkman

Russian Federation State Research Center Institute of Biomdical Problems, Russian Academy of Sciences

P14. Seasonal variation in blood pressure and orthostatic intolerance in Parkinson's disease

Yuki Niimi[1], *Yasuhiro Hasegawa*[2], *Satoshi Iwase*[3], *Tomoko Yamana*[1], *Takao Yagi*[1], *Kazuo Mano*[4], *Yasuo Koike*[2]

1. Department of Neurology, Tsushima City Hospital
2. College of Life and Health Science, Chubu University
3. Department of Physiology, Aichi Medical University
4. Department of Neurology, Japanese Red Cross Nagoya Daiichi Hospital

P15. Lung surfactant system in C57BI/6 mice after long-term space flight onboard BIONM1 and ISS

Andrey A. Kavunenko, Anastasia G. Volkova, Nagtalia N. Vasilieva, Irina G. Bryndina

Izhevsk State Medical Academy, Izhevsk, Udmurtskaja Respublika, Russia

P16. Body fluid distribution during artificial gravity using a segmental bioelectrical impedance analysis

Naoki Nishimura[1], *Satoshi Iwase*[2], *Yoshihisa Masuo*[2], *Kunihiko Tanaka*[3], *Tadaaki Mano*[3]

1. Faculty of Sport Sciences, Nihon Fukushi University, Okuda, Mihama, Aichi, Japan
2. Department of Physiology School of medicine, Aichi medical University, 1-1 Yazakokarimata, Nagakute, Aichi, Japan
3. Graduate School of Health and Medicine, Gifu University of Medical Sciences, 795-1 Ichihiraga, Nagamine, Gifu, Japan

P17. The influence of different HDACs on MuRF-1 and MAFbx mRNA expression in rat soleus upon 3- day hindlimb unloading.

E.P. Mochalova, S.P. Belova, B.S. Shenkman, T.L. Nemirovskaya

Institute of Biomedical Problems, RAS, Moscow, Russia

Abstracts

Endothelial Dysfunction Induced by Simulated Weightlessness

M.A. Custaud[1,6], R. Murphy[2], I. Larina[3], C. Gharib[4], G. Gauquelin-Koch[5], N. Navasiolava[6]
[1] Laboratoire Mitovasc, UMR CNRS 6015 - INSERM U1083, Angers University, France
[2] Dublin City University, Ireland
[3] Institute for Biomedical Problems, Moscow, Russia
[4] Université Claude Bernard, Lyon, France
[5] CNES, Paris, France
[6] Clinical Research Center, Angers Hospital, France

Endothelium plays a central role in vascular regulation. Endothelium dysfunction is one of the earlier mechanisms of vascular impairment. Studies about endothelium remains a challenge because the endothelium differs from each organ. Functional tools are difficult to operate (flow mediated dilation, iontophoresis) and biological testing remains very global.
However, we underlined, after simulated weightlessness, the importance of microcirculatory changes and in particular of endothelial dysfunction. Indeed, 56 days of bed rest in healthy women induce a decreased vasodilator response to acetylcholine (iontophoresis coupled with laser Doppler) associated with an increase in the number of circulating endothelial cells. Seven Days of dry immersion also alters the endothelial dependent vasodilation and induced a significant increase in circulating endothelial-derived microparticles together with a decrease in the soluble VEGF (Vascular Endothelial Growth Factor) while the E-Selectin is preserved. This endothelial damage would be linked to a resting state of the endothelium rather than to its activation.
Using those models, we identified potentially interesting biomarkers to assess endothelial function in sedentary situation such as microparticles and soluble endothelial factors. We have shown that our models consistently induced a decrease in soluble CD146, an endothelial adhesion molecule whose functions are not fully understood but could become another biomarker of endothelial functions.
Endothelial damage can thus be directly induced by simulated weightlessness. Studying models of weightlessness allows a better understanding of vascular disorders related to physical inactivity.

Body Fluid Changes, Cardiovascular Deconditioning and Metabolic Impairment induced by 5-Day Dry Immersion

Marc-Antoine Custaud[1], *Elena tomilovskaya*[2], *Irina larina*[2], *Claude Gharib*[3], *Guilmette Gauquelin-Koch*[4], *Nastassia Navasiolava*[5]
[1] Angers University, France
[2]IBMP, Moscow, Russia
[3]Université Lyon 1, France
[4]CNES, Paris, France
[5]Clinical Research Center, CHU Angers, France

Dry immersion is an effective and useful model for research in physiology and physiopathology. The focus of this study was to provide integrative insight into renal, endocrine, circulatory, autonomic and metabolic effects of dry immersion. We assessed if the principal changes were restored within 24 h of recovery, and determined which changes were mainly associated with immersion-induced orthostatic intolerance. Five-day dry immersion without countermeasures, and with *ad libitum* water intake, standardized diet and a permitted short daily rise was performed in a relatively large sample for this experiment type (14 healthy young men). Reduction of total body water derived mostly from extracellular compartment, and stabilized rapidly at the new operating point. Decrease in plasma volume was estimated at 20% - 25%. Five-day immersion was sufficient to impair metabolism with a decrease in glucose tolerance and hypercholesterolemia, but was not associated with pronounced autonomic changes. Five-day immersion induced marked cardiovascular impairment. Immediately after immersion, over half of the subjects were unable to accomplish the 20-min 70° tilt; during tilt, heart rate and total peripheral resistance were increased, and stroke volume was decreased. However, 24 hours of normal physical activity appeared sufficient to reverse orthostatic tolerance and all signs of cardiovascular impairment, and to restitute plasma volume and extracellular fluid volume. Similarly, metabolic impairment was restored. In our study, the major factor responsible for orthostatic intolerance appeared to be hypovolemia. The absence of pronounced autonomic dysfunction might be explained by relatively short duration of dry immersion and daily short-time orthostatic stimulation.

Head-down tilt as a model for intracranial pressure changes during spaceflight

Charles A. Fuller, *Tana M. Hoban-Higgins*
Department of Neurobiology, Physiology & Behavior, University of California, Davis. Davis, CA, 95616 USA

Introduction: In addition to the unweighting of the muscle and skeletal systems which normally counteract Earth's gravity, entry into the microgravity environment of an orbiting spaceflight removes the hydrostatic gradient, which produces a pronounced cephalic fluid shift. This study used the well-established rat hind-limb (HLS) suspension model to investigate the hypothesis that this fluid shift results in an increased intracranial pressure (ICP). Increased ICP could, in turn, underlie some of the ocular changes seen in astronauts during and following long-duration spaceflight. Methods: The HLS model, functionally equivalent to human head-down bedrest, was used to examine the relationship between cephalic fluid shifts, the regulation of intracranial pressure (ICP) and ophthalmic responses. In this model, a tail-suspension apparatus maintains the animal in a ~30° head down orientation. The forelimbs remain in contact with the floor and a pulley system allows the rat free access to all areas of the cage. Long Evans rats served as subjects for this project. Four groups of rats were studied to examine the effects of gender, age and hypercapnic exposure on the responses. In this presentation, we report on the results from rats 9 months of age at the start of the experiment. This group mimicked the age of the older astronauts studied in whom the prevalence of ocular changes was more prevalent. Subjects were studied for 180 days consisting of 90 days of HLS and then a 90-day post HLS recovery period. An additional population of age-matched animals were similarly studied as cage controls. All animals had *ad lib* access to food and water. A 12:12 LD cycle was present. Biotelemetry (DSI) was used to record ICP, EEG, body temperature and activity. MRI imaging of the brain and eyes was performed at baseline and every 45 days thereafter. Intraocular pressure (IOP) was also measured. Ophthalmological measures included: OCT, fundoscopy, refraction and measurement of the length of the eyeball axis. Results and Conclusions: The 90-day duration of the HLS was chosen to mimic an exploration class mission. A sustained small increase in ICP was seen during HLS. These observations agree with recent data collected from human subjects showing a small increase in ICP during spaceflight. Similarly, a small elevation of IOP was seen in the HLS subjects. MRI revealed reversible shifts in structures, including possible dilation of the optic nerve sheath and a dorsal shift in the location of the eyes. Differences in refraction, similar to those seen in astronauts, were also evident. (Supported by NASA Grant NNX13AD94G)

Cooling effects of wearer-controlled vaporization for extravehicular activity

Kunihiko Tanaka
Gifu University of Medical Science

Introduction: The extravehicular activity suit currently used by the United States in space includes a liquid cooling and ventilation garment (LCVG) that controls thermal conditions. Previously, we demonstrated that self-perspiration for evaporative cooling (SPEC) garment effectively lowers skin temperature without raising humidity in the garment. However, the cooling effect is delayed until a sufficient dose of water permeates and evaporates. In the present study, we hypothesized that wearer-controlled vaporization improves the cooling effect.

Methods: Six healthy subjects were subjected to cyclic ergometer under loads of 30, 60, 90, and 120 W for durations of 3 min each were studied. Skin temperature and humidity on the back were measured continuously. Subjects wore and tested three garments: 1) Spandex garment without any cooling device (Normal), 2) simulated LCVG (s-LCVG) or Spandex garment knitted with a vinyl tube for flowing and permeating water, and 3) a garment that allows wearer-controlled vaporization (SPEC-W).

Results: The use of s-LCVG reduced skin temperature by 1.57 ± 0.14 °C during 12 min of cooling. Wearer-controlled vaporization of SPEC-W effectively and significantly lowered skin temperature from the start to the end of cycle exercise. This decrease was significantly larger than that achieved using s-LCVG. Humidity in SPEC-W was significantly lower than that in s-LCVG.

Discussion: These preliminary study suggest that SPEC-W is effective in lowering skin temperature without raising humidity in the garment. The authors think it would be useful in improving the design of a cooling system for extravehicular activity.

The German space life sciences program

Christian Rogon, Markus Braun, Peter Gräf,
DLR, Deutsches Zentrum für Luft-und Raumfahrt, Aerospace Center, Space Administration, Department of Microgravity Research and Life Sciences, Koenigswinterer Str. 522-524, 53227 Bonn, Germany

The Microgravity Research and Life Sciences department is part of the DLR Space Administration in Germany (German Space Agency). The Agency is acting on behalf of the German Government and is responsible for the definition of the German Space Program and its implementation. The budget of the agency is provided by the Ministry of Economic Affairs and Energy (BMWi). The Space Administration is taking care of the international representation and coordination.
The DLR department of Microgravity Research & Space Life Sciences has three major tasks:

1. Research-Funding Management (scientific projects in the fields of Biology, Medicine, Physics and Material Sciences)
2. Project Management for the development of experiment hardware and provision of flight opportunities
3. International representation and coordination in ESA-Boards and international and bilateral working groups (Russia, China, CNES, NASA etc.)

Within the field of Life Sciences, DLR Space Administration focusses on topics such as exploring nature (Gravitational, Radiation and Astro-Biology), improving health (Integrative Physiology, Diagnosis and Therapy, Telemedicine) and enabling exploration (Biological Life Support Systems, Health and Performance in Astronauts).
The talk will focus on the department´s engagement in Space Life Science projects and give an overview on major German research topics and on-going facility developments for the ISS and other microgravity platforms and flight opportunities such as sounding rockets and parabolic flights. Some examples will be described in more detail. It will become obvious that ISS research is accompanied by the utilization of other flight opportunities and that all space research is embedded in and complemented by terrestrial research, e.g. by simulation, isolation and bedrest studies. The important role of international co-operations will be outlined. The status of future perspectives for the German Life Science Program will be discussed.

Efficacy of lower body compression garments during the first 24 hours after long-duration spaceflight.

<u>Staurt Matthew Clark Lee</u>[1], L. Christine Ribeiro[1], Steven S. Laurie[1], Brandon R. Macias[1], Marissa J.F. Rosenberg[1], Igor S. Kofman[1], Ajitkumar P. Mulavara[1], Jacob J. Bloomberg[2], Millard f. Reschke[2], Michael B. Stenger[2]
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INTRODUCTION

Orthostatic intolerance is a well-recognized consequence of spaceflight, particularly in the first 24 hours following long-duration (4-6 month) missions. An extensive evidence base exists regarding the use of lower body compression garments to protect against hypotension during re-entry and landing, including NASA's Anti-Gravity Suit that was worn by Space Shuttle astronauts and the Russian Kentavr compression garment that is worn by cosmonauts and astronauts on the Soyuz. The next-generation compression garment in development by NASA, the Gradient Compression Garment (GCG), has protected against signs and symptoms of orthostatic intolerance after 2 weeks of bed rest and following short-duration Space Shuttle missions (10-14 days). However, NASA has not systematically evaluated the efficacy of these garments in the hours after landing following International Space Station missions. This information is critical to the continued development of the GCG for future NASA spaceflight missions.

METHODS

Preflight testing was conducted approximately 90 and 60 days before launch for US Orbital Segment (USOS) astronauts. None of the subjects wore compression garments during preflight testing. The GCG was custom constructed in the months before flight to fit each crewmember, and proper fit was verified before launch. Astronauts wore the GCG during postflight tests conducted 3 times during the first 24 hours after landing: (1) either in the medical tent adjacent to the Soyuz landing site in Kazakhstan (~1 hr after landing) or after transportation to the Karaganda or Kustanai airports (~4 hr after landing) (R+0A); (2) at the refueling stop during return to Houston (~12 hr after landing; R+0B); and (3) upon return to NASA Johnson Space Center (~24 hr after landing; R+0C). Heart rate (HR; Holter monitor) and finger blood pressure (photoplethysmography) were measured while the subjects were prone for 2 min and during 3.5 min of standing. The HR and mean arterial pressure (MAP) responses to standing (mean standing value – mean prone value) were calculated for each test. Postflight responses to standing while wearing the GCG were compared to results from the last preflight measurement without compression garments.

RESULTS

The HR response to standing in astronauts wearing the GCG during postflight tests (R+0A: 13 ± 7; R+0B: 13 ± 9, R+0C: 7 ± 8 bpm; mean ± SD) was not different than L-60 (12 ± 11 bpm, p=0.36). Similarly, the MAP response to standing in these astronauts during postflight tests (R+0A: 4 ± 11; R+0B: 6 ± 12, R+0C: 2 ± 14 mmHg) was not different than L-60 (5 ± 12 mmHg, p=0.75).

CONCLUSIONS

Wearing the GCG prevented the tachycardia that astronauts normally experience while standing on landing day without compression garments, and MAP was well maintained during this short stand test. Results from this study and previous work by our group support the use of GCG in NASA's future spaceflight missions, with modifications to improve overall fit and comfort during re-entry, landing, and the reconditioning period following landing.

Functional capacity after long-term spaceflights experiment “FIELD TEST”

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It is evident that physical capacity of cosmonauts is significantly reduced after long-duration space flights. The most impaired systems and functions are those that rely on gravity, particularly vertical posture and gait. Because of the disturbances in activity of these systems, motion sickness induced by spaceflight can be observed. While the severity of particular symptoms varies, disturbances in spatial orientation and alterations in accuracy of voluntary movements are persistently observed after long-term space flights. All currently available data are purely descriptive and are not suitable for predicting operational impacts of such decrements upon landing on planetary surfaces or asteroids. There are no data on the recovery dynamics of functionality of various body systems; hence it is difficult to model and simulate the cosmonauts’ activity after landing and to develop countermeasure methods and prescriptions. However, the videos of cosmonauts and astronauts walking and performing other tasks shortly after return from space flight speak volumes about their level of deconditioning. A joint Russian-American team is carrying out a study which is addressed specifically to the aforementioned data gaps. The study consisted of 11 tests directed to evaluation of sensory-motor and cardiovascular systems’ state at different stages of recovery period after long term space flight. Most of them are based on rather simple natural movements which will be necessary to perform after landing on the surfaces of space objects – transition from seated and prone positions to standing, walking, stepping over obstacles, tandem walking, etc. Most of the tests are performed in the medical tent at the landing site, some other which require a big amount of special equipment are performed later in Star City and Johnson Space Center. The results of the studies with participation of 42 crew members of ISS long term missions revealed significant decline of functional capacity, orthostatic tolerance, accuracy of voluntary movements, and high level of motion sickness on the landing day. Around 20% of the participants were not able even to start the testing at the landing site; 40% started to perform the test but didn’t succeed it due to the motion sickness; 40% succeeded the full battery of tests. The recovery process continued during the next 2 weeks, however, the accuracy of complicated locomotor test (tandem walking test) was altered even on the 12th day after landing.

The study is supported by the Russian Academy of Sciences and NASA.

Atmosphere selection of Lunar surface modules to minimise Decompression Sickness probability and improve crew performance during EVAs

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As envisioned by the Global Exploration Roadmap (GER), Moon permanent settlements will be a reality in future years. A Lunar outpost supporting In-Situ Resources Utilisation (ISRU) is considered as a case study. Differently from Apollo missions, new physiological problems arise, affecting crew performance and safety. Frequent Extravehicular Activities (EVA) for operations and maintenance are required and this may compromise crew health, as Decompression Sickness (DCS) may occur. For this reason, a new strategy that optimises the pressurisation levels and atmosphere composition of the system is required to improve crew performance in EVA, reducing the risk of embolism and bends for the astronauts.
In this paper, the results of the trade-off among the solutions obtained through the optimisation of the atmosphere composition and pressure level of the manned surface modules is presented. Human physiology is considered as the main driver of the study. The probability of DCS, the crew pre-breathe time and the performance of the crew during EVA, evaluated through the work efficiency index, have been optimised, while taking into account the amount of leakage due to the inert gas selected for the atmosphere composition. Three different gases have been considered in the process, with regard to half-time and density, having an impact respectively on DCS risk and leakage rate. Design space boundaries in terms of total pressure levels and Oxygen Volume fraction in the modules have been set according to the Normoxic equivalent level and the Hypoxic level, by reference to NASA standards. suit pressure, tissue ratio and flammability have been included. Suit pressure levels are based on current and under development technologies, while a maximum value of tissue ratio is fixed to 1.4 to obtain an acceptable probability of DCS. Finally, the flammability threshold constrains the Oxygen Volume fraction in the cabin to 35%. An optimisation is then carried out to minimise DCS probability, EVA crew pre-breathe time, leakages and to maximise EVA crew performance. Moreover, a trade-off among the set of optimal values obtained has been performed to select the solution of the design that better suit the analysed case study.
Eventually, a new approach has been considered for the transfer between the LOP-G and the Moon surface, performed by the Manned Transfer Vehicle (MTV). The aim is to study a MTV with a variable pressure system, capable of equalising the pressure level of the cabin during the transfer, without performing pre-breathing time and reducing risk of DCS, removing the need of an airlock. The choice between the variable pressure MTV and the airlock solution has been performed with a trade-off. The result of this trade-off is presented, highlighting advantages and disadvantages of both architectures.
The SEEDS program is an international program conducted by Politecnico di Torino (Italy), ISAE-Supaero (France) and University of Leicester (UK). It aims at studying technologies and operations to sustain future human space exploration relying on Moon resources utilization and LOP-G. The program is supported by Thales Alenia Space Italy, the European Space Agency (ESA), and the Italian Space Agency (ASI).

Development of Space Pant Factories in Controlled Ecological Life Support Systems

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Introduction: Plant culture in space has recently been of greater concern as the possibility of realizing manned space flights over a long duration is increasing. The feasibility of achieving long-duration manned space missions will be highly dependent on the controlled ecological life support systems (CELSS) centered upon crop production in space farming. Plants will play important roles in food production, CO_2/O_2 conversion and water purification. Space farming will require the establishment of appropriate plant culture facilities with scheduled crop production and high yields with a rapid turnover rate. Here I will outline topics on fundamental knowledge about expected plant functions in the CELSS and on development of closed plant culture facilities such as plant factories in space including problems for growing healthy plants over a complete ontogenetic cycle in space farming. Possible effects of low gravity conditions on plant growth and reproduction were summarized in a view of heat and gas exchanges between plants and atmosphere. The challenges regarding environmental control under low gravity conditions in space are also considered. It is concluded that proper control of air movement would be essential to enhance the heat/gas exchanges between plants and the ambient air and thus to promote growth of healthy plants especially under microgravity conditions in space.

Molecular mechanisms of plant growth and development in the ISS, a closed environment under microgravity

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Introduction: Our life is sustained by nutrients, and sustainable plant growth is a key component to accomplish long-termed manned mission in space. To the Mars, it will take more than 300 days to reach Mars, and each astronaut consume approximately 1.5 kg of foods per day. Thus, our demands on “Space Agriculture” are increasing to accomplish our next missions in the space, and establishment of effective material-use loops to promote sustainable development on the earth. Plant science studies and trials in space ships and the international space station (ISS) revealed that environments in these closed space, micro-gravity, cosmic rays and extremely high CO_2 around 3000 to 7000 ppm elevated by the crew’s exhalation, obviously affect plant growth and development. In the ISS, spindly growth and less fertility, leading lesser productivity were often observed in many kinds of the plant species tested. Thus, impacts of “space stresses against plants” should be precisely studied to clarify the underlying molecular mechanisms, and practical application of overcoming techniques to incarnate sustainable food production are awaited.

Results and Conclusions: To clarify the underlying molecular mechanisms and improve plant growth and development in space, we focused on the molecular mechanisms underlying gravity sensing, and the impacts of extremely high CO_2 conditions. Monitoring and the following pharmacological and genetic analysis of the Ca^{2+}-signal induced by gravistimulation (changes in direction and amplitude of gravity) in Arabidopsis seedlings revealed that MS channels play a central role in the early stage of plant gravity sensing. Growth assays and a transcriptome analysis of Arabidopsis seedlings suggested that extremely the high CO_2 conditions in the ISS, rather than the microgravity, strongly promoted spindly growth. The spindly growth under the high CO_2 conditions is clearly attenuated by a simple modification of a hydroponic nutrient supply, with an increase in flesh weights, improving the yield. Furthermore, Ca^{2+}-signals against some kinds of biotic and abiotic stresses are modulated in high CO_2-grown seedlings. (Supported in part by JSPS KAKENHI Grant Number 15K07025, 21026009, 23120509, 25120708, 26291026, 15K18560, 24770041, 23870013, a grant from Japan Space Forum, and MEXT KAKENHI Grant Number 16H01650)

Effects of inhibition of reactive oxygen species on the properties of rat soleus muscle during hindlimb suspension

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Effects of daily *i.p.* injection of recombinant manganese superoxide dismutase {rMnSOD, 0.08 mg in 100 µl phosphate-buffered saline (PBS) per kg body weight} or PBS during 16-day hindlimb suspension (HS) on the properties of soleus were studied in adult male Wistar Hannover rats. The SOD activity in blood plasma was increased by rMnSOD treatment in cage controls. It was maintained at the control level by rMnSOD treatment, although it was decreased in HS group with PBS injection. rMnSOD treatment attenuated muscle fiber atrophy and fiber type shift from slow- to fast-twitch phenotype, induced by HS. Although succinate dehydrogenase (SDH) activity was decreased in the HS-PBS group, such decrease was attenuated by rMnSOD injection. Significant changes were not observed in apoptosis- and autophagy-related protein contents. Myonuclear number in single muscle fibers was decreased in the PBS-injected HS group, but was maintained at the control level by rMnSOD treatment. It was suggested that the enhancement of antioxidative capacity by rMnSOD treatment attenuated atrophy and fiber type shift, which were associated with maintenance of protein synthesis capacity (myonuclei) and SDH activity in soleus muscle of rats.

Brain Freedom from Body:
Enhancement of Neuronal Activity by Brain-Machine Interface (BMI) in the Rat

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The present study focuses on brain-body interaction and reports how neuronal activity is enhanced when it is directly rewarded without body movements. The core method is a high-performance brain–machine interface (BMI) system, which uses long-term stable recording of multiple neuronal activity and real-time spike-sorting with independent component analysis (ICA) and newly developed multi-electrodes. Here we present our results showing selective enhancement of firing frequency and synchrony of multiple neighboring neurons in rat motor cortex and hippocampus. Rats were trained to engage in a free-operant task in which nose-poke behaviors were rewarded in session 1, and firing rates and synchrony above preset criteria were rewarded in sessions 2 and 3, respectively. The firing rates of motor cortical and hippocampal neuron groups were found to increase rapidly in session 2 similarly to the nose-poke behavior in session 1. Placing contingency of reward on firing synchrony resulted in selective enhancement of firing synchrony of only hippocampal neurons in session 3. These results suggest that direct reinforcement of neuronal activity without body movement enhances firing rate and synchrony rapidly. We argue that this type of research using BMI can elucidate interactions of brain activity and bodily muscle movements.

Oxygen and silicon extraction from lunar regolith simulant

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A manned lunar station is planned to be used as the Mars exploration rocket launch base. Oxygen is used as a manned space activity, and combustion assistant for the rocket engine as well. Silicon is used as a photovoltaic cell generating electricity, indispensable for the manned base. Both oxygen and silicon must be produced from resource of the Moon.
Production methods of oxygen and silicon from lunar regolith were thus proposed by our group and conducted by using an electrochemical cell as a first attempt. Chloride mixture was selected as an electrolyte, because the existence of chloride salt in the Moon would be expected to be as rock salt or hydrated salt. An electrochemical cell with three electrodes system was used for the evolution of oxygen gas and the extraction of silicon. Dissolution of lunar regolith simulant in the electrolyte was confirmed by the data of electrochemical measurements. Based on the electrochemical results, potentiostatic electrolysis was conducted to obtain oxygen gas and silicon in the cell. From the x-ray data of the samples, pure silicon film was electrochemically deposited on the metal substrate. Gas evolution was observed from the anode electrode and the gas was determined to be pure oxygen gas by the data of the gas analysis.
Considering construction of the Moon base as well as the Mars base in far future, in situ resource utilization must be established. The present data showed the feasibility for building the life support system of the manned base.

Modulation of leg muscle activity during treadmill walking by varying body weight unloading

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Lower body positive pressure treadmills have been recently developed to reduce body weight loading during walking. Since somatosensory inputs from load receptors are considered to play an important role in muscle activities for walking, changes in lower limb muscle activity while walking with different levels of body weight unloading were investigated in this study. Subjects walked at a speed of 3.5 km/h on a lower body positive pressure treadmill under five conditions of body weight unloading (100%, 80%, 60%, 40%, and 20% of their body weights). The level of body weight unloading was controlled by the air pressure in the chamber of the treadmill. The walking duration in each body weight condition was 2 minutes. The body weight conditions were randomly changed. The muscle activities in the lower limb during walking were recorded by a wireless surface electromyographic (EMG) measurement system. The decreased EMG activities in the vastus medialis and soleus muscles were observed by increased body weight unloading, whereas the activation levels in the biceps femoris and tibialis anterior muscles were unchanged with all five different levels of body weight unloading. Thus, body weight unloading during walking resulted in decreased muscle activity in the extensor muscles of the knee and ankle joints.

Study of Human Gait Characteristics under Different Low-Gravity Conditions

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Future space exploration missions have already been scheduled, both for the Moon and Mars. A better understanding of the gait characteristics under low-gravity conditions is required in order to design better equipment for the astronauts and better countermeasure methods for the bone density and muscle mass losses they experience. Our laboratory had already performed low-gravity gait studies during which the ground reaction forces and joint angle values were measured through a wearable device for motion analysis. This new study had for objective to design a model allowing us to implement experimental data recorded by the wearable device on the simulation software SIMM. The subjects' ground reaction forces and gait patterns were recorded using the wearable device while they performed 5 walking trials on the AlterG anti-gravity treadmill, each with a different body weight ratio: 100%, 80%, 60%, 40%, and 20%. We were able to recreate a simplified version of the subjects' gait patterns from the experimental data. We then loaded it on SIMM to run inverse dynamics simulations while using the software's parameters to adjust the value of the gravity constant. We studied different outputs, including muscle activations, muscle forces, and joint moments. The results observed for 100% of body weight were in accord with the ones commonly observed for the walking gait on Earth. For lower gravities the subjects presented the expected evolution of the gait, with a longer swing phase and overall longer gait cycle.

Effects of denervation-related inhibition of antigravity activity during growing period on the properties of hindlimb bones in rats

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Effects of complete inhibition of antigravitational activity during growing period on the properties of hindlimb bones and joints were studied in 18 male Wistar Hannover rats. Ranges of movement in ankle and knee joints were examined in 6 rats under anesthesia with *i.p.* injection of sodium pentobarbital (5 mg/100 g body weight), as the pre-experimental controls (7 days after birth). Further, the hindlimbs were saved bilaterally after sampling of various muscles. The bones were cleaned by submerging in 5% papain solution at ~50°C, followed by water. Then, the dry weight and length, as well as the shape, of femur and tibia + fibula were analyzed. The denervation of sciatic nerve at the gluteal region was performed bilaterally in 6 rats. The remaining 6 rats served as the normal controls. Three rats were housed in a cage with 28 x 17 cm and 12 cm height and supplied water and solid diet *ad libitum* for 6 weeks. Then, the same analyses and samplings, as was explained above, were performed. As the results, growth-associated increase of the femur and tibia length was inhibited by ~6.7 and 7.3% by denervation, respectively. Inhibition of dry weight increase in femur and tibia + fibula was ~42 and 43%, respectively. Further, slight external bend of the shaft was noted in the proximal region of tibia. Such phenomena may be related to continuous pulling by ankle dorsi-flexors, tibialis anterior and extensor digitorum longus. Even though these muscles are also denervated, they are slightly stretched due to ankle plantar-flexion. As for the range of movement, dorsi-flexion of ankle joints was inhibited to ~60° vs. 0° noted in the pre-experimental and age-matched cage controls.

Effect of 9-week exercise training regimen on expression of developmental genes in adipose-derived stem cells of rats

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[Introduction] It is well known that physical inactivity results in the change of body composition. For example, prolonged bed-rest, which represents an extreme form of inactivity, reduces muscle mass but increases body fat accumulation: this experimental approach is often used to verify the effects of spaceflight on the human body. Vigorous physical activity, on the other hand, can increase muscle mass with reduction of fat mass. However, the impact of either physical activity or inactivity on body fat distribution remains to be unknown exactly. Body fat distribution is determined by regional differences in the accumulations of white adipose tissue, which is classified in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). SAT and VAT exhibit different intrinsic expressions of developmental genes, and some of these genes exhibit changes in expression which closely correlate with the pattern of fat distribution. Our recent study showed that *Hox* genes expressions in response to exercise training vary among different WAT types. However, the impact of habitual physical activity on the expressions of the developmental genes in adipose tissue-derived stem cell (ADSC) remains unknown. ADSC has a potential role in supplying new adipocytes to maintain adipocyte turnover under a normal metabolic state.

[Methods] Five-week-old male Wistar rats were randomly divided into two groups: a sedentary control and the exercise trained group. The trained rats were subjected to exercise on a treadmill set at a 5-degree incline 5 days per a week for 9 weeks, and the rats were sacrificed at 36 h after the last exercise session. Adipocytes and ADSC were separated from one subcutaneous (inguinal) fat depot and two intra-abdominal (epididymal and retroperitoneal) fat depots. The mRNA expression levels of developmental gene (homeobox family, Glypican-4, T-box15) and bone morphogenetic protein (BMP) family genes were determined using real-time PCR.

[Results] Habitual physical activity altered the expressions of the developmental genes tested in a fat depot-specific manner.

Tilt-Translation ambiguity problem in normal and cerebellectomized goldfish evaluated by the vestibulo-ocular reflex

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According to Einstein's equivalence principle, the otolith organ, which is a biological accelerometer in the inner ear, cannot distinguish translational motion from tilt relative to gravity. This is called the tilt-translation ambiguity problem. Humans can correctly perceive these two types of self-motion even in the dark without visual information. In theory, the tilt-translation ambiguity problem can be solved by a differential equation consisting of linear and gravitational accelerations and angular velocity (Angelaki et al., 1999). A previous study showed in monkeys that the cerebellum solves the tilt-translation discrimination problem as Purkinje cells which are cerebellar output neurons code only translational acceleration information in response to combined tilt-translation stimulations (Yakusheva, et al., 2007). However, it is still unknown how the cerebellar neuronal circuitry solves the tilt-translation ambiguity problem and selectively outputs the translation component.

The vestibulo-ocular reflex (VOR) counter-rotates the eyes in the orbit to stabilize visual images projecting on the retina when head is translated and/or tilted. In primates, lateral translation and roll tilt induce different types of VOR even when both head motion cause identical otolith responses. Namely the former induces horizontal (Yaw) VOR, while the latter induces torsional (roll) VOR. Therefore, the VOR can be a physiological indicator of tilt/translation discrimination.

The goldfish has been a popular animal to study the vestibular system, and their neuronal circuitries subserving the vestibular and oculomotor functions including those in the cerebellum have been well identified. This was a major reason for them to be used in a space experiment (IML-2, STS-65) to clarify causes of space motion sickness which may be related to the tilt-translation ambiguity problem.

Presently, we evaluated the VOR in normal and cerebellectomized (cb) goldfish during various tilt (roll)-translation (lateral) combination stimulations. We first developed a vestibular stimulator for goldfish by which tilt and translation stimulations can be applied in synchrony or independently while recording their eye movements. Especially we tuned the stimulator to enable the tilt minus translation stimulus which cancels the linear acceleration produced by translation with that produced by tilt so that the response of the otolith organs is minimized. We initially hypothesized that in normal goldfish roll tilt motion induces vertical VOR, and lateral translation motion, horizontal VOR which may stabilize visual field during each motion in light, and in cb goldfish both stimulations induce the same type of VOR. As predicted by the hypothesis, cb goldfish showed vertical VOR in response to both tilt and translation stimulation. When tilt minus translation stimulation was applied, their vertical VOR was minimal. Interestingly, normal goldfish showed quantitatively the same behavior as cb goldfish during solo tilt/translation and their combined stimulations. These results suggest that lateral eyed goldfish do not have to distinguish roll tilt and lateral translation to stabilize their visual scene by VOR, thus the cerebellum is not required for this purpose. Further, it is suggested that they utilize only otolith information to generate proper VOR.

Keywords: Cerebellum, Otolith, Vestibulo-ocular Reflex, Goldfish, Eye movement

Sleep Homeostasis during Long Duration Cephalic Fluid Shifts

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Introduction: It has been proposed that one underlying role of sleep is to drive metabolite clearance from the adult brain via the glymphatic system. Previously, we have shown that long term hind-limb suspension (HLS) in the rat leads to a reduction in slow wave sleep consolidation over the 24-hour day. This study tested the hypothesis that hypothalamic orexin levels, which promote sleep-wake consolidation, were altered during HLS. This study used the well-established rat HLS model to investigate the relationship between sleep and hypothalamic orexin neurons after HLS. Methods: In the HLS model, a tail-suspension apparatus maintains the animal in a ~30° head down orientation, producing a cephalic fluid shift mimicking that seen in the microgravity spaceflight environment. While the hindlimbs are elevated, the forelimbs maintain contact with the floor and a pulley system allows the rat free access to all areas of the cage. The HLS model is the equivalent of human head down bed rest. Male Long Evans rats, 9 months of age at the start of the experiment, served as subjects. Experimental subjects were studied for 90 days of HLS. An additional population of age-matched non-suspended animals acted as cage controls. All animals had *ad lib.* access to food and water. A 12:12 LD cycle was present. Biotelemetry (DSI) was used to record electroencephalogram (EEG). EEGs were sampled at 250 Hz, pre-filtered 0.1 to 64 Hz, and artifacts removed by detecting peaks and troughs outside the normal EEG range. EEG Delta power was obtained from 4-second FFTs binned between 0.5 and 4 Hz. Results and Conclusions: The 90-day duration of HLS was chosen to produce a chronic fluid shift. EEG frequency domain power analysis demonstrated a reduction in delta power (0.5-4 Hz) indicating a decrease in slow wave sleep. Further, there was also a reduction of delta EEG consolidation as evidenced by a reduced diurnal rhythm amplitude. The hypothalamic orexin cell count was also reduced by ~30% ($p < 0.0139$). The loss of orexin producing cells is consistent with the demonstrated reduction in sleep levels and sleep consolidation. (Supported by NASA Grant NNX13AD94G)

The role of MAP-kinase p38 activation in the m.soleus slow-to-fast fiber type shift during gravitational unloading

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During the last decades it has been discovered that muscle fiber type is determined by the relative content of slow and fast myosin heavy chain (MyHC) isoforms in the fiber. Several transcription factors, such as NFATc1, MEF-2D and TEAD1 can regulate slow MyHC expression. NFATc1 activity is regulated by dephosphorylation/phosphorylation, leading to its' translocation into the myonuclei or from the myonuclei. p38 MAPK can phosphorylate NFAT (Braz et al, 2003) and TEAD1 (Lin,2017), causing their exit from the nucleus and inactivation of NFATc1- and TEAD1- dependent transcription. It was observed that during gravitational unloading the level of p38 activity increases (Derbre et al,2012), so we suggested that pharmacological inhibition of p38 MAPK may prevent unloading-induced slow-to-fast fiber-type shift by affecting NFATc1 and/or TEAD1 nuclear content.
Male Wistar rats were randomly assigned to vivarium control (C), 3-days unloading (3HS) and
3-day unloading with daily oral supplementation of 10 mg/kg p38 MAPK inhibitor VX-745 (3HS+VX). The hindlimb unloading was performed by Morey-Holton method. After the experiment, the rats were sacrificed by tribromethanol overdose, and their soleus muscles were dissected and immediately frozen in liquid nitrogen. NFATc1, MEF-2D and TEAD1 in rat soleus nuclear extract were determined by Western-blotting. MyHC I, MCIP1.4 and Myh7 beta expression levels were determined by real-time PCR. 3 days of hindlimb unloading caused significant decrease of MyHC I and Myh7 beta mRNAs expression as well as the decrease of NFATc1-dependent MCIP1.4 mRNA expression. The levels of NFATc1, MEF-2D and TEAD1 myonuclear contents in unloaded group were slightly decreased compared to control, although these decreases were not significant. In 3HS+VX group expression of MyHC I beta did not differ from control group and Myh7 beta and MCIP1.4 mRNAs expression levels was significantly higher than in 3HS group. Nuclear contents of NFATc1 and TEAD1 were significantly higher than in 3HS group and even higher than in control group (by 16% and 67% respectively). At the same time the decrease of MEF-2D nuclear content in 3HS+VX group was even more profound than in 3HS group.
Therefore, we found that p38 inhibition prevents unloading-induced MyHC I expression decrease and leads to increases of NFATc1 and TEAD1 nuclear contents and activation of NFATc1-dependent transcription in unloaded soleus muscles. Basing on these facts we can conclude that unloading-induced activation of p38 contributes to slow-to-fast fiber-type shift after three days of hindlimb suspension, possibly by NFATc1 and TEAD1-dependent mechanisms.
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The effect of varying gravity levels on postural control - neuromechanics of compensatory responses during perturbation

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INTRODUCTION

Spontaneous changes in gravity play a significant role in interplanetary space missions. Astronauts will be exposed to surface space walks and extravehicular activities in differing hypo- or hyper-gravitational conditions. Unknown habitats with gravitational conditions to which humans are not adapted could affect the astronauts' ability to safely perform their operations and may even lead to mission-threatening situations. To preserve the astronauts' capability to execute mission-critical tasks and reduce the risk of injury in transit and on planetary surfaces, a comprehensive understanding of the neuromuscular control of postural responses after balance in hypo- or hyper-gravity conditions is essential. Therefore, this study aimed to evaluate the effect of acute gravitational variation induced by parabolic flight on postural adjustments in response to perturbations.

METHODS

Postural set was manipulated by randomly providing unilateral left, bilateral or split perturbations which require balance corrections to restore postural stability. So far, in six subjects, postural reactions were recorded after anterior and posterior surface perturbations for progressively increased gravitational conditions ranging from 0.25 to 1.75 g. Ankle and knee joint kinematics and electromyograms (EMG) of eight leg muscles were recorded prior (PRE) and during perturbation. Muscle activation onset latencies and amplitudes in the short-, medium- and long-latency responses (SLR, MLR, LLR) were determined.

RESULTS

Results demonstrate an increased muscle activity ($p<0.05$) and co-contraction in the lower extremities ($p<0.05$) prior to perturbation in hypo- and hyper-gravity. After perturbation, reduced muscle onset latencies ($p<0.05$) and increased muscle activations in the MLR and LLR ($p<0.05$), concomitant with an increased co-contraction in the SLR, were characteristic in a progressive rise in gravity. Ankle and knee joint deflections remained unaffected, whereas angular velocities increased ($p<0.05$) with increasing gravitation. Effects were more pronounced in bi- compared to unilateral or split perturbations ($p<0.05$). Neuro-mechanical adaptations to gravity were more distinct and muscle onset latencies were shorter in the perturbed compared to the non-perturbed leg.

DISCUSSION

In conclusion, timing and magnitude of postural reflexes important for stabilization of bipedal stance are shown to be gravity-dependent. The primarily linear relationship between gravity and perturbation induced EMG amplitudes or muscle onset latencies indicates that the central nervous system is able to correctly predict the level of gravity. Moreover, it accurately determines the mechanics of the antigravity muscles, functionally important to counterbalance the gravitational pull and to regain upright posture after disturbance. Importantly, unilateral perturbations evoked fast reflex responses in the synergistic muscles of the non-displaced contralateral leg suggesting a synchronized inter-limb coordination mediated by spinal circuitries.

Locomotion task of stepping over the obstacle after long duration space flight

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Background. In the future humans will have to stay and work on the surface of space objects, which may present a challenge due to the negative effects of long-duration exposure to microgravity.

Research question. The goal of our study was to gain a better insight into gravity-related changes in motor control of crewmembers after long-duration spaceflights by exposing them to the stepping over obstacle test.

Methods. Our paper presents kinematic characteristics of locomotion of twenty Russian crew members that performed space missions of 115 – 340 days in duration aboard the International Space Station. The test was performed 60-30 days before flight and during first hour after landing and day 3, 7 and 12 of recovery. To perform the stepping over obstacle test, subjects stood up from a seated position and walked straight for approximately 4 meters as quickly as possible, then turned around, walked back to the chair while stepping over an obstacle (horizontal bar) that had been placed in their path, and sat down. The test was repeated 3 times and the obstacle height increased with each subsequent trial. Inertial measurement unit sensors (IMUs) were used for recording body kinematics, which allowed us to examine changes in astronauts' gait as well as head and trunk coordination. The following parameters were measured: time period of one-foot stance, height and length of the step of the leading and trailing limb and clearance between the leading and trailing foots and the obstacle.

Results. Immediately after landing the height of foot was increased significantly for obstacle 5, 10 and 15 cm for leading limb and trailing limb compared with the preflight results for leading and trailing limbs ($p \leq 0,02$). The step length decreased significantly compared with the preflight results for the 5, 10, 15 cm obstacle during the first hour after the landing ($p \leq 0,05$). There weren't any differences compared with the pre-flight results for the height and length of the step on the day 3, 7 and 12. The clearance between the leading foot and the obstacle and the clearance between the trailing foot and the obstacle weren't different for the obstacle 5 and 10 cm before and after flight. For the obstacle 15 cm clearance for the trailing foot was significant greater than clearance for the leading foot ($p \leq 0,02$) before flight. There weren't significant difference between clearances for the trailing and leading foots during the first hour after the landing, but for the day 3, 7 and 12 after landing clearance for the trailing foot was significantly greater than clearance for the leading foot ($p \leq 0,02$). The duration of one-foot stance increased significantly for the obstacle 5, 10 and 15 cm during the first hour after the landing ($p \leq 0,05$).

Concslusion. It can be concluded that changes in multi-sensory integration following long-duration exposure to microgravity we can see in the first hour after landing.

Recovery of kinematic characteristics of locomotion almost occurs on the day 3 after the landing.

This study was supported by the Russian Academy of Sciences (63.1).

A gender comparison of the loss of muscle mass during a 10-day normoxic and hypoxic bed rest: the FemHab project

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ABSTRACT

Introduction

While the adverse effects of unloading on muscle wasting in normoxic conditions are well established, the potential additive role of systemic hypoxia remains unclear. This study was part of a programme of research initiated by the European Space Agency, investigating the effects of hypoxia on the known processes of adaptation of physiological systems to normoxic microgravity-related unloading. Specifically, we sought to determine the effects of hypoxic unloading/inactivity on body composition, and skeletal muscle function in healthy female participants.

Methods

Healthy adult females (N=12) and males (N=11) took part in one or more of three 10-day interventions: Hypoxic Ambulation (HAMB); Hypoxic Bed Rest (HBR) and Normoxic Bed Rest (NBR). The continuous normobaric hypoxic stimulus during HAMB and HBR was equivalent to an altitude of 4,000m. Prior to and immediately following each intervention, body composition, thigh and calf cross sectional area, isometric muscular strength, haematology, and resting energy expenditure were recorded.

Results

Participants lost whole body mass (HAMB: male -1.5 ± 0.9kg, female -1.9 ± 0.7kg; HBR: male-2.0 ± 1.8kg, female -2.4 ± 0.8kg and NBR: male -1.4 ± 1.3kg, female -1.4 ± 0.9kg) and % fat free mass (HAMB: male -3.9 ± 3.0%, female -3.4 ± 2.0%; HBR: male -4.0 ± 4.4%, female -4.1 ± 2.0 and NBR: male -4.0 ± 3.4%, female -2.2 ± 2.7%) as a result of each intervention with no between-condition differences or groups. Knee extension strength (HAMB: male -0.2 ± 9.1%; female 1.3 ± 4.9%, HBR: male -7.8 ± 10.3%; female -3.3 ± 10.9% and NBR: male -14.5 ± 11%; female -3.4 ± 6.9%) was significantly lower for males in NBR compared to HAMB. Additionally, the loss of MVC in knee extensors was significantly different for males and females. There were no other significant changes noted following the experimental interventions. There were no differences recorded between sexes in the change in maximal MVC for either of the elbow flexors/extensors, plantar flexors or dorsi flexors. REE did not significantly change following the experimental interventions.

Cross sectional area of the calf significantly decreased following bed rest (HAMB: 4.9 ± 2.0%, HBR: 9.9 ± 2.6%, NBR: 8.0 ± 1.6%).

Conclusions

These findings suggest that a 10-day period of profound inactivity within a simulated altitude of ~4,000 m does not exert any additionally significant functional effect on the weight bearing muscles in healthy males or females compared to those noted following 10 days of inactivity under normoxic conditions.

Acknowledgements
Supported by the European Space Agency (ESA) Programme for European Cooperating States (Planetary Habitat Simulation: The Effect of Prolonged Bedrest on Physiological Responses in Females; ESA Contract No. 4000111808/14/NL/NDe).

Using the video analysis of movements and analysis of EMG in the assessment of the functional state of the musculoskeletal system at gravitational unloading

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The paper analyzes the results obtained in studies of the state of the musculoskeletal system (MSS) in human under conditions of gravitational unloading. All studies were performed on identical hardware used with methods identical registration, analysis and statistical processing.

In the period 2007-2019 at different stages of work, the following studies were performed.

Long-term space flights. The study was conducted with the participation of cosmonauts members of the expeditions to the ISS (n=18). The purpose of the study is to assess the effectiveness of various physical training regimes of cosmonauts during SF to the ISS on the biomechanical parameters of walking. Before and after SF, cosmonauts performed a test – walking at a pace of 90 steps/minute. Analyzed values of angles and phase trajectories of leg joints, EMG-activity of TA, Sol, GM, double step length. The results showed that cosmonauts who used locomotor training in interval mode, after SF, observed smaller changes in the double step length, angles in the leg joints and a smaller increase in EMG-cost of walking compared to pre-flight level.

Head-down- and head-up bedrest. The volunteers stayed for 21 days in HDBR of -6 degrees (n=10) and a three-week sequential human exposure to the conditions of 5-day HDBR and 16-day HUBR of +9.6 degrees (n=12). Locomotor test – walking at a pace of 60 and 120 steps/minute was performed on a treadmill. The results showed that staying in HDBR was accompanied by the development of more pronounced changes in the kinematics and motor skills of walking as compared to HUBR. Significant changes in the magnitudes of the joint's angles in push-back phase were revealed, which is a consequence of an increase in the EMG-activity of the leg muscles and increase in the "physiological cost" of walking.

Locomotion in conditions of reduced weight load on the MSS. In a study involving volunteers (n=48), a comparative analysis of locomotion on a treadmill was performed: walking and running with external support of 100, 70, 38 and 17% of body weight. In addition to the kinematic and EMG characteristics, we analyzed the space-time parameters and ground reaction forces. The change in gravitational load on the MSS by the vertical hanging significantly changes the motor pattern of walking, which is reflected in the extremes and the shape of EMG-profiles of the leg muscles in double step cycle. Reducing the gravitational load to 38% and 17% of body weight leads to changes in kinematic characteristics and strategy of locomotion. Changes in the kinetic energy associated with flexion and extension in the legs joints during walking with different weight loads are revealed.

Ground-based simulations of stay under lunar gravity. The volunteers were in the conditions of HUBR for 7 days on a unique lodgment, allowing to fully simulate a human stay on the lunar surface by creating an appropriate weight load and body inclination at an angle of +9.6 degrees relative to the horizon. A test on a bicycle ergometer was used as a test exercise. Analyzed the EMG-activity of VL, RF, BF, TA, Sol and GM, as well as the spectral characteristics of the EMG. Staying under the conditions of a 7-day HUBR leads to an increase in the EMG-cost of the shin muscles during testing on a bicycle ergometer.

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Cartilage biology and morphology during long-duration space flight – first results of “CARTILAGE”

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Introduction: Musculoskeletal homeostasis of the human body is greatly affected by changes to the mechanical environment. Prolonged exposure to microgravity causes catabolic processes in muscle and bone tissue. The objective of the ESA CARTILAGE study was to investigate the effect of microgravity exposure on articular cartilage health in astronauts. Articular cartilage health relies on appropriate mechanical stimulation of the tissue and immobilization because of injury or disease can cause both thinning and softening of cartilage tissue. We hypothesized that a 4-6 months exposure to microgravity negatively affects cartilage biology and morphology.

Methods: 12 USOS (United States orbital segment) astronauts with an International Space Station (ISS) mission length of 4 – 6 months participated in the study. Magnet resonance imaging (MRI) of the knee joint was performed pre-flight (launch (L)-60 days) and post-flight (return (R)+7, R+28, R+365 days). Urine and blood samples were scheduled on the same days as the MRI with an additional data collection time point on R+1. Cartilage thickness and volume were analyzed from MRI images for defined regions of the articulating surfaces of femur, tibia and patella. Serum concentrations of biomarkers of cartilage health (COMP, C2C, CPII, CS-846) were determined using commercially available enzyme-linked immunosorbent assays (ELISA).

Results: Preliminary results from the quantitative MRI analysis indicate sub-regional changes of mean cartilage thickness at the exterior (+3.1%, $\alpha<0.05$) and posterior (+3.0%, $\alpha<0.05$) medial tibia (N=12). There are high inter-individual differences in the morphological adaptation to microgravity. Serum COMP concentration is not different from L-60 to R+1, however, values increase to R+7 (+ 20.7%, $p<0.05$) and even further to R+30 (+ 24.0%, $p<0.05$). Serum C2C, CPII and CS-846 levels show increased levels on R+1, R+7 and R+30 and serum C1,2C on R+1 and R+7, but at this stage of analysis (N=6) with no statistical significance.

Discussion: This study is the first to provide data on the effect of microgravity on articular cartilage health in astronauts. Changes in cartilage morphology and serum levels of cartilage biomarkers suggest that articular cartilage is sensitive to prolonged exposure to microgravity and that reduced mechanical loading during microgravity has the potential to initiate catabolic processes. The high inter-individual differences in the response of cartilage specific markers to microgravity suggest the investigation of risk factors in order to identify individuals at risk for developing cartilage degeneration.

Metabolic signals and their sensors are involved in the control of slow myosin expression in unloaded rat soleus muscle

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The contractile properties of fast and slow muscle fibers are determined by the predominant myosin heavy chain (MyHC) isoform expression. It is well-known that under conditions of the real or simulated microgravity the slow-to-fast shift in MyHC isoform expression is usually observed in the mammalian postural muscle [for review see Shenkman, 2016]. The significant drop in mRNA expression of slow MyHC isoform was found as early as after 24 hours of rat hindlimb unloading (HU) [Giger et al., 2009; Vilchinskaya et al, 2017]. This drop was completely prevented by activation of AMP-activated protein kinase (AMPK) accompanied with the histone deacetilase 4 myonuclear export [Vilchinskaya et al., 2017]. The similar effect was demonstrated when the ATP cleavage was stimulated by means of β-guanidin propionic acid, the competitive phosphocreatine inhibitor. Obviously, the fast ATP accumulation in disused muscle could induce the AMPK dephosphorylation, HDAC4 translocation from cytoplasm to nuclei and reduced slow MyHC mRNA expression. Interestingly, the nuclear content of HDAC4 decreased as early as after 3 days of HU and does not differ from the control levels. But its main target MEF2D, the transcription factor involved in the slow MyHC mRNA expression control, is exported from myonuclei. Simultaneously, from the first day of HU and after the first week of HU we observed the significant decrease of the nuclear content of the NFATc1, the transcription factor triggering the slow myosin mRNA expression. We found some kinds of machinery providing the NFATc1 export from the myonuclei (myonuclear GSK3β activation) or preventing its myonuclear translocation (elevated calsarcin-2 expression). It was revealed that during the first week of HU both of these mechanisms as well as myonuclear HDAC4 content were dependent on nitric oxide muscle content, which decreased in disused rat soleus muscle. Thus altered AMP/ATP ratio and NO content in the unloaded rat soleus muscle may be considered as the metabolic signals triggering the machinery of reduction of slow myosin expression at the early stages of unloading. The study was supported by Russian Science Foundation Grant #18-15-00107.

Effects of support withdrawal on the spine and trunk muscles size: Dry Immersion results

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Backpain phenomenon is regularly observed in cosmonauts during the first days of space flight (SF) and early postflight period (Wing P.C. et al., 1991). Exposure to weightlessness is also followed by height increase. Both phenomenon can be accurately reproduced under simulated weightlessness conditions - Dry Immersion (DI) and anti-orthostatic bedrest (Baum K. et al., 1999). The aim of the study was to investigate the changes of characteristics of spine and trunk muscle size under conditions of simulated microgravity.

8 healthy volunteers subjects took part in 5-days DI study. On the 3d and 5th days of DI spinal MRI scannings were performed, the transverse stiffness of back extensor muscles, height of the subjects and subjective backpain evaluation were registered.

Data analysis revealed the decrease of back extensors transverse stiffness on the 3d day of DI. At the same time according to MRI data at the level of L4-L5 the cross-sectional area of m. quadratus lumborum decreased to the level of 86,68 $\pm$ 13,32% from baseline values (F (2, 9) = 8,748; P=0,0078). The analogous changes were obtained in m. multifidus - decrease to 88,21$\pm$11,79% (F (2, 15) = 14,11; P=0,0004). On the day 5 of DI the mentioned values showed the tendency to recover and consisted 90,99$\pm$9,01% and 91,99$\pm$8,01% from the baseline values (p=0,006).

Back column length increased on average for 0.32±0.12 cm for neck, 0.49±0.26 cm for thoracic and 0.89±0.45 cm for lumbar part of the spine. At the same time the height of intervertebral discs increased 0.36$\pm$0.03 on average. Neck kyphosis was flattered for 6.6±3.29 degrees, thoracic for 6.0±2.58 degrees and lumbar lordosis was flattered for 6.6±3.29 degrees.

It can be suggested that the original cause of back pain and height increase under microgravity conditions can be the flattering of spine curvature and increase of height of intervertebral discs due to axial unloading and atonia of back extensor muscles.

The study is supported by the Russian Academy of Sciences (63.1).

Alteration in the biomechanical characteristics of arbitrary walking after long space flights

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Locomotor disturbances are a natural consequence of space flight (SF). The marked gait instability of the crew members is observed even after relatively short expeditions (from 72 hours to 16 days): at the same time, the walk remains unstudy, the legs are wide apart, the hands are spread, the body rolls from side to side in the tact of steps. Studies carried out in zero gravity and under microgravity simulations revealed a wide range of changes in characteristics of both the muscular periphery (atony, weight reduction, reduction in the power and speed characteristics of anti-gravity muscles), and the leading sensory inputs - supporting, muscular, vestibular, each of which may adversely affect the operation of the motor control systems and be a factor in the development of disturbance of postural regulation, precision movement control , locomotor coordination (A. I. Grigoriev, 2004; I. B. Kozlovskaya, 1990, 2002).

The purpose of the study was to obtain quantitative data of alterations in the biomechanical characteristics of arbitrary walking after long (mission duration 146 ± 73 days) space flights.

The work was carried out according to the program of the «Field Test» Russian-American space experiment. 5 Russian cosmonauts in the study performed the arbitrary walking test (5 meters) twice prior to the SF: 60 and 30 days before launch and post-flight once in the medical tent at the landing site approximately 1 hour after the flight, and then - on the 3-4th, 6-8th and 12th days after the SF completion.

The data obtained indicate a significant change in the walking pattern at the landing site: the single support phase is shortened (by 11,24±1,23%) due to the increase in the duration of the double support phase (by 10.32±0,76%), the gait cycle is shortened by 5.34±0,21%, so that the frequency of the steps inreased, as well as the values of ground reaction forces (GRF) diminished by 19.51±2,03%, that reflect a decrease in muscle forces on the soles from the support surface.

Thus, the results of the studies reveal in the first hours after landing alterations in the activities of the control locomotor systems and in particular, a great decline of the GRF. It can be assumed, that the later could be resulted in an increase of the density and timing of the soles with the support surface.

The study is supported by the Russian Academy of Sciences (theme 63.1) and NASA.

Molecular markers and functional characteristics of human skeletal muscle deconditioning after short-term dry immersion.

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Muscle deconditioning is a well-described phenomenon characterized by a loss of strength mainly due to a loss of muscle mass. This study aimed to identify the early functional and molecular markers of muscle deconditioning after a 5-day dry immersion (DI) period on humans. The DI model, by suppressing all support zones, accurately reproduced the effects of microgravity. Eighteen healthy volunteers completed 5 days of DI by remaining strictly immersed in a supine position, in a controlled thermo-neutral water bath. Muscle biopsies were taken off from vastus lateralis before (Pre) and after (Post) deconditioning conditions and treated either for skinned fiber measurements or muscle proteome western blot analyses. Muscle phenotype was identified using myosin heavy chain (MHC) isoform expression.
Muscle contraction was registered on more than 400 isolated skinned muscle fibers and calcium-tension relationships were established for all muscles identified by their MHC expressions.
In parallel of the muscle fiber atrophy in most of the subjects, a good correlation between the functional data and the muscle proteome changes were seen after DI.
We suggested that the short-term DI period was sufficient to activate muscular variations and that this study could contribute to the importance of characterizing early changes and biomarkers of skeletal muscle deconditioning, mainly to implement strategies to limit muscle modifications.

This study was funded by the French spatial agency "Centre National d'Etudes Spatiales" (CNES).

The effectiveness of plyometrics as an integrated countermeasure: evidence from bed rest, parabolic flights and artificial gravity studies

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Background**:** Physical inactivity and prolonged space missions both result in loss of bone and muscle mass as well as neuromuscular and cardiovascular deconditioning. An integrated countermeasure that is effective against these negative effects is important for astronauts and sedentary populations alike. Plyometrics, i.e., exercises consisting of different types of jumps, have been shown to exert very high forces on the leg muscles and bones, resulting in high strains and strain rates. Thus, plyometrics are a promising candidate for an effective integrated countermeasure. Here, we present results of several studies (bed rest, parabolic flights, lab, and artificial gravity elicited by centrifugation) that provide evidence for the effectiveness of plyometrics in preventing or at least greatly reducing the deteriorating effects of physical inactivity on the human body.

Methods**:** Results from the following studies are included: (1) The RSL bed rest study at DLR Cologne (60 days of six-degree head-down tilt bed rest, 24 young healthy male participants, half of them performing plyometrics for 5-6x per week). (2) Several parabolic flight campaigns assessing the prerequisites of plyometrics in a microgravity environment, namely the stretch reflex, which is important for the stretch-shortening cycle. (3) A study on the short-arm human centrifuge at DLR Cologne, where we assessed the feasibility of plyometrics during centrifugation with different gravity levels (14 young healthy male participants). (4) Several lab studies assessing the metabolic demand of different jump exercise sessions as well as the suitability of plyometrics with short rest intervals as a form of high-intensity interval training, both in young healthy participants as well as in an elderly population.

Results**:** The jump training used in the 60d bed rest study was successful in preventing the pronounced deconditioning effects observed in the control group (bone mineral density, muscle mass, maximal leg extensor strength, maximal aerobic capacity, resting heart rate). The parabolic flight campaigns established that the neuromuscular system and the stretch-shortening cycle are not compromised in microgravity, i.e., plyometrics are physiologically possible in a microgravity environment. The study assessing the feasibility of jumps on the centrifuge showed that in principle, jumps can be combined with artificial gravity, but that the peak forces are significantly reduced compared to jumps in a normal gravitational environment. Last, the results of the lab studies indicate that with sufficiently short rest intervals, plyometrics elicit near maximal oxygen uptake and heart rate.

Conclusion**:** Taken together, these studies show that plyometrics are an effective and efficient integrated countermeasure for bone, muscles and cardiovascular system. In addition, the physiological prerequisites for the feasibility of plyometrics in a microgravity environment are met, and in principle, jumps can be combined with centrifugation.

Adaptation to changes in the gravitational field occurs more successfully at repeated spaceflight

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A comparative assessment of the mechanisms of action and the effectiveness of means and methods of countermeasures to hypogravitational disorders for cosmonauts who performed first and repeated space flights made it possible to clarify the concept of a system for preventing the negative effects of weightlessness for exploratory flights. The hypothesis was tested that the adaptation of the organism to the conditions of weightlessness and the adaptation to the conditions of the Earth in repeated flights are easier than in the first flight. It is important that during the repeated mission the astronauts performed physical exercises similar to the first flight.

In terms of space flight, a comparative analysis of the level of physical work capacity of cosmonauts performing first and repeated flights was estimated by the physiological cost of physical activity in the locomotor test with an increasing load. The test was performed in a passive mode of motion of the treadmill belt and included five steps. The physiological cost was calculated as the ratio of the increase in heart rate in comparison with a rest to the product of the speed of the fast run and the value of the axial load. The test was carried out 60-30 days before the flight, and twice during the flight. Group A included cosmonauts at first flight (n = 15), group B — cosmonauts for whom it was repeated flight (n = 10). After space flight, the comparison of the readaptation processes to the conditions of gravity of the Earth in the groups of cosmonauts who performed the first and repeated flights was carried out based on the assessment of changes in the electromyographic cost of walking and the maximum voluntary force in comparison with the value of these indicators before the flight. The maximum amplitude of the smoothed inverted electromyogram (iEMG) of the leg muscles soleus, m. gastrocnemius medialis, m. tibialis anterior was estimated. The maximum voluntary force of the legs muscles was assessed by the results of isokinetic testing.

During space flight, the physiological cost of running at maximum speed using leg strength to the second flight test was significantly higher in the group of cosmonauts making the first flight, compared with the participants of the repeated mission. After the flight, an increase in the electromyographic cost of walking and maximal voluntary muscle strength of the flexors of the thigh is also significantly greater in the group of astronauts who performed the first flight. Thus, the hypothesis was confirmed that the repeated adaptation to a change in the gravitational field is successful. Knowledge of the more successful adaptation processes during the repeated action of the weightlessness factor is the contribution to the concept of preventing hypogravitational disorders for exploratory flights and can serve as a basis for the selection of crews for interplanetary missions.

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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 — as well as the deep space gateway.

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Spaceflight deconditioning impairs several systems, including neurovestibular, fluid/electrolyte, cardiovascular, musculoskeletal, bone metabolism, and autonomic regulation. We examined whether the artificial gravity by short arm centrifuge with exercise ameliorate these deconditioning induced by simulated microgravity. The experimental position during centrifuge was lying down position with their legs up. The diameter of the device was 2.8 m, the loaded G was 1.4G at the heart level, and the intermittent G load was 30 min per day. Fluid shift was compared using bio-impedance. Before and after the bedrest, the anti-G score was calculated as the sum of [the loaded G] x [endured time in second], and the countermeasure group exhibited significant higher score compared with the control. Muscle sympathetic nerve activity in post-experimental level was enhanced in basal levels as compared with the pre-experimental level. Cardiac output showed no significant changes between pre- and post-experimental levels of Myatrophy and bone metabolism showed no significant difference between the pre- and post-experimental level, however, post-experimental level of the both was decreased in the control. In addition, artificial gravity induced by short radius centrifuge with ergometric exercise 30 min per day was effective in improving the orthostatic tolerance. In 2017, a novel artificial gravity project was proposed. A centrifuge device with exercise is to be installed in the H-II Transfer Vehicle X (Konotori) attached to the International Space Station of the Japanese area of KOBO. This device will validate the effectiveness of artificial gravity under actual weightlessness in space. This device is to be also proposed to be installed in the deep-space gateway station, and projected to be established on the moon as the base, as well as to be installed in a spacecraft for the return voyage to the Mars.

Hypergravity and simulated microgravity affects adipocytes and fat metabolism

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Gravity is a well-known physical force that exerts a continual downward vector on the Earth. It is the one constant pressure all life on Earth has evolved to cope with. Because of this, life on Earth has developed specific physiological adaptations to survive. However, as we move away from Earth and into the vastness of space or even other planets, new pressures, or lack thereof, occur. Varying the amount of gravity an organism experiences leads to adaptations in the physiology of that organism. One particular alteration occurs to adipocytes and fat metabolism. Animals exposed to microgravity (µg) or simulated µg have displayed a tendency toward increased fat mass, whereas those exposed to hypergravity (hg) had the opposite effect or decreased adipose content.

In this study we explored the effects of simulated microgravity and hypergravity on three types of invertebrates, Daphnia magna, Nasonia vitripennis, and Folsomia candida. Each of these organisms was representative of a different type of environment found on Earth, namely aquatic, air, and terrestrial. Their fat content was measured using Nile red staining and fluorescence or by weight, before and after immersion in ether for two days. The results indicated that exposure to simulated microgravity can indeed increase the amount of fat content observed within an organism. It was also suggested that exposure to hypergravity can have the opposite effect; decreasing fat content, although this was not true for all organism's tested. The effects of hypergravity were also tested on differentiating 3T3-L1 adipocytes. The results denoted that an increase in the amount of lipid droplets occurred as measured with fluorescence of a Nile Red stain. Understanding how the composition of fat in a body changes upon exposure to these forces is key in the development of long duration space missions where understanding functioning of the human body is important, but also for developing sustainable renewable food sources using bio-regenerative life support systems. This study may also have possible applications for the future application of in-flight chronic acceleration as crew countermeasure against the microgravity environment.

Long-term space flight simulation studies provide a different view on the principles of salt and water metabolism in humans

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Space medicine and new technology such as magnetic resonance imaging of tissue sodium stores (23NaMRI) have changed our understanding of human sodium homeostasis and pathophysiology. It has become evident that body sodium comprises 3 main components. Two compartments have been traditionally recognized, namely one that is circulating and systemically active via its osmotic action, and one slowly exchangeable pool located in the bones. The third, recently described pool represents sodium stored in skin and muscle interstitium, and it is implicated in cell and biologic activities via local hypertonicity and sodium clearance mechanisms.
I will review space science-derived evidence that large amounts of Na^+ are stored in the skin in animals and humans. Humans increasingly store Na^+ in skin and muscle as they age. Skin Na^+ storage is linked to both primary and secondary hypertension, while muscle Na^+ storage is associated with insulin resistance and diabetes. These unexpected findings brought about a novel view on the principles of salt and water metabolism, opening new research avenues for understanding the pathogenesis of arterial hypertension, diabetes mellitus, autoimmune disease, and left ventricular hypertrophy.
Our observations raise new clinical questions regarding the role of salt and water metabolism as a cardiovascular risk factor in the general population, environmental conditions triggering Na^+ storage, and local mechanisms regulating fluid metabolism in skin and muscle.

Individual variation of the psychological responses to hypoxic bedrest

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Introduction The anticipated environment within future space crafts on missions to deep space and within the habitats on the Moon or Mars will likely be hypobaric and hypoxic, to enable extravehicular and extrahabitat activities without the need for lengthy and complex decompression procedures. Previous studies (PlanHab, LunHab, FemHab) comparing the effects of hypoxic and normoxic bed rest (Stavrou *et al.*, 2015; Stavrou *et al.*, 2018) have revealed a significant effect of hypoxia and inactivity on the total mood disturbance (TMD). The aim of the present retrospective study was to assess the factors that contribute to TMD.

Methods. Data from four projects were combined for this analysis, in which subjects participated in three interventions, as shown in Table 1.

Table 1: Projects from which data were used for the present analysis

Project	**Funding agency**	**Gender**	**Duration (days)**	**Number of subjects**	**Interventions**		
PlanHab	EC FP7	Male	21	14	Normoxic bedrest	Hypoxic bedrest	Hypoxic ambulation
LunHab	ESA	Male	10	16			
FemHab	ESA	Female	10	15			
HECS	ARRS	Male	10	14	Hypoxic ambulation & Exercise		

Subjects participated in each of these 3 interventions in a randomised manner with a cross over design. The hypoxic stimulus was normobaric and equivalent to ~ 4,000m ($PiO2$ ~89mmHg). The participants completed the POMS and PANAS instruments prior to and following each of the campaigns (Pre, Post) and on days 5(Mid), 10(Late) (10-day studies), 14 (Mid) and 21 (Late) (21-day study) during the interventions. The data were analysed with the Bayesian generalised linear mixed model for global effect with gender, activity level (bedrest, ambulation or exercise), duration (10 or 21 day) and condition (hypoxia or normoxia) as fixed effects and subjects as random effects.

Results Total mood disturbance (TMD) indicated a significant disturbance during the intervention with a significant increase in the individual variability (SD changed from Pre (10.8), Mid (13.3), Late (13.4) to Post (11.0). The participants displayed a decrease in the Positive Affect of PANAS from Pre to Mid and Late intervention, with mood becoming more positive in the Post phase. The variability of the responses narrowed from Pre (8.0) to Mid (7.1) and Late (7.0). The variability increased again by Post (7.6)

There was a significant gender influence on POMS TMD and PANAS positive and negative affect state. Similarly, duration also had a significant influence on the

psychological responses for both POMS and PANAS. Activity level only exhibited a strong effect on POMS TMD with no significance found for PANAS.
Conclusions. The observed large individual variabilities of mood state, were driven by the activity level (bed rest or exercise), gender and duration of exposure.

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Neurocognitive performance is enhanced during short periods of microgravity

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Previous studies have demonstrated no, or a slightly negative impact, of space flight on cognitive performance but were not able to distinguish between the primary effects of weightlessness and any secondary effects of stress and confinement. When isolating the effect of weightlessness, achieved by parabolic flights, reaction time while solving an arithmetic task was shown to be enhanced. This effect was found to be stronger with increasing task complexity and was independent of previous experience of weightlessness as well as anti-nausea medication. Analysis of event related potentials showed a decreased amplitude of the N100-P200 complex in weightlessness but was not able to distinguish a possible effect of task complexity.
The present study aimed to extend this previous work, by comparing behavioral (reaction time) and neurological (event related potentials analysis) performance to a simple (oddball) and a complex (mental arithmetic + oddball) task during weightlessness.
28 participants participated in two experiments. 11 participants performed a simple oddball experiment in the 1G and 0G phases of a parabolic flight. A further 17 participants were presented a complex arithmetic task in combination with an oddball task again during the 1G and 0G phases of a parabolic flight. Reaction time as well as event related potentials (ERP) were assessed.
Results revealed a reduced reaction time ($p < .05$) for the complex task during 0G. No gravity effects on reaction time were found for the simple task. In both experiments a reduction of typical ERP amplitudes was noticeable in weightlessness, whereas latency remained unaffected.
As task complexity plays a major role, which casts a possible effect of arousal into doubt, it is assumed that the weightlessness induced fluid shift to the brain is positively affecting neuro-behavioral performance.

Spaceflight associated neuro-ocular syndrome: ISS vs analog

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Introduction

Spaceflight-associated neuro-ocular syndrome (SANS) affects ~40% of astronauts on long-duration spaceflight missions, as determined by one or more findings: optic disc edema, hyperopic shifts, globe flattening, cotton-wool spots, or choroidal folds. Ocular Health and Fluid Shift studies demonstrate that International Space Station (ISS) astronauts develop significant peripapillary retinal and choroid tissue thickening in both eyes early in the spaceflight and this effect persists throughout the mission. Additionally, we recently demonstrated that healthy test subjects develop optic disc edema during 30 days of 6° head-down tilt (HDT) bedrest in 4 mmHg PCO_2 environment, which is an analog of spaceflight (Laurie et al 2019). We hypothesize that a chronic headward fluid shift within the venous and cerebrospinal fluid compartments leads to optic disc edema, yet the primary initiating factor may differ for bed rest and spaceflight. The purpose of this study was to determine if the time course of retinal changes are similar for bedrest subjects and astronauts.

Methods

Images from radial and circle optical coherence tomography (OCT) (Spectralis, Heidelberg Engineering) centered over the optic nerve head were acquired from test subjects before and during 30 days of strict head-down tilt (HDT) bedrest (n=11 subjects), and from astronauts before and during spaceflight (between 22-63 days on the ISS; n=20 astronauts). Retinal changes were quantified by peripapillary total retinal thickness (TRT) measured from Bruch's membrane to the internal limiting membrane on OCT images. Two independent observers verified segmentation of retinal layers for all OCT scans, and TRT was calculated over a region from Bruch's membrane opening outward 250 µm (Patel et al 2018). Additionally, a circle scan was used to quantify choroid thickness after manually segmenting Bruch's membrane and the choroid scleral border. The values from each observer varied by <10% and the average value was used for analysis. Mixed-effects regression, including subject-specific random intercepts, was performed to determine statistical significance.

RESULTS

After 30 days of strict HDT bedrest, 5 of 11 subjects were diagnosed with optic disc edema as determined by analysis of fundoscopy images, whereas only one astronaut received a similar diagnosis after about 30 days of spaceflight (Fisher's exact test $P = .02$). TRT increased by an average of 54 µm (35-73 µm: mean [95% CI]) in bedrest subjects, which was significantly greater than the average 17 µm (2-32 µm, $P = .01$) increase in TRT in astronauts during spaceflight. Peripapillary choroidal thickness increased significantly in astronauts (30 µm [19 to 40 µm]), but not in bedrest subjects (3 µm [-6 to 12 µm]).

Discussion

The higher greater incidence of optic disc edema, greater increases in TRT, and smaller changes in choroid thickness during 30 days of HDT bedrest than during a similar duration of spaceflight suggests that the underlying mechanisms contributing to these findings differs in each condition. Interestingly, neither the bedrest subjects nor astronauts developed any additional SANS signs or symptoms. Identifying factors that contribute to the differences in ocular adaptations between astronaut and bedrest subjects is critical for understanding the mechanism of SANS and will help guide the development of countermeasures. Possible differences between these groups that require more research include the role of exercise, gravitational vectors during bedrest, and the influence of intrathoracic pressure on fluid distributions.

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Jugular veins demonstrate enhanced constriction and structural remodeling following spaceflight in mice

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Approximately 60% of astronauts on long-duration missions to the International Space Station (ISS) are reported to have experienced some impairment in visual acuity. These visual disturbances have been hypothesized to result from increases in intracranial pressure. Increases in intracranial pressure associated with spaceflight are thought to occur through the cephalad shift of intravascular and cerebrospinal fluids due to the loss of the head-to-foot gravity vector present on Earth, as well as possible impairment of fluid outflow from the cranium due to venous congestion or diminished lymphatic drainage. The purpose of this study was to determine whether spaceflight alters venoconstrictor responsiveness or structure of cervical veins in a manner that may contribute to impaired outflow of blood from the head. Twenty 16-wk old male C57BL/6J mice were flown on the ISS for a 30-day mission and euthanized 33-41 hr after return to Earth. Internal jugular veins were excised from Spaceflight (SF), Habitat Control (HC) and Vivarium Control (VC) mice for *in vitro* determination of 1) venoconstrictor responses to norepinephrine (10^{-9}-10^{-4} M), 2) active pressure-diameter responses (2-12 cmH_2O) in the presence of 10^{-4} M norepinephrine, and 3) passive pressure-diameter response (2-12 cmH_2O) in Ca^{+2}-free buffer solution. Venoconstrictor responses to norepinephrine were greater in jugular veins from the SF mice relative to that in HC and VC animals. Likewise, jugular veins from the SF mice demonstrated smaller normalized diameters across a range of intravascular pressures in the presence of 10^{-4} M norepinephrine. Jugular vein maximal passive diameter at 4 cmH_2O was smaller in SF mice relative to that in the control groups. These data suggest that spaceflight enhances venoconstriction across a range of intravascular pressures and induces remodeling of neck veins. Such adaptations could serve to promote venous congestion in the head and contribute to possible elevations in intracranial pressure.

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Transient cerebral blood flow responses during microgravity

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INTRODUCITON

Numerous studies described central cardiovascular changes caused by changing gravity levels as they occur e.g. during parabolic flight. Limited data exists on the effect of microgravity on the cerebrovascular system and brain perfusion, although intracranial pressure is increased during microgravity (Lawley et al., 2017). It was hypothesised that this might be caused by an increased inflow and reduced outflow of blood towards and away from the brain; however, recent studies could not confirm an increase in cerebral blood flow during microgravity. Unfortunately, previously cerebral blood flow was only averaged over 10 second intervals and possibly missing transient changes during microgravity (Ogoh et al., 2015). This study aimed to investigate the underlying kinetic of cerebral blood flow during the 22s of microgravity during parabolic flights.

METHODS

Middle cerebral artery blood flow velocity (MCAv) of 16 participants (37±11 years) was continuously monitored on a second-by-second basis during 15 consecutive parabolas (1G, 1,8G, 0G, 1,8G). Time aligned central cardiovascular parameters (heart rate, mean arterial blood pressure, cardiac output) were simultaneously assessed.

RESULTS

Results revealed an immediate central cardiovascular reaction to the changed gravity levels. In contrast, changes in MCAv were only in accordance with a normal cerebral autoregulation during an initial phase of the gravitational changes. Whereas all of the measured central cardiovascular parameters reached steady state after approximately 8 seconds of microgravity, MCAv, after an initial reduction with the onset of microgravity, increased again during the second half of the microgravity phase.

CONCLUSION

These findings suggest that the increase in MCAv during the second half of the microgravity period reflects a decrease of cerebrovascular resistance caused by a pressure driven increased venous outflow and/or a contraction of precapillary sphincters in order to avoid hyperperfusion of the brain.

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Impact of space flight or simulated microgravity combined with space radiation exposure on retinal oxidative damage

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The purpose of this study was to determine whether space flight or combined exposure of unloading and space radiation can induce deleterious effects on retina and retinal endothelium in mouse models. For flight study, the eyes were evaluated in C57BL/6 male mice (n=12) after Space-x returned from a 35-day mission using mouse Habitat Cage Unit (HCU) in the Japan Aerospace Exploration Agency (JAXA) 'Kibo' facility in the International Space Station (ISS). Mice were under ambient microgravity (µg) or in a centrifugal habitat unit permits 1 gravity (1g). Habitat control (HC) and vivarium control mice housed similarly in HCU cage or regular cages, respectively. Within 48 hours of landing, the spaceflight mice were euthanized and their eyes were removed for characterization of cellular damage. Retina tissues were also isolated to evaluate protein expression profiles using quantitative proteomics analysis. For ground-based study, In order to simulate the broad energy spectrum of a large solar particle event (SPE), we irradiated male C57BL/6 mice (n=8/group) whole body with fully modulated beams 150 MeV protons containing particles from 0 energy to 150 MeV and a uniform dose versus depth profile. The mice were also hindlimb-unloaded (HLU) by tail suspension. Mice were unloaded for 7 days, irradiated at 50 cGy, unloaded an additional 7 days and then sacrificed for tissue isolation at 4 days and 30 days after the combined treatment. Quantitative assessment of ocular tissue demonstrated that µg group in the flight study induced significant apoptosis in the retina vascular endothelial cells compared to all other groups ($p<0.05$) and was 64% greater than that in the HC group. Proteomics analysis showed that many key pathways responsible for cell death, cell repair, inflammation, and metabolic stress were significantly altered in µg mice compared to HC animals. Additionally, there were more significant changes in regulated protein expression in the µg group relative to that in the µg+1g group. For ground-based study, increases in the number of apoptotic positive cells were seen in radiation-only and radiation + HLU mice compared to control at both days 4 and 30 ($p<0.05$). Most robust changed were observed in combination group, suggesting a synergistic (or additive) response of radiation and unloading. In summary, the data provided the first evidence that low dose radiation and spaceflight conditions induce apoptosis and changed in retinal protein expression related to cellular structure, immune response and metabolic function in the retina. These retinal cellular response may have long term impact on visual function and increase the potential risks of late retinal degeneration.
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Space Experiments for "Cancer Progression" in the International Space Station

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Space radiation can cause damage to the DNA, such as double-stranded breaks, resulting in chromosome aberrations or gene mutations. Consequently, exposure to space radiation may lead to an increased risk of cancer incidence and mortality after long-duration and exploration spaceflights. Interestingly, simulate microgravity (μG) experienced during spaceflight with hind-limb unloading (HU) significantly increased tumor growth and caused greater splenic atrophy in wild-type mice, whereas immune-deficient mice showed no difference in these phenomena. Additionally, splenic and thymic atrophy were observed in mice flown in space, and thymopoiesis was reduced in healthy astronauts in space, compared with ground controls. However, the effect of spaceflight on cancer progression due to immune system dysregulation and how to prevent these adverse effects remain unknown.

We previously demonstrated that mice subjected to HU had significantly greater tumor growth, lung metastasis, and splenic and thymic atrophy compared with mice in constant orthostatic suspension and standard housing controls. Furthermore, we discovered a means of preventing cancer progression during HU. HU mice undergoing temporary loading (2 h/day) demonstrated no difference in cancer progression and immune organ atrophy compared with controls. Our findings suggest that temporary loading can prevent HU-induced cancer progression and immune organ atrophy [1].

Here, we propose a future space experiment using the newly developed mouse habitat units and *in vivo* deep bioluminescence imaging system [2] in the International Space Station. We anticipate that the findings of our proposed experiments will be helpful for human adaptation to the μ*G* and radiation environment during long-term space travels.

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Evident biological effects of space radiation in astronauts

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Astronauts in space are exposed to cosmic radiation that consists primarily of protons and other heavy charged particles. Potential health risks associated with space radiation exposure include cancer, damage in the central nerve system, degenerative tissue effects and acute radiation syndrome. Spaceflight environmental factors other than radiation may also contribute to some of these identified radiation risks. Consequently, it is difficult to determine whether some of the diseases in astronauts, such as cancer, are caused by space radiation or, say, immune dysfunction, or both. Nevertheless, space radiation exposure is considered to be the primary cause for three reported physiological and biological effects in astronauts; i) Light flash first observed in Apollo astronauts on their trip to the Moon. Scientists reproduced this phenomenon on the ground by exposing the eyes of test subjects to high-energy charged particles. Although whether light flashes are associated with permanent eye damage is unknown, they could be a psychological stress factor since the astronauts are constantly reminded of the radiation environment during space mission. ii) Development of cataracts. Like the general population, astronauts will develop cataracts as they age, but exposure to space radiation caused an earlier onset cataracts. iii) Chromosome damage in astronauts' lymphocytes detected after long-duration space missions. Since the beginning of International Space Station (ISS) missions, chromosome aberrations in the lymphocytes of ~40 crewmembers pre- and post-ISS missions have been analyzed to estimate the biological dose received during flight. Additionally, blood samples were collected pre-flight and exposed *ex vivo* to a set of gamma ray doses in order to determine the individual sensitivity. Statistical analysis of the pre- and post-flight chromosome aberration data indicate that the post-flight chromosome damage depends on the blood-forming organ (BFO) dose. The differences between individuals in post-flight damage can be partially explained by differences in the background number of chromosome aberrations and the radiosensitivity as determined in preflight samples. In addition, the relative biological effectiveness (RBE) is about 3 when compared to the *ex vivo* dose response to gamma irradiation. These three effects can be related, directly or indirectly, to the various health risks associated with spaceflight.

The influence of the shifted balance of high-energy phosphates to AMPK dephosphorylation and expression of slow myosin at the early stage of gravitational unloading

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Mechanical unloading of postural muscles results in both muscle atrophy and a slow to fast fibre type transition. The cause of such changes is a reduction in slow-type MyHC isoform expression and an increase in fast-type MyHC isoform expression. It is believed that calcineurin/NFAT signalling pathway and AMP-activated protein kinase (AMPK) are involved in the regulation of slow-type MyHC isoform. Previously we showed that AMPK phosphorylation is significantly decreased in rat soleus at the early stage of mechanical unloading. We found, that stimulation of AMPK activity at the early stage of gravitational unloading prevents a decline of slow MyHC expression. We assumed that a decrease in AMPK activity in rat soleus at the early stage of gravitational unloading could be associated with changes in the ratio of intracellular high-energy phosphates (ATP/ADP). The aim of the study was to investigate the impact of high-energy phosphates ratio changes on AMPK activity and slow-type MyHC isoform expression in rat soleus muscle at the early stages of gravitational unloading.
To verify the hypothesis, we used administration of β-guanidinopropionic acid (βGPA), before (6 day) and during 24-h hindlimb suspension. The content of phospho-AMPK, phospho-ACC, phospho-PKD, HDAC4/5 in rat soleus was determined by Western-blotting. The amount of MyHCI(β) pre-mRNA and mRNA was evaluated by RT-PCR.
After 24-h HS we observed a decrease ($p<0.05$) in phospho-AMPK content *vs.* control group, but in HS+ βGPA group didn't differ from the control. After 24-h unloading we found a significant increase in the content of nuclear HDAC4 in the HS group, but in the HS+ βGPA group the content of nuclear HDAC4 didn't differ from the control group.
24-h unloading resulted in a decrease in MyHCI(β) pre-mRNA and mRNA expression *vs.* the control group. The expression level of MyHCI(β) pre-mRNA and mRNA in HS+ βGPA group didn't differ from the control.
Thus βGPA administration prevents a decline in AMPK phosphorylation. Therefore, we can conclude that at the early stage of gravitational unloading an accumulation of high-energy phosphates (ATP, ADP and creatine phosphate) takes place and leads to reduced AMPK activity and a slow to fast myosin fibre type transition. The study was supported by RSF grant # 18-15-00107.

HDACI regulate Atrogin-1/MAFbx mRNA expression in unloaded rat soleus muscle.

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Abstract
It is known that E3 ubiquitin ligases MuRF-1 and atrogin-1/MAFbx are upregulated in rat soleus muscle under unloading conditions. We aimed to determine the role of histone deacetylase 1 (HDAC1) in the activation of MuRF-1 and MAFbx mRNA expression in rat soleus muscle at the early stage of hindlimb suspension (HS). To this end, male Wistar rats (195-215 g) were divided into 3 groups (n=8/group): control (C), 3-day HS (HS) and 3-day HS + HDAC1 inhibitor CI-994 (1 mg/kg/day) (HS+CI). Protein content and mRNA expression levels of regulatory molecules were assessed by WB and RT-PCR. CI-994 treatment prevented HS-induced increase in HDAC1 nuclear content. As expected, 3-day HS induced a significant increase in the mRNA expression levels of MAFbx, MuRF-1 and ubiquitin. CI-994 administration resulted in an attenuation of unloading-induced up-regulation of MAFbx and ubiquitin but had no effect on MuRF-1 mRNA expression. A decrease in histone acetyltransferase p300 nuclear content in the HS group was prevented by CI-994 administration. There were no significant differences in the content of phosphorylated anabolic signaling molecules between HS group and HS+CI group. Thus, inhibition of HDAC1 prevented hindlimb suspension-induced up-regulation of MAFbx and ubiquitin, but did not affect MuRF-1 mRNA expression.
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Morphology and motility of mice sperm after long-term modeling microgravity

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Change of the external mechanical field has a significant impact on different types of cells and can lead to the reorganization of the cytoskeleton, as well as the formation of its adaptation pattern. However, data concerning changes in male germ cells under gravitational unloading are very few.

The purpose of this work was to evaluate the parameters of mice spermogram, as well as to study the content of cytoskeletal proteins and sperm-specific proteins, their mRNA in sperm and testes cells. During the experiment, the animals were subjected to 30-day antiorthostatic suspension and subsequent 12-hour recovery, and received essential phospholipids at a dosage of 500 mg/g/day (groups 30HSE and 30HSE + 12h) or a similar dose of placebo (groups 30HS and 30hs + 12h). Accordingly, control groups (group CE and group C) were formed.

The obtained spermogram data showed a decrease in the number of sperm in the 30HS and 30HS+12h groups. The administration of essential phospholipids increased the number of mature forms in the CE group (by 34% compared with the group C ($p<0.05$)). Moreover, spermogram parameters in the 30HSE+12h group did not differ from those in the control group. In all groups of the study the proportion of viable sperm and sperm with normal morphology correlated with the number of mobile sperm. Thus, despite the decrease in the number of mature sperm forms in the suspension groups, the cells remain mobile.

The relative content of cytoskeletal proteins in sperm was relative to the control, with the exception of group 30HS+12h, where the alpha-actinin-4 level was 13% greater than the control ($p <0.1$). In the testes tissues alpha-actinin isoforms had multidirectional dynamics: the relative content of ACTN1 in the 30HS group was significantly higher than in group C by 17% ($p <0.05$) and decreased after 12 hours of recovery, while the content of ACTN4 was lower than control by 20% ($p <0.05$) and increased to the control after a 12-hour recovery. At the same time, in the group with administration of essential phospholipids, the relative content of ACTN1 and ACTN4 did not differ from the control. However, in sperm the relative content of beta-tubulin was reduced in groups 30HS and 30HS+12h and such reduction was not prevented by the administration of essential phospholipids. In the testes tissues changes of the beta-tubulin content were not observed. Summarizing, it can be concluded that the administration of essential phospholipids made it possible to prevent changes resulting from antiorthostatic suspension and subsequent recovery both in the testes and in sperm, with the exception of the content of tubulin in germ cells. But, at the same time, the relative mRNA content of the genes encoding the studied cytoskeletal proteins changed more pronounced, which may indicate that the protein content is regulated mainly at the translation level.

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Cytoskeleton structure and according genes' expression in the testes and duct deference tissues of mice under space flight.

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Studies of the influence of weightlessness on the structure and function of male germ cells not only are applied research but also are of fundamental importance to the understanding of the evolutionary aspects of ontogenesis. However, on Earth, all such studies have limitations that are inherent to modeling experiments. In this study, we had the opportunity to study the duct deferens and the testes of mice that were exposed to space flight conditions for 21-24 days (experiment Rodent Research-4, SpaceX-10 mission, February 2017, USA) and were euthanized there. The samples, in accordance with the NASA-Roskosmos protocol "Utilization Sharing Plan on-board ISS" (signed on July 18, 2013), were delivered to Russia on dry ice without defrosting.

The results suggested that there was no change in the levels of the studied cytoskeletal proteins (beta- and gamma-actin, alpha-actinin 1 and 4, beta-tubulin and desmin) in the flight group, although there was a decrease in *Actn1* mRNA in the duct deferens and in beta-tubulin in the testes. In the heart and lungs after a 34-37-day flight (in the Rodent Research-1 experiment that also performed tissue fixation under weightlessness), there were practically no changes in the levels of cytoskeletal proteins, although there were significantly more changes in the mRNA levels. However, it should be noted that testes tissues have high levels of cellular heterogeneity, which does not allow for the identification of the contributions of individual cell types to the results that we obtained. Therefore, we decided to examine the content of germ cells of varying degrees of maturity, both in the duct deferens and in the testes.

In the duct deferens, we did not observe changes in the protein levels and mRNA levels of the gene, marker for early spermatogonia KDM5B. However, in the testes, despite the absence of a change in the protein level, the mRNA level of this gene increased in the flight group, which may indicate a decrease in the differentiation potential and, accordingly, in the mature forms of sperm. However, in this case, we did not note changes in the levels of protamines, the expression of which is highest in spermatids, as well as sperm lysozyme-like protein 1, which is localized to the acrosome of mature spermatozoa. Perhaps this is because more mature forms of sperm are localized in the caudal epididymis, which was not available for analysis.

Thus, despite the absence of changes in the levels of cytoskeletal and sperm-specific proteins, changes in the expression of some proteins were observed. The reasons for the changes in expression could be associated with a wide range of factors, such as histone modifications, but for higher mammals, it is most likely because of a change in the DNA methylation levels of CpG-islands in the promoter regions of genes.

This work was financially supported by the program for fundamental research SSC RF – IBMP RAS; program "Cell and Molecular Biology" of the RAS Presidium; Russian Academic Excellence Project 5-100.

We Propose Again the Importance of Sexuality for Establishing a Happy and Peaceful Space Human Society

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Introduction: In 2005 we stated at IAC – 05 in Fukuoka that *"It is now the time to start systematically the study of sexuality, particularly reproductive activity in the space life."* However, since that time this topic has not been investigated from the viewpoint of systematical approach nor medical science. Sexuality is an important factor to establish the basic property of the human being and to make a background for the human life and human society. It is basically recognized that sexuality is composed of sex, gender, identity, sexual orientation, sexual behavior, sexual activity, eroticism, emotional attachment, and reproduction. During the past two decades space development has been more accelerated and the human population in the space is proposed to increase greatly in this century. **Matters of Discussion:** We discussed carefully from various points about the following problems. 1) Issues related to sexuality in the space life, 2) The most fundamental problems of our attitude when the human being try to approach this topic, 3) Prospective effects of the space environment on human reproduction, 4) Counter-measure against prospective or possible effects, 5) Application of the space environment to reproductive medicine on the ground. **Summary:** We suggest again that to construct a peaceful human society in the space environment, a deep understanding of sexuality is essential and it is now the time to promote the study on this topic.

Analysis of femurs from mice embarked on board BION-M1 biosatellite reveals a decrease in immune cell development, including B cells, after one week of recovery on Earth

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ABSTRACT:

Bone loss and immune dysregulation are among the main adverse outcomes of spaceflight challenging astronauts' health and safety. However, consequences on B-cell development and responses are still under-investigated. To fill this gap, we used advanced proteomics analysis of femur bone and marrow to compare mice flown for one month on board the BION-M1 biosatellite, followed or not by one week of recovery on Earth, to control mice kept on Earth. Our data revealed an adverse effect on B lymphopoiesis one week after landing. This phenomenon was associated with a 41% reduction of B cells in the spleen. These reductions may contribute to explain increased susceptibility to infection even if our data suggest that flown animals can mount a humoral immune response. Furthermore, it appears that future studies should investigate the quality/efficiency of produced antibodies and whether longer missions worsen these immune alterations.

KEY WORDS: lymphopoiesis, bone, immunity, proteomics, gravity

Socio-environmental stressors encountered during spaceflight partially affect the murine TCRβ repertoire and increase its self-reactivity

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ABSTRACT:
Spaceflights are known to affect the immune system. In a previous study, we demonstrated that hypergravity exposure during murine development modified 85% of the TCRβ repertoire. Here, we investigated whether socio-environmental stressors encountered during space missions affect T lymphopoiesis and TCRβ repertoire. To address this question, pregnant mice were subjected to CUMS, a model used to mimic socio-environmental stresses encountered during space missions, throughout gestation. Then, newborn T lymphopoiesis and TCRβ repertoire were studied by flow cytometry and high-throughput sequencing, respectively. No change in thymocytes maturation or TCR expression were noted. TCRβ repertoire analysis revealed that 75% of neonate TCRβ sequences resulted from the expression of 3 Vβ segments and that this core repertoire is not affected by CUMS. However, the minor repertoire, representing 25% of the global repertoire, was sensitive to CUMS exposure. We also showed that the V(D)J recombination process is unlikely affected. Finally, we noted that CUMS neonatal minor repertoire is likely more self-reactive than the one of control pups. These findings show that socio-environmental stressors such as those encountered during space missions affect a fraction (25%) of the TCRβ repertoire and that these stressors could increase self-reactivity.

Key words: T cell receptor, space, stress, lymphopoiesis, immunity.

β

Murine bone marrow progenitor cells from proximal and distal hindlimb bones after antiorthostatic suspension

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The main aim of biological experiments in space flights and ground-based experiments is to study the fundamental mechanisms of adaptation of living beings to microgravity and radiation. Musculoskeletal, nervous, endocrine, cardiovascular, vestibular systems react sharply to space flight factors. Negative changes in rodent bones after space flight and simulation studies were shown. There was resorption of spongy bone and slowing of renewal of compact bone. Mainly, damage occurred in bone areas that are subjected to mechanical stress during walking and static poses (distal extremities). Bone marrow and bone tissue are closely interconnected. Their morphological and functional parameters are in dynamic equilibrium. Despite a bulk of data on the effect of unloading on different parts of the bone system, the data on the state of bone marrow progenitor compartment of different locations only begin to appear.

The goal of this work was to study the effect of 30-day hindlimb suspension on bone marrow progenitor cells of distal and proximal murine hindlimb bones. Male of C57Bl/6N mice in the following groups were used: VC – vivarium control animals (n=7), HS – animals after the 30-day hindlimb suspension (n=7). The cellularity, immunophenotype, proliferation, clonogenicity and differentiation capacities of murine bone marrow stromal and hematopoietic progenitors from femur and tibia bones were examined.

Immunophenotype was characterized using the markers: CD106, CD44, Sca-1, CD157, CD45, CD90, CD34, Ter-119. CFU-f number was determined at day 14 of culture after crystal violet staining. The proliferative activity was determined as a number of population doublings (PD). The calculation was conducted by the equation PD=log2 (N/No), there N_0 and N are initial and final cell number. Spontaneous osteo- and adipocomitment of stromal progenitors were evaluated in primary cultures of bone marrow cells. In the samples revealed The myeloid colony forming units (CFU-GEMM, CFU-GM, CFU-M, CFU-M, BFU-E) were studied using semi-liquid selective media. The colonies were counted after 10 days of cultivation.

The number of mononuclear cells isolated from the femur exceed tibia in 1.5 folds. The cellularity of tibia bone marrow was 2-folds and femur - 1,2-folds less after prolonged unloading. There was the decline in the proportion of stromal and hematopoietic cells in the femur and tibia after suspension. The number of CFU-f from tibia and femur after suspension was decreased by 1.5 times, moreover number of CFU-f tibia was 1.3 times more compared to the femur. Cell growth of mononuclears from femur practically did not change after suspension, while in the tibia decreased by 2 times. The decline of the alkaline phosphatase activity was more pronounced in stromal precursors from tibia. The intensity of spontaneous adipogenesis was similar in all cells. The total number of detected hematopoietic progenitors from tibia was 1.3 time more than that from femur. After suspension in the bone marrow from both bones, there was a decrease in the total number of CFU, with the most significantly reduced number of CFU-GEMM, CFU –M and BFU-E.

Our results indicated that the proportion of stromal and hematopoietic progenitor cells in the distal parts of the murine hind limbs was greater than in the proximal part. In this regard, the more pronounced negative impact of the unloading was observed in murine bone marrow progenitors cells from the distal parts of the hind limbs.

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Anti-allergic effects of hypergravity are associated with restoration of Th1/Th2 balance and decrease in innate lymphoid type 2 cells

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Abstract

Gravity influences biological and physical processes, with specific effects on the immune system. *Dermatophagoides pteronyssinus (*Dp*)* is a well-known, typical house dust mite involved in the induction of allergic asthma. Recently, type 2 innate immune response has been shown to play an important role with adaptive immune response in mechanisms underlying Dp-induced allergic asthma. However, the effect of hypergravity therein remains unknown. In this study, we induced allergic asthma via intranasal administration of a Dp extract (during sensitization and challenge periods), which elicited robust Th2-polarized airway inflammation. We evaluated the immunological responses of BALB/c mice concurrently exposed to Dp and hypergravity (Dp+HG). These mice experienced decreased eosinophilic inflammation and Th2-related immune responses than mice that were only administered Dp. In addition, the proportion of innate lymphoid type 2 cells (ILC2s) was decreased in the Dp+HG group. The levels of interleukin-33 and thymic stromal lymphopoietin, which are cytokines associated with ILC2s, were also significantly reduced by hypergravity. These findings suggest that hypergravity exerts beneficial effects on the clinical treatment of diseases such as allergic rhinitis and asthma-controlled type 2 innate immune response by reducing the release of interleukin-33 and thymic stromal lymphopoietin.

Immune-cell responses under μG -Stress – a biomechanical view

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Altered gravitational stress, especially microgravity, can induce immune dysfunction in long-term, manned spaceflight and space exploration. Leukocytes and endothelial cells (ECs) are highly sensitive to mechanical forces and contribute to the microgravity-induced impaired immune function.
To explore the underlying mechanisms, we first used a rotary cell culture system (RCCS) to elucidate the effects of simulated microgravity on polymorphonuclear neutrophils (PMN)-like HL-60 cells[1]. Although the up-regulation of cytokine secretion such as interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemotactic protein 1 (MCP-1) was observed for the cells cultured in RCCS, the reduced rolling speed and decreased adhesion of PMN-like cells on ECs under shear stress indicated that PMN recruitment might not be effective under microgravity. The rolling rate of peripheral blood mononuclear cells (PBMCs) on P-selectin glycoprotein ligand-1 (PSGL-1)/intercellular cell adhesive molecule-1 (ICAM-1) immobilized substrate was also decreased under microgravity in a parabolic flight (PF) experiment, suggesting the impaired interactions between leukocytes and ECs.
Next we cultured EA.hy926 ECs on board the SJ-10 Recoverable Scientific Satellite and analyzed the related morphological and functional changes of ECs in space[2]. Space microgravity suppressed the glucose metabolism, reduced the expression of ICAM-1s and vascular cell adhesion molecule-1s (VCAM-1s), increased the expression of CD44s, and depressed the release of pro-angiogenesis and pro-inflammation cytokines. Meanwhile, it also induced the depolymerization of actin filaments and microtubules, promoted the vimentin accumulation, restrained the extracellular matrix (ECM) deposition, regulated the mechanotransduction through focal adhesion kinase and Rho GTPases, and enhanced the exosome-mediated mRNA transfer.
This work furthers the understandings of the mechanobiological responses of immune cells under microgravity and provides useful information for optimizing the countermeasures to immune suppression in future spaceflight.

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Poster session

Neuronal responses to the modulated gravity in vestibular nucleus

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Objective: To examine the effects on the neuronal activities in vestibular nucleus (VN) after exposed to short (24hours) and long (4weeks) hypergravity (4G)
Background: The VN is the first central brain area directly receiving the incoming stimulation through the vestibular end organ. To perform its role for postural balance and spatial orientation, the organ senses the changes related with head rotations and its linear transmissions. The gravity is one of the dramatically changing factors to disrupt the vestibular functions in the space, causing the space sickness. Based on our previous animal behavior experiments, however, the abnormal behaviors disappeared as the exposed time increased, indicating the neural plasticity. In this study, it was examined if the plasticity could be demonstrated by the resting firing rate (FR), in the VN. **Method:** Four rats (SD, 290-350g, M) were used in two groups (2 animals for each group) depending on the length of the exposed times to hypergravity (4G); 4-weeks (Group A) and 24-hours (Group B). For the control, eight animals were recruited with no exposure to hypergravity. The hypergravity was generated in a large diameter centrifuge facility, and the range of the modulated gravity was from 1 to 10G with a resolution of 0.01G. The animals in Group B were continuously exposed for 24 hours, while those in Group A were done for 4 weeks with a 30-minute break per day. The neuronal activity was obtained by an extracellular neural recording in vestibular nucleus (VN), using a tungsten electrode (5MΩ, A-M systems, US). Using the rotation and the linear transmission of the head, the origin of the recording neuron was identified. The neuronal recordings started immediately (<0.5-hour) after the animals were removed from the facility, and all recordings were completed within four hours. The obtained data were filtered (bandpass, 0.3-5 kHz) and stored at a sampling rate of 40 kHz (Plexon, US). Data analysis was performed off-line using a custom-made program in Matlab (MathWork, US). After specifying the data in time (10 sec), the averaged FR (aFR) was computed, and the results were compared among the control, A, and B. **Result:** Seventeen neurons from the control, 10 from Group A, and 3 neurons from Group B were measured. Most units (8/10) of Group A were canal-related, and all in Group B were the otolith-related units. Also, all neurons in the control were identified as canal-related. For the control, the aFR (±STD) was 18.92 (±12.10) spks/sec, and its range was between 2.67 and 47.81 spks/sec. For Group A, the canal-related aFR was 17.60 (±6.11) spks/sec, and the otolith-related aFR was 13.58 (±7.77) spks/sec. Their ranges were 10.55-26.16 and 8.08-19.07 spks/sec,

respectively. The aFR in Group B was 15.69 (±12.24) spks/sec (range: 2.27-26.24 spks/sec). **Conclusion:** The aFRs were compared among three groups with the different exposing time to hypergravity to understand the effects on the neuronal activities by the plasticity in VN. However, there was no significant difference in the aFR, and the results indicated that there was no relation between the plasticity and the aFR.

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Comparison of the serotonin receptor expression in vestibular nuclei between short-term and long-term hypergravity stimulation

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Background: Motion sickness occurs as a result of a mismatch or conflict between the information arising from the vestibular system, the visual and proprioceptive inputs. Many different environments can cause motion sickness including travelling on land, sea or space. The activation or blockade of serotonin receptors is known to be closely related with migraine and motion sickness. Some drugs related to serotonin receptors which modulates serotonins are effective in treating the vestibular symptoms of motion sickness. However, the molecular mechanisms by which serotonin modulates vestibular function remain unclear

Objectives : In this study, we studied the expression of serotonin receptors and compared the changes in expression with duration of the hypergravity stimulation.

Methods:

we use our gravity system named Inha G-simulator which is a centrifuge device for animal. With 4G hypergravity stimulation was exposed for 24hrs, 1week, 2weeks and 4weeks with SD male rats(aged 7~8weeks, weighing 250-300g). We checked the vestibular function with animal rotator (VOR responses). And we did western blotting and immunohistochemistry analysis to quantify the protein expression of serotonin receptors (5-HT1B, 2A, 1D, 1F) in medial and lateral vestibular nuclei.

Results

Under 4G for 24hrs stimulation for 4weeks, the gain at 0.04, 0.08,0.16, 0.32 HZ were 0.463±0.113, 0.524±0.455, 0.494±0.093, 0.509±0.198 respectively. They showed significant reduction on VOR gain compared to the control group. Decreased VOR gains were recovered to normal range on 3~4days after stopping the hypergravity stimulation. According to the duration of the exposure, longer exposure group showed lower gain values than the other groups.

The expressions of 5HT1B, 5HT1F receptor protein were increased significantly compared to control group in vestibular nuclei. Also 4weeks exposure group showed higher expression than 24hrs and 2weeks exposure group significantly. In the immunohistochemistry exam, 5HT1B & 5HT1F-positive neurons were significantly increased compared with control group on both medial and lateral vestibular nuclei. However, the expression of 5HT2A receptor protein did not show the significant change compared to control group. The 5HT1D was highly expressed on 24hrs stimulation group compared to control group and 4weeks stimulation group.

Conclusion:
By observing the data, hypergravity stimulation affect the vestibular function and modulation of gene expression in vestibular nuclei. We can induce the results that long-term hypergravity stimulation causes remarkable changes on VOR gain in rats. And this altered gravity also induced differential modulation of several target gene expression according to the duration of stimulation in vestibular nuclei in rats.

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Tissue adhesive hydrogel glue for the bleeding control in space

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In this study we prepared tissue-adhesive hemostatic glue which is applicable in space for bleeding control and assessed hemostatic effects on hepatic bleeding. animal model. Alginate was used as primary polymer for the fabrication hemostatic glue, oxidized for the introduction of the tissue-adhesive Schiff base forming aldehyde and then encoded with mussel-inspired dopa. In addition, polyallylamine (PAA) was selected for as an intra-structuring polymer which ensures the gel strength and allows instantaneous glue formation on the bleeding spot. Primary glue (OA glue) was quickly formed within 5 to 10 seconds after the mixing oxidized alginate (OA) with PAA. The degree of oxidation and the mixing ratio of OA and PAA were precisely determined based on glue formation time and gel strength. And the extend of dopa conjugation on OA was determined by the tissue adhesion force and the elasticity of the glue (Dopa-OA glue). Especially, elasticity of Dopa-OA glue was significantly enhanced after introduction of dopa to OA. Functional assay of Dopa-OA glue on hepatic bleeding animal model showed much enhanced hemostatic action. Dopa-OA glue is expected to provide novel injectable tissue adhesives for the treatment of accidental bleeding or surgery procedure in space.

Keywords: Space medicine, Hemostatic glue, Schiff base, Surgery in space, Tissue adhesive.
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Effects of centrifugation-induced hypergravity on the hypothalamic feeding-related neuropeptides gene expressions in mice via vestibular inputs

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Feeding behavior is well known to be regulated by anorexigenic and orexigenic neuropeptides in the hypothalamus in mice and rat. In the present study, corticotrophin-releasing hormone (CRH) in the paraventricular nucleus and proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) in the arcuate nucleus were chosen as anorexigenic neuropeptides, and neuropeptide Y (NPY)/agouti-related peptide (AgRP) in the arcuate nucleus and melanin-concentrating hormone (MCH)/orexin in the lateral hypothalamic area were chosen as orexigenic neuropeptides. These hypothalamic feeding-related neuropeptides gene expressions in mice with/without bilateral vestibular lesion were examined after centrifugation-induced hypergravity (2*g*), using *in situ* hybridization histochemistry. Several neuropeptides gene expressions were changed significantly after centrifugation-induced hypergravity (2*g*) in mice without bilateral vestibular lesion, while some of these changes were attenuated significantly in mice with bilateral vestibular lesion. These results suggest that vestibular inputs to the hypothalamus may be involved in the regulation of the gene expressions of anorexigenic and orexigenic neuropeptides in mice during/after centrifugation-induced hypergravity in mice.

The effect of beta-GPA treatment on AMPK/mTORC1 signaling in rat soleus muscle at the onset of simulated microgravity

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It is well established that exposure to real or simulated microgravity results in a significant atrophy of mammalian postural muscles, such as soleus. However, potential molecular mechanisms that could trigger the development of atrophic changes in postural muscles remain undefined. Since unloading-induced skeletal muscle inactivity leads to alternations in intramuscular phosphagen levels, we hypothesized that energy-sensing enzyme AMP-activated protein kinase (AMPK) would play an important role in regulation of anabolic responses in postural muscles. For example, activated AMPK is known to inhibit anabolic signaling and concurrently activate catabolic pathways in skeletal muscle. Beta-guanidinopropionic acid (β-GPA) is able to competitively inhibit cellular creatine uptake leading to reductions in high-energy phosphates and subsequent AMPK activation.

The purpose of the study was to examine a response of AMPK/mTORC1 signaling to β-GPA treatment in rat soleus following 1-day mechanical unloading. Male Wistar rats were randomly assigned to the following 4 groups (n=8/group): 1) cage control + saline (C), 2) cage control + β-GPA pre-treatment for 7 days (C+GPA), 3) hindlimb unloading (HU) for 1 day + saline (HU), 4) HU+ β-GPA pre-treatment for 6 days before HU and during 1-day HU (HU+GPA). β-GPA was administered daily via I.P. injections (400 mg/kg).

The content of phosphorylated and total signaling proteins was assessed by Western-blotting. Pre-treatment with β-GPA did not induce any changes in phosphorylation status of AMPK, acetyl-CoA carboxylase (ACC), ribosomal protein S6 kinase (p70S6K) and translation initiation factor 4E-binding protein 1 (4E-BP1) in the C+GPA group. One-day HU resulted in a significant decrease in phosphorylation of AMPK (Thr172), ACC (Ser79), 4E-BP1 (Thr37/46) and an increase in p70S6K (Thr389) phosphorylation. However, pre-treatment of the unloaded rats with β-GPA prevented a decrease in AMPK (Thr172), ACC (Ser79) and 4E-BP1 (Thr37/46) phosphorylation as well as returned p70S6K phosphorylation to the control levels. Collectively our results suggest that AMPK activity may play an important role in the modulation of mTORC1-signaling in rat soleus muscle at the early stage of simulated microgravity.

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Changes in the mice bone tissue elements content under hypergravitation

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Studies conducted by scientists from many countries on board space biosatellites and stations have shown that the skeleton is one of the most important part of the body affected by microgravity. Many elements are represented in the bone tissue, but the percent of calcium is the most significant. Therefore, at the early period of regular space flights, the indicators of calcium balance in the body were used to assess the state of bone tissue. It was clarified to what extent the negative calcium balance reflects the degree of skeletal demineralization by use of the intravital densitometry methods [V.S. Oganov, 2014].

The tests with animals in microgravity and ground model experiments (penile hypokinesia, antiorthostatic hypodynamia - "hanging") have being conducted to confirm the proposed hypotheses and to develop countermeasures. The use of discontinuous centrifugation was proposed as a means to create artificial gravity in space flight [I.B. Krasnov et al., 2006].

The objects of our osteological study were the calvaria bones of mice exposed to a 30-day rotation on a centrifuge (2,0 g). The experiment was conducted at the Jean Monnet University (Saint-Etienne, France).

Three groups consisted of 10 animals were formed. Mice of the control group were kept in vivarium conditions for 30 days.

Animals of the "2g" group were exposed to permanent effect of acceleration 2g during 30 days. The biomaterial was taken from them immediately after the centrifugation.

In the "2g + 12h" third group, animals were also exposed to a 30-day acceleration, but the biomaterial was taken 12 hours after its completion to assess the early period of re-adaptation.

To determine the content of elements (Ca, Mg, P, Zn) in the bones, a laser-spark rapid analyzer of laser- spark emission spectrometry (LSES) elemental composition developed by the SPA "Typhoon" was used. To study the elemental composition of biological samples using LSES, the measurement technique is specially adapted. The results are statistically processed with the use of dispersion analysis.

A decrease in the percentage of all detectable elements in the calvaria bones was reported at hypergravity. The similar losses were noted by the elements composing hydroxyapatite (Ca, P): by Ca content - a decrease from 46.26% to 40.57%, and by P content - from 4.05% to 3.59% (12% and 11%, respectively). Losses of Mg and Zn were more significant and equal (20% each). Differences between the control group and the hypergravity group are statistically significant for all studied elements ($p < 0.05$)

Short-term re-adaptation (12 hours) revealed interesting facts: Mg recovered by 7.2% during this time, and P also showed relatively quick recovery (6.4%). Zn was recovered twice slower (3.3%). The rate of Ca recovery was the lowest (1.2%). However, a statistically significant difference between the hypergravity and re-adaptation groups was not found.

It can be assumed that the cause of the mineral components loss was the redistribution of fluids in the body under the influence of an acceleration of 2.0 g. To confirm this working hypothesis, further investigations are needed to study the content of elements in other regions of the skeleton.

Effects of denervation-related inhibition of antigravity activity during growing period on the properties of hindlimb bones in rats

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Effects of complete inhibition of antigravitational activity during growing period on the properties of hindlimb bones and joints were studied in 18 male Wistar Hannover rats. Ranges of movement in ankle and knee joints were examined in 6 rats under anesthesia with *i.p.* injection of sodium pentobarbital (5 mg/100 g body weight), as the pre-experimental controls (7 days after birth). Further, the hindlimbs were saved bilaterally after sampling of various muscles. The bones were cleaned by submerging in 5% papain solution at ~50°C, followed by water. Then, the dry weight and length, as well as the shape, of femur and tibia + fibula were analyzed. The denervation of sciatic nerve at the gluteal region was performed bilaterally in 6 rats. The remaining 6 rats served as the normal controls. Three rats were housed in a cage with 28 x 17 cm and 12 cm height and supplied water and solid diet *ad libitum* for 6 weeks. Then, the same analyses and samplings, as was explained above, were performed. As the results, growth-associated increase of the femur and tibia length was inhibited by ~6.7 and 7.3% by denervation, respectively. Inhibition of dry weight increase in femur and tibia + fibula was ~42 and 43%, respectively. Further, slight external bend of the shaft was noted in the proximal region of tibia. Such phenomena may be related to continuous pulling by ankle dorsi-flexors, tibialis anterior and extensor digitorum longus. Even though these muscles are also denervated, they are slightly stretched due to ankle plantar-flexion. As for the range of movement, dorsi-flexion of ankle joints was inhibited to ~60° vs. 0° noted in the pre-experimental and age-matched cage controls.

Effect of 9-week exercise training regimen on expression of developmental genes in adipose-derived stem cells of rats

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[Introduction] It is well known that physical inactivity results in the change of body composition. For example, prolonged bed-rest, which represents an extreme form of inactivity, reduces muscle mass but increases body fat accumulation: this experimental approach is often used to verify the effects of spaceflight on the human body. Vigorous physical activity, on the other hand, can increase muscle mass with reduction of fat mass. However, the impact of either physical activity or inactivity on body fat distribution remains to be unknown exactly. Body fat distribution is determined by regional differences in the accumulations of white adipose tissue, which is classified in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). SAT and VAT exhibit different intrinsic expressions of developmental genes, and some of these genes exhibit changes in expression which closely correlate with the pattern of fat distribution. Our recent study showed that *Hox* genes expressions in response to exercise training vary among different WAT types. However, the impact of habitual physical activity on the expressions of the developmental genes in adipose tissue-derived stem cell (ADSC) remains unknown. ADSC has a potential role in supplying new adipocytes to maintain adipocyte turnover under a normal metabolic state.

[Methods] Five-week-old male Wistar rats were randomly divided into two groups: a sedentary control and the exercise trained group. The trained rats were subjected to exercise on a treadmill set at a 5-degree incline 5 days per a week for 9 weeks, and the rats were sacrificed at 36 h after the last exercise session. Adipocytes and ADSC were separated from one subcutaneous (inguinal) fat depot and two intra-abdominal (epididymal and retroperitoneal) fat depots. The mRNA expression levels of developmental gene (homeobox family, Glypican-4, T-box15) and bone morphogenetic protein (BMP) family genes were determined using real-time PCR.

[Results] Habitual physical activity altered the expressions of the developmental genes tested in a fat depot-specific manner.

Effects of low frequency electromyostimulation on characteristics of reflex excitability of calf extensor muscles

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One of the main reasons for changes in motor system following exposure to weightlessness is considered to be the alterations in afferent systems' activity in particular a decrease of volume of proprioceptive afferentation activating motor neurons of skeletal muscles. Electromyostimulation (EMS) can be used as a countermeasure mean to maintain contractile activity of skeletal muscles and additional proprioceptive afferentation.

With regard of this the aim of the study was to investigate the effects of low frequency EMS on reflex responses in calf extensor muscles in human during 5-day Dry immersion (DI).

Experiment was conducted with participation of 20 male subjects aged from 21 to 43 years old. According to the tasks of the study subjects were divided in two groups: in the first one (group "Immersion") the subjects were exposed to DI for 5 days without any additional influences; in the second one (group "Immesrion+EMS") a low frequency EMS of both legs' thigh and calf muscles (anterior tibial muscle, triceps surae muscle, quadriceps and back thigh muscle) was applied daily for 4 hours in the course of DI with the use of "Stimul-01 NCH" device. Electrical stimuli were applied to all the stimulated muscles simultaneously; stimulation train lasted for 1s and was followed by rest period of 2 s. Stimulator generated bipolar 1 ms impulses with frequency of 25 Hz. Stimulation amplitudes were tuned up to the threshold of tolerance.

Effects of EMS training were evaluated by amplitude characteristics of H-reflex in m. soleus and m. gastrocnemius lat. H-reflex was elicited by single 1ms electrical pulses applied to tibial nerve. Reflex responses testing was performed before immersion, on the 3d and 5th days of DI and on the 2nd day after DI completion. Reflex threshold and maximal peak-to-peak amplitudes of responses were evaluated.

Under conditions of DI a distinct hypersensitivity of spinal reflex mechanisms developed which was expressed by valuable decrease of reflex thresholds and increase of response amplitudes. In the "Immersion" group H-reflex threshold decreased by 29% ($p<0.05$) in m. soleus and by 28% ($p<0.05$) – in m. gastrocnemius lat. In the "Immersion+EMS" group this parameter changed during DI in the same manner: in m. soleus and in m. gastrocnemius lat. H-reflex threshold decrease amounted 29% ($p<0.05$). After DI completion in both groups a tendency to recovery of H-reflex threshold in the studied muscles was seen.

Amplitudes of reflex responses in "Immersion" group significantly increased by the 5th day of DI: in m.soleus – by 30% ($p<0.05$), in m. gastrocnemius lat. – by 37%. In "Immersion+EMS" group in the course of DI response amplitudes slightly increased on the 3d day of DI but by the 5th day of DI they tended to decrease.

The data obtained confirms that under conditions of support unloading excitability of motoneurons of calf extensor muscles increases due to reflex changes. Additional proprioceptive stimulation with the use of low-frequency EMS training leads to lessen this effect, i.e. helps to maintain the level of motoneurons excitability.
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The influence of a sustained 10 day bed rest with hypoxia on cartilage and subchondral bone in females: the FemHab study

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The present study assessed the influence of 10-day bed rest and hypoxia on cartilage and subchondral bone density across the patello-femoral joint (PFJ) in healthy women. Female subjects (N=11) participated in this prospective, randomised and controlled study comprising three 10-day interventions: hypoxic ambulation (HAMB), normoxic bed rest (NBR) and hypoxic bed rest (HBR). Venous samples were collected pre-intervention (day -2), during the intervention (days 2 and 5), immediately before reambulation (R0) and 24 hours after reambulation (R1). Blood samples were analysed for the following serum biomarkers: aggrecan, hyaluronan, amino-terminal propeptide II procollagen (PIIANP) and cartilage oligomeric matrix protein (COMP). Total bone mineral density (BMD) in 8 regions (2 mm x 10 mm) across the PFJ was determined before and after each intervention with peripheral quantitative computer tomography. The three interventions (HAMB, HBR and NBR) did not induce any significant changes in the serum cartilage markers: COMP, hyaluronan or PIIANP. Aggrecan increased during the HAMB trial to 2.29 fold the Pre value by the end of the condition. There were significant differences in BMD measured across the PFJ: cortical patellar bone was the densest (735 to 800 mg/cm^3) and femur trabecular bone the least dense (195 to 226 mg/cm^3). However, there were no significant relative changes in BMD from Pre to Post bed rest in any of the ROIs. The results of this 10-day interventions suggest that there are no significant effects inactivity/unloading on cartilage and subchondral bone. Further, there was no effect of hypoxia recorded on these markers.

Key words: Bed rest, Hypoxia, Inactivity, Cartilage, Serum markers, Subchondral bone, Bone density

Effect of simulated lunar gravity on function of respiratory system in humans

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Introduction. The study of physiological adaptation to lunar gravity equal to 1/6 of Earth's gravity is an important task of space medicine.

The aim of the paper is to assess the effect of 7-day simulated lunar gravity on the parameters of external respiration and pulmonary gas exchange.

Methods. Thirty two healthy male volunteers aged 20-36 years took part in the study. The ground-based analogue developed by Research Institute for Space Medicine of FSCC of FMBA of Russia was used to simulate the physiological effects of lunar gravity. This analogue is based on using of head-up bed rest at +9.6º angle (HUBR) with a supporting load on the musculoskeletal system equal to 1/6 of the body weight (Baranov MV et al., 2015).

Sixteen subjects were in the HUBR for 7 hours, and six of them remained for 7 days. Respiratory tests were performed before the study in a sitting position (baseline) on the 7th hour (1st day), the 3rd and 7th days of investigation. The data obtained in 7 hours of HUBR were compared with physiological responses in 7 hours of supine (horizontal) position (10 subjects) and head-down bed rest (HDBR) at -6º angle (6 subjects).

The spiroergometry system "MetaLyzer 3B" ("Cortex Biophysik") was used for registration of spirometry and pulmonary gas exchange parameters. Results were processed statistically. Changes were considered significant at $p<0.05$.

Results. In 7 hours of HUBR the following changes in lung volumes and airflow velocity compared with baseline were found: significant decrease ($p<0.05$) in tidal volume, VC (~6%), FVC (~7%), FEV_1 (~8%), forced expiratory flow at 25-75% of FVC (FEF_{25-75}) (~12%) and a decreasing trend in peak expiratory flow (PEF) and MVV. Further to the 7th day of HUBR, the values of these parameters tended to recover but did not exceed their baseline level. On the 1st day of HUBR a significant ($p<0.05$) increase in IRV (~16%) followed by a decrease by the 7th day was also noted. At the same time, the dynamics of the FEV_1/FVC remained without significant changes. Lung ventilation had a decreasing trend in the early period of exposure to HUBR with subsequent recovery to 7th day. Significant changes in oxygen consumption and CO_2 production were not obtained.

Uni-directional early changes in respiratory parameters were observed in the horizontal position and HDBR. Changes in most parameters were less pronounced under conditions of horizontal position, and more pronounced in HDBR than in HUBR. However, the differences did not reach statistical significance.

Conclusion. Exposure of subjects to 7-day simulated lunar gravity does not lead to significant disturbance of function for respiratory system. The most noticeable changes were found on the first day of the lunar bed-rest analogue. These changes were transient and tended to baseline level to the end of the 7-day study.

Sphingolipids are involved in disuse muscle atrophy: effects of inhibitor of acid sphingomyelinase clomipramine

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It is known, that sphingolipids are the class of molecules playing a pivotal role in wide range of intracellular processes, e.g. survive and apoptosis, cell circle arrest and senescence, regulation of insulin signaling and membrane raft formation, etc. We have shown in our previous works (Bryndina et al., 2012-2018) that sphingolipids backbone molecule ceramide accumulates in disused skeletal muscle after short-term (6-12 h) or more prolonged (4-30 days) hindlimb unloading (HU). Clomipramine, an inhibitor of acid sphingomyelinase (aSMase) prevented a number of effects caused by disuse including activation of prooxidant signaling, lipid rafts disruption and formation of ceramide enriched membrane domains (Bryndina et al., 2017, 2018). In the present work we studied morphological and biochemical changes in soleus muscle of rats suspended during two weeks with clomipramine or vehicle pretreatment. A typical atrophic alteration has been found in muscles of rats suspended with vehicle administration. We observed a decline of muscle mass and morphological signs of atrophy. Simultaneously a substantial increase in ceramide and aSMase in plasma membrane and detergent resistant membrane domains have been shown. Clomipramine treatment (Anafranil, Novartis Pharma, i/m, for 5 days before HU and every other day during HU), partially prevented morphological and biochemical changes revealed in disused soleus muscle. Earlier Salaun with co-authors (2015) used myriocin (an inhibitor of serine palmitoyltransferase) to decrease ceramide biosynthesis in rats subjected to 7-day hindlimb unloading (HU). Although this treatment managed to decline the enhanced ceramide level in rat soleus muscle it did not prevent its atrophic changes. So the authors concluded that de novo ceramides synthesis is not involved in skeletal muscle atrophy induced by short-term mechanical unloading. Our data evidence that the mechanisms leading to the development of atrophic changes in disused muscle could involve ceramide formation induced by activation of aSMase. Thus, inhibition of sphingomyelinase hydrolysis with clomipramine partially prevents the development of muscle atrophy in two weeks muscle disuse. *This work was supported by RSF (grant # 16-15-10220)*

Signaling consequences of p70S6K upregulation in rat soleus muscle at the early stage of mechanical unloading

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Unloading-induced skeletal muscle atrophy results from an increase in proteolysis and a decrease in protein synthesis. To date, signaling mechanisms that initiate the development of disuse-induced muscle atrophy remain largely understudied. It was previously shown that p70S6K phosphorylation is paradoxically elevated in rodent soleus muscle at the early stage (12 h, 1- and 3 days) of mechanical unloading (Mirzoev T.M., 2016). However, the consequences of such p70S6K hyperphosphorylation on proteolytic and anabolic markers are poorly defined.

In order to analyze the signaling effects of increased p70S6K phosphorylation at the initial stage of unloading, a specific mTORC1 inhibitor rapamycin was used. It was shown in C2C12 cells that ceramide induced p70S6K hyperphosphorylation mediates negative feedback to phosphorylate insulin receptor substrate 1 (IRS-1) at Ser636/639, which in turn causes decreased AKT Ser473 phosphorylation (Hsieh C.T., 2014). A decrease in AKT Ser473 phosphorylation can lead to forkhead box O3 (FOXO3) dephosphorylation and myonuclei import resulting in the increased expression of E3 ubiquitin ligases muscle RING finger 1 (MuRF-1) and muscle atrophy F-box (MAFbx/atrogin-1) (Brunet A.,1999; Sandri M., 2004). Therefore, we hypothesized that rapamycin-induced downregulation of p70S6K phosphorylation under 24-h HU would lead to the reduced mRNA expression of E3 ubiquitin ligases.

Methods. The mechanical unloading was performed via hindlimb suspension (HS). 21 male Wistar rats were randomly divided into 3 groups: intact control (C), 1-day HS (HS), and 1-day HS with rapamycin (mTORC1 inhibitor) treatment (1.5 mg/kg) (HSR). Anabolic and catabolic markers were assessed using WB and RT-PCR.

One-day HS resulted in a significant 65% increase in p70S6K Thr389 phosphorylation relative to the C group. Rapamycin treatment during one-day HS (the HSR group) abolished this increase in the level of p70S6K Thr389 phosphorylation. The content of total IRS-1 in the both unloaded groups was significantly reduced compared to the control values. At the same time, IRS-1 phosphorylation on the negative regulatory site Ser639 significantly increased following 1-day HS vs. the C group and inhibition of mTORC-1 signaling with rapamycin resulted in a decrease in IRS-1 Ser639 phosphorylation vs. the HS group. The content of phosphorylated AKT (Ser473) was significantly reduced in the HS and HSR groups as compared with the C group (Fig 5A). A similar pattern of changes was observed for FOXO3 Ser253 phosphorylation. The level of MuRF-1 and MAFbx mRNA expression was significantly upregulated in the HS group vs. the C group. However, rapamycin treatment led to a significant reduction in MuRF-1 and MAFbx mRNA expression compared to the HS levels. Moreover, rapamycin treatment attenuated unloading-induced increase in MuRF-1 protein expression. In addition, rapamycin administration during 1-day unloading resulted in a significant increase in the nuclear HDAC5 content relative to the HS group.

Overall, the present findings demonstrate that mTORC-1/p70S6K signaling pathway in rat soleus muscle is activated following 24-h mechanical unloading. Increased activity of the mTORC1/p70S6K pathway at the initial stage of hindlimb unloading leads to the upregulation of E3 ligases MAFbx/atrogin-1 and MuRF-1 via nuclear export of HDAC5.

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Seasonal variation in blood pressure and orthostatic intolerance in Parkinson's disease

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A seasonal variation in blood pressure (BP) has been described, with BP being lower in summer than in winter, and orthostatic tolerance becomes worsen in summer and ameliorated in winter, for example, in elderly people, patients with hypertension, and others. It is not known whether patients with Parkinson's disease (PD), those have some autonomic dysfunction, show seasonal variation in BP and orthostatic intolerance, or not. To examine whether PD patients have seasonal variation in BP and orthostatic intolerance, we measured BP in patients with PD during a year. Subjects were thirteen (eight men and five women) PD patients with age of 77±6 yrs, disease duration of 3.5±1.7 yrs, and Hoehn-Yahr's stage of 3.0±0.6 (mean±SD). Once a month in during a year, subjects were asked to visit to the outpatient clinic, where they were required to sit on chair for 5 min, and then to stand-up actively within 5 s. The intermittent systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate (PR) were measured with sphygmomanometer. As a result, we observed a biphasic seasonal variation in blood pressure. The lowest level of SBP was observed in July (122.6±13.1mmHg) and the lowest level of DBP was observed in June (72.2±11.2mmHg). SBP was significantly higher in December (133.2±14.5mmHg) than in July and the highest level of DBP was observed in December (82.5±13.3). DBP decreased with standing in August and September. The PR had no seasonal variation. We concluded that patients with PD have seasonal variation in blood pressure and orthostatic intolerance.

Lung surfactant system in C57Bl/6 mice after long-term space flight onboard BIONM1 and ISS

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Lung surfactant (LS) is one of the most important components in respiratory system. The main function of LS is to reduce the surface tension at the air-liquid interface and, thus, prevent the expiratory collapse of the alveoli. Along with this, LS affects the oxygen transfer through alveolar-blood barrier, water exchange and functioning of the lungs innate immunity cells. It is still unknown how the long space flight affects LS properties. In the ground experiments (tail suspension model), we have shown earlier (Bryndina et al., 2013) that, in the early period of HS, surfactant functions are activated leading to the decrease of surface tension of bronchoalveolar lavage fluid (BALF), whereas long-term exposure to suspension significantly declines these properties. In BION-M1 mission we did not find a significant disturbance of LS function in 30-day space flown mice (Bryndina et al., 2014). In the present work we compared the effect of 30-day space flight onboard the BION-M1 biosatellite and a 37-day flight onboard the ISS (Rodent Research program, RR-1) on the composition, functional properties of pulmonary surfactant and expression of some enzymes regulating phospholipids metabolism in lung. The experiments were performed on C57Bl6 and C57Bl6 / J mice, the biomaterial (lungs) was kindly provided to us by IBMP and NASA. In both experiments, animals were divided into 4 groups: 1 - vivarium control (VC); 2 - basal control (BC, transportation to the spaceport and back); 3 – ground control (GC, 30- or 37-day stay in conditions similar to those in flight), and 4 - space flight (SF). All studies in the BION-M1 program were performed after 12 hr after landing, whereas in the RR-1 mission dissection was carried out directly onboard the ISS. In the BION-M1 project, there was a possibility to investigate BALF and lung tissue; in the RR-1 project, only lung tissue (frozen onboard the ISS and delivered to Earth) was studied.

In SF mice in BION-M1 group the activity of alveolar surfactant did not change, the amount of phospholipids in BALF increased, and expression of choline phosphate cytidylyltransferase as the main enzyme regulating phosphatidylcholine biosynthesis did not differ from the control level. The significant changes in the gravimetric indices were observed as well: dry residue was decreased, wet to dry lung mass and total lung fluid were increased. In RR-1 project we have shown the increased amount of total phospholipids in lung tissue of SF mice. At the same time the increased expression of the main enzymes involved in phospholipids metabolism was not found, although it does not exclude the changes in their activity. In the phospholipids spectrum, the increase of PEA and LPL as well as enhanced LPL / PC coefficient was detected. Simultaneously a decrease in the expression of beta 2-adrenoreceptors was found in lung tissue of SF animals. It is interesting to note that all detected changes were significant only in SF or GC groups in comparison with VC or BC experiments. At the same time, no differences

were found between VC and BC or GC and SF groups. The obtained results evidence that, in long-term space flight, LS is prone to alterations. Some effects may be considered as adaptive, but the negative consequences of LPL accumulation cannot be excluded.
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Body fluid distribution during artificial gravity using a segmental bioelectrical impedance analysis

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In recent years, many of the astronaut have stayed for long periods on the International Space Station, and astronauts would be exposed to a microgravity environment for an unprecedented period. Long term exposure to microgravity increases the risks of spaceflight deconditioning including cardiovascular, musculoskeletal, bone metabolism and neurovegative disorder. The cause of the spaceflight deconditioning has not been clarified, however, it appears to be mainly caused by cephalad fluid shift and gravitational unloading of the lower limbs. We examined the segmental body fluid distribution using a segmental bioelectrical impedance analysis (BIA) during artificial gravity of 1.0G at the heart level for 10 min by short arm centrifuge device. The impedance of the chest and upper arm area increased transiently after the onset of centrifuge. This signifies that the body fluid was decreased. Thereafter, body fluids remained low level throughout the centrifuge period for 10 min. On the other hand, the impedance of the abdomen area decreased (body fluid was increased) transiently after the onset of centrifuge and remained throughout the centrifuge period. The impedance of the thigh area gradually decreased (body fluid was increased) during centrifuge period. Body fluid distribution after artificial gravity quickly returned to the initial level. Therefore, we were able to confirm the fluids shift to the leg direction during artificial gravity. We were able to confirm the fluids shift during artificial gravity using segmental BIA and provide more information on the indices of orthostatic intolerance after spaceflight.

The influence of different HDACs on MuRF-1 and MAFbx mRNA expression in rat soleus upon 3- day hindlimb unloading.

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Muscle unloading leads to its atrophy development. The MuRF-1 and MAFbx E3-ligases expression is increasing under this condition. It has been shown that the histone deacetylases 1, 4 and 5 may regulate the expression of MuRF1 and MAFbx. We investigated the HDAC-dependent mechanism, which is involved in control of E3-ubiquitin ligases gene expression under at the early stage of muscle unloading. For this reason HDAC1 inhibitor (CI-994), HDAC4/5 inhibitor (LMK-235) and HDAC4 inhibitor (Tasqinimod (Tq)) were used. There is no specific HDAC5 inhibitor. The method of hindlimb suspension was described by Morey-Holton E & Globus R (2002). 40 males Wistar rats (180-200 g) were divided into 5 groups (n=8 in each): C-control, HS+CI-hindlimb suspension with CI-994 (i.p. 1 mg/kg), HS+LMK (i.p. 20 mg/kg), HS+Tq i.p. 10 mg/kg and HS -hindlimb suspension group with placebo administration. The animals were anaesthetized with an i.p. injection of tribromoethanol. (240 mg kg-1), soleus muscles were surgically excised, frozen in liquid nitrogen (Vilchinskaya NA et al., 2017). RT-PCR analyzes were done (three replicates of each sample). The Statistical analysis was performed using REST 2009 v.2.0.12 and Bio-Rad CFX Manager programs at the significance level set at 0.05. The significant differences between groups were statistically analyzed using Kruskal-Wallis rank test. All PCR data are expressed as median and interquartile range (0.25-0.75). CI-994 administration resulted in an attenuation of unloading-induced up-regulation of MAFbx ($p < 0.05$) but had no effect on MuRF-1 mRNA expression (which were equally increased in both unloaded groups relative to the C group. LMK administration resulted in an attenuation of MuRF-1 mRNA expression up-regulation, while MAFbx mRNA expression was increased dramatically compared to control (by 2 fold, $p<0.05$). Tasqinimod administration did not affect *E3-ligases* expression. MuRF1 and atrogin-1/MAFbx mRNA expression were significantly increased (by 1,5 and 4.0 Fold respectively, $p<0.05$) in both unloaded groups (HS and HS+Tq) relative to the C group. It could be concluded that 1) inhibition of HDAC1 prevented hindlimb suspension-induced up-regulation of MAFbx, but did not affect MuRF-1 mRNA expression; 2) The inhibition of HDAC4/5 take part in the regulation both MuRF1 and MAFbx E3 ligase expression; 3) the inhibition of HDAC4 alone did not influence on both E3 ligase mRNA expression under 3-days soleus unloading. Perhaps, HDAC 1 and 5 are more involved in the regulation of MuRF1 and atrogin-1/MAFbx mRNA expression than HDAC 4. This work was supported by Russian Science Foundation (grant № 18-15-00062).

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