PROCEEDINGS OF THE 2019 ISGP MEETING

Nagoya University, Japan - May 26-31, 2019

frontiers Event Abstracts

2019 ISGP MEETING

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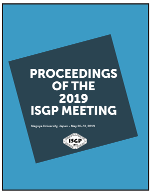

May 26-31, 2019, Nagoya University, Japan

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Welcome to the 2019 ISGP Meeting

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

We are very happy to welcome you this year for the 40th Annual Meeting of ISGP together with the "Space Life Science and Medicine" in Japan at Nagoya University.

see:
https://www.frontiersin.org/events/40th_ISGP_annual_meetingThe_International_Society_for_Gravitational_Physiology/6802

THE LOCAL ORGANIZER

Prof. Satoshi Iwase

LIST OF ORGANIZERS

Charles Fuller, University of California, Davis, United States,
Martina Heer, University of Bonn, Bonn, Germany.
Marc-Antoine Custaud, Université d'Angers, Angers, France

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

Our 40th ISGP annual meeting, together with the Space Life Science and Medicine, was hosted by Nagoya University at Noyori Conference Hall in Japan from May 25th to 31st 2019.

This conference was integrated with the Japan Association of Physiology, the Japan Association of Aerospace and Environmental Medicine and the Japan Aerospace Biological Society.

During this meeting, recent topics of microgravity-associated systemic changes were extensively discussed in space medicine and physiology.

Effects of microgravity (μG) on human physiology

Effects of microgravity (μG) on human physiology has been investigated for several decades, which includes the neurovestibular, cardiovascular, musculoskeletal, bone metabolic, and immune-hematological systems. Recent studies will be briefly discussed.

1. **Neurovestibular system**
   Space motion sickness has been a potential obstacle after the exposure to μG. Among mechanisms, four hypotheses have been proposed, 1) sensory conflict, 2) fluid shift, 3) otolith asymmetry, and 4) orientation adaptation. Until now, it was unclear which of these hypotheses was most likely, however, evidence from Space Shuttle missions suggests that 3) & 4) are unlikely.

2. **Cardiovascular system**
   The changes in the cardiovascular system begin solely with the fluid shift associated with μG, followed by the decreased blood volume, cardiac size, and aerobic capacity. The reduced blood volume after adaptation to μG is the result of 1) a negative balance of decreased fluid intake and smaller reduction of urine output, 2) fast fluid shifts from the extravascular to intravascular space, 3) fluid shifts from intravascular to muscular interstitial space due to the decrease in the muscle tension.

   In addition to the well-known deconditioning, spaceflight-associated neuro-ocular syndrome (SANS), and brain structural shrink during spaceflight.

3. **Musculoskeletal system**
   Disuse muscle loss has been concerned since Skylab and Space Shuttle missions, whose main cause is the disappearance of mechanical constraints under μG, due to gravitational influence on the genes regulating the protein synthesis of muscle protein degradation enzymes. Both splint and aerobic trainings are necessary to prevent the muscle loss of type II white fibers and type I red fibers respectively. Recent studies have suggested the mechanism of disuse

amytrophy, especially oxidative stress, to be an important regulator of pathways leading to amytrophy during periods of disuse. Redox disturbances, such as those in myotubes, increase the expression of key components of the proteasome proteolytic system, which is a prominent factor in protein degradation in disused muscles. Another hypothesized mechanism is the degradation of muscle proteins resulting from their ubiquitination. These molecular mechanisms underlie protein degradation during disuse.

4. **Bone metabolic system**

Bone development and restructuring Bone tissue contains osteocytes, which develop from osteoblasts, and are changed into osteoclasts by the action of RANKL. The balance of bone formation and resorption is controlled by the balance of hormonal changes in calcitonin and parathormone (PTH). Several studies suggested that mechanical impact per se is not the direct stimulus for bone remodeling, while PTH seems to be responsible for the balance of bone formation and resorption. Although chronic increases in PTH increase bone resorption, intermittent stimulation accelerates bone formation. PHT stimulates osteoclast formation by binding to PTH receptor 1 on stromal/osteoblastic cells and thereby increases the production of receptor activator of RANKL and M-CSF and suppresses the RANKL decoy receptor osteoprotegerin. Moreover, PTH controls the production of osteoblasts through actions on osteocytes through Wnt signaling in osteoblastogenesis. The action of osteocytes, which can directly sense a mechanical unloading stimulus, increased the expression of both inhibitors of bone formation and stimulators of bone resorption through Wnt signaling. These results support the hypothesis that intermittent mechanical impacts induce osteocyte action, which inhibits bone formation and stimulates bone resorption, and that an intermittent increase in PTH controls the production of osteoblasts. Although bisphosphonates administration might be a promising candidate to prevent space bone loss, a combination of PTH should be considered in addition to the mechanical impact countermeasures.

5. **Immunology and hematology**
   Mechanical impact reduction also results erythropoietin levels during spaceflight, which induces "space anemia" after long duration of spaceflight. Since RBC life span is 120 days, countermeasure for this anemia should be considered. Another problem is T-helper cell suppression under microgravity. Viral infections or tumor production should be concerned.

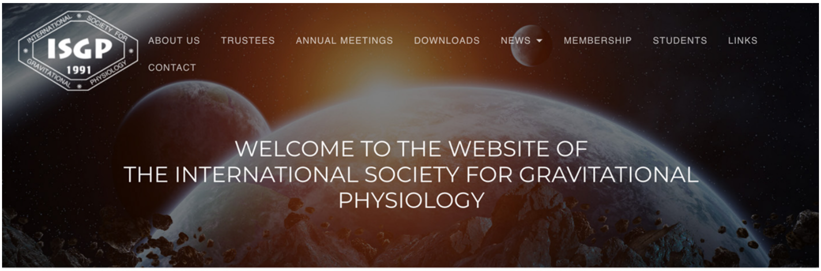
6. **Autonomic nervous system**
   Under microgravity, it has been demonstrated that sympathotonic condition probably due to vagal suppression or stress-induced sympathoexcitation occurs continuously. This might cause cardiovascular and thermoregulatory deconditioning. This should be ameliorated by vagal activation using respiratory control, meditation or aerobic training.

7. **Artificial gravity**
   We have been reporting that the artificial gravity (intermittent or continuous) by centrifuge with exercise suppresses the above mentioned deconditioning. Effective protocol should be established in the near future. In application of artificial gravity with exercise, there would be two ways to apply artificial gravity, one is to put a rotational centrifuge device on board spacecraft, and the other is to rotate spacecraft itself. We think that we should adopt the method of rotating spacecraft itself, since it is rejected because it spoils the structure of the ISS. For that, we should promote the project of rotating the Gateway itself.

FOR THE LOCAL ORGANIZING TEAM

Satoshi Iwase, MD & PhD
Department of Physiology,
Aichi Medical University,
Japan.

You are invited to visit our ISGP web-site!



About ISGP

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

www.isgp-space.org

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- Get news such as books, events, ...
- Register for free membership
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- Download *Journal of Gravitational Physiology* archives
- And a lot more!

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

Final Programme

Sunday, May 26, 2019

19:00 – 21:00 Welcome reception & 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], 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]

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], 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], Nobutaka Tsujiuchi[1,3]

1. Graduate School of 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], 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

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], Tetsuya Izawa[1,2,3]

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2. Faculty of Health and Sports Science
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4. Organization for Research Initiatives and Development, Doshisha University, Kyotanabe City, Kyoto 610-0394, Japan

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: Neuroscience13:30 – 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], *Patrick M. Fuller*[2]

1. Department of Neurobiology, Physiology, & Behavior, University of California, Davis. Davis, CA, 95616 USA
2. 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-tofast 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]

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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]

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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 Physical 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, Frankfurt/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 Biomedical 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,

09:00 – 09:30 **The Immune-cell skeleton under µG -Stress – a biomechanical view**

N.Li, M.Log

09:30 – 10:00 **B lymphopoiesis under simulated microgravity, anexample of accelerated aging?**

J.P. Frippiat

10:00 – 10:30 **Neural pathways regulate immune cell-gateways in the CNS**

M. Murakami:

10:30 – 11:00 **Coffee break**

11:00 – 11:45 **Hypersensitivities in Space and in Space Analogues**

A. Choukèr

11:45 – 12:30 **Interdisciplinary/Operational versus Specific Immunological Countermeasures for Deep Space Exploration**

B. Crucian

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

15:45 – 16:00 **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]

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

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

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], *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

Oral 12: 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 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], 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 TCRrepertoire and increase its self-reactivity

Coralie Fonte[1], Sandra Kaminski[1], Anne Vanet[2,3], Stéphanie Ghislin[1], 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, 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], *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] *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 C57Bl/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

Extended Abstracts of 2019 ISGP Meeting

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

Davide Barbero[1], Matteo Devecchi[1]*, Lorenzo Rabagliati[1], Guillaume Thirion[2]

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INTRODUCTION

Given the current global movement towards the Moon driven by the Global Exploration Roadmap, a Moon permanent settlement will be a reality in the near future. A Lunar outpost supporting In-Situ Resource 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 pressure 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, and to decrease the mission cost, in order to have efficient operations.

METHODOLOGY

The selection of the design atmosphere composition and pressure level of a pressurised structure is a problem which falls into multi-objective optimisation and multi-criteria decision analysis (Figure 1). In order to obtain the design solution, the starting point is the definition of the design space, that is the space in which the variables can vary within the boundaries set (Table 1). Then an optimisation is performed. Three different gases, Nitrogen, Helium and Neon, are analysed. After the optimisation, a set of optimal solution is obtained and then a trade-off of the solutions obtained is performed, from which the design point is extracted (Table 2).

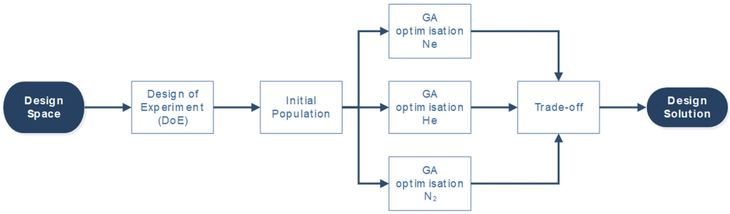

FIGURE 1: Methodology workflow.

Table 1: Design space boundaries

Variable	Lower bound	Upper bound	Unit
Cabin Pressure	50.39	101.33	kPa
Suit Pressure	25.90	57.20	kPa
Oxygen Concentration	0.17	0.35	-
Supersaturation ratio	1	1.4	-

Table 2: Atmosphere selection results

Parameter	Value	Unit
Cabin Pressure	62.37	kPa
Suit Pressure	43.87	kPa
Oxygen Concentration	29.50	%
Supersaturation ratio	1.002	-
DCS probability	0.09	%
Pre-breathing time	0.27	Minutes
Leakages	30.23	$kg/s/m^2$
Emergency time	9.63	minutes
Cost	$6.41*10^7$	$

Starting from the design space, different boundaries are defined. Four variables are used: Oxygen percentage, total pressure of the crewed modules, EVA suit pressure and Supersaturation ratio after pre-breathing. First of all, oxygen percentage and total pressure are bounded by the normoxic equivalent, that is defined as the oxygen partial pressure in the cabin that will keep the same alveolar oxygen pressure as the one at sea level on Earth. These two variables are defined also by the hypoxic equivalent, in which the alveolar partial pressure is the one before hypoxia occurs. The oxygen percentage is also limited by material flammability, that is a constraint on the materials. The maximum percentage of oxygen is set to 35%, according to the literature (Abercromby, 2014). Boundaries are also defined in terms of EVA suit pressure, based on current technologies, ranging between EMU and Mark III suit, and Supersaturation ratio after pre-breathing, setting the maximum acceptable probability of Decompression Sickness.

Multi-objective optimisation based on genetic algorithms is used to obtain the set of optimal solutions that minimise or maximise the objective functions. The chosen objective functions for this study are: DCS probability, pre-breathing time, that is an inefficient crew time, leakage rate, that is a loss that will have consequences on logistics and on cost, and the crew performance, defined as glove dexterity. Indeed, this last point is one of the most challenging aspects to increase the efficiency of astronauts on EVA.

From the set of optimal solutions obtained from the optimisation for the three gases, there is a need to extract the design point. In order to do so, a trade-off is performed through a multi-criteria decision analysis method, the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS). Six figures of merit are identified and prioritised with Analytic Hierarchy Process methodology (Table 3). The most important are DCS probability, cost, related

Table 3: Trade-off figures of merit weight

Figures of merit	Weight
Cost	15.64%
DCS criticality	56.46%
Emergency capability	10.38%
EVA performance	11.10%
Sound pressure level	2.46%
Voice frequency	2.31%
Comfort	1.64%

to leakages and pre-breathing time, and emergency time, defined as the time before the hypoxic level is reached when a failure in the sealing occurs or there is a puncture due to a micrometeoroid. Other figures of merit considered are EVA performance, sound pressure level, voice frequency and comfort.

RESULTS

Neon has shown to be the best gas in term of DCS probability thanks to its low solubility in the body fluids, in compliance with the literature. As a consequence, Neon is probably the safest inert gas and that confirms some preliminary studies that a Neon-Oxygen mixture leads to a lower danger of bends compared to the Nitrogen-Oxygen one. Wash-out time is lower if Helium and Neon are used as inert gases instead of Nitrogen.

The total pressure inside the cabin is 62.37 kPa (Figure 2), which is much lower than the total pressure at sea-level (SL) condition. At standard conditions on Earth (21% Oxygen concentration), this total pressure is at an altitude of 3909.6 m (12824.8 ft). However, the concentration of Oxygen is much higher than in SL condition, being approximately 30%, so that the equivalent altitude on Earth would be about 1200 m (3950 ft). As a consequence, the crew could face Acute Mountain Sickness (AMS) symptoms due to a rapid

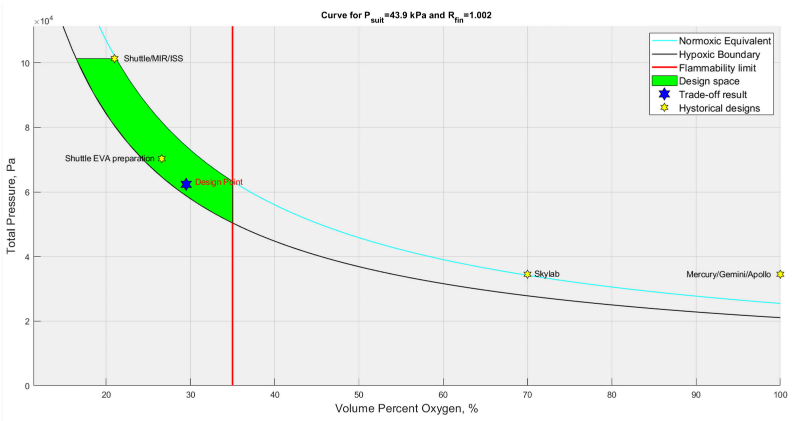

FIGURE 2: Trade-off result compared to historical data.

pressure change between the lunar ascent and descent module and the Lunar Ground Segment.

The consequences of the figures of merit are described hereafter. The trade-off has identified a level of suit pressure equal to 43.87 kPa. This level of pressure is much higher than in Apollo suits and in the current EMU suits, but it allows to maintain the likelihood of DCS and serious DCS very low, without pre-breathing time. Solutions exist if a better dexterity is needed. Concerning the leakages, the selection of Neon leads to a benefit on steady-state leakage compared to the commonly used Nitrogen. Pre-breathing time and leakages have been considered in terms of cost. Neon has shown to be a cost-effective solution, despite being the most expensive gas. This is due to the fact that there is a zero pre-breathing time. Moreover, the leakages in mass are lower for Neon than for Nitrogen. Since Neon has a similar density of Nitrogen, there is no distortion of the voice, as occurs in case of the Helium-Oxygen mixture. However, a total pressure of 62.4kPa inside the cabin leads to a noise level increases due to the higher fan work and the sound pressure level, which is directly proportional to the capacity of the air drum to hear the sounds, decreases.

CONCLUSIONS

As Neon has been selected because of its engineering advantages, it has some open points. First, the lack of Nitrogen in the atmosphere may alter physiological processes that require it. Moreover, there is no experience in long term exposure of humans to a mixed Oxygen/Neon atmosphere. However, the first problem can be addressed through an atmosphere composed of three gases, namely Oxygen, Neon and Nitrogen, if Nitrogen is shown to be essential. The second issue can be analysed through testing in a controlled environment, starting from experiments with cells cultures. Another concern may be the psychological acceptance of the crew to the new gas mixture.

Another problem may be the high-pressure suit, as it may affect the performance of astronauts during EVA activities. This, however, can be tackled through different technologies. For example, a variable pressure suit, in which the pressure is reduced for a short time during the most demanding activity without increasing the DCS probability, can be used.

Keywords: Decompression Sickness, Multi-objective optimisation, Atmosphere selection, Moon habitat, Neon

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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 (Figure 1). 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. Importantly, techniques and devices to farm cultivars in spacecraft, perfectly closed environment, could be very useful to develop plant industries, especially in the polluted area on the earth as well.

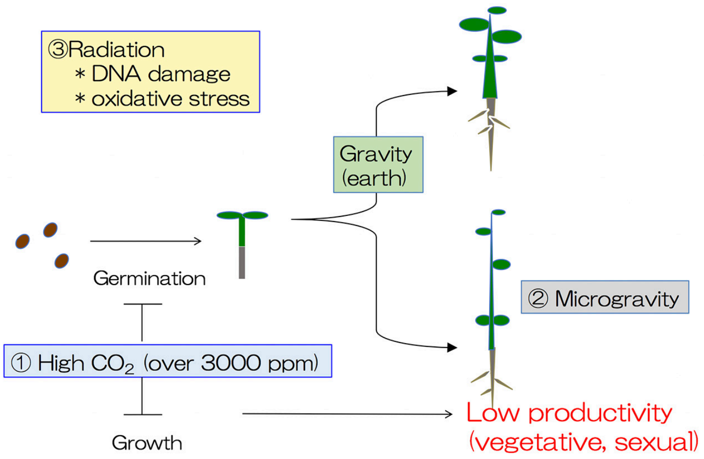

FIGURE 1: Environmental factors that affect plant growth and development in the space farm.

RESULTS AND CONCLUSIONS

To clarify the underlying molecular mechanisms and improve plant growth and development in space, we focused on two of key issues; the molecular mechanisms underlying gravity sensing, and the impacts of extremely high CO_2 conditions. To demonstrate the role of Ca^{2+}-signaling in plant gravity sensing, monitoring of the changes in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) induced by gravistimulation in Arabidopsis seedlings expressing aequorin, a Ca^{2+}-reporting photoprotein were performed. By turning from upside-up to upside-down position (+180°), the seedlings showed a biphasic $[Ca^{2+}]_c$-increase, that is composed by a fast-transient and a following slow $[Ca^{2+}]_c$-increases [1]. The following pharmacological and kinetic analysis revealed that the first, fast-transient $[Ca^{2+}]_c$-increase depends on the rotational velocity but not on the rotational angle, whereas the second, slow $[Ca^{2+}]_c$-increase depends on the rotational angle but not the rotational velocity, and MS channels play a central role in the gravistimulation-induced increased in $[Ca^{2+}]_c$-increase [1, 2]. Among the known MS channels in Arabidopsis, MCA1 has been functionally characterized as a plasma membrane Ca^{2+}-permeable MS cation channel [3, 4], that could be potentially involved in the gravity sensing and the following morphological changes in Arabidopsis seedlings.

Elongation of petioles, one of the events of spindly growth, is apparently promoted in Arabidopsis seedlings that grown under the extremely high CO_2 conditions (4000 ppm), with a reduction in fresh weights (approximately 20 %). To clarify the underlying molecular mechanisms, we performed transcriptome analysis of Arabidopsis seedlings, and found that some key element genes that promote spindly growth are up-regulated under the high CO_2 condition. Based on those data, we performed growth assays with different composition of hydroponic nutrient supply, and revealed that a simple modification could attenuate the spindly growth with an increase in flesh weights, improving the yield. Furthermore, Ca^{2+}-signals against some kinds of biotic and abiotic stresses and contents of antioxidants are up-regulated in high CO_2-grown seedlings, suggesting that extremely high CO_2 conditions in ISS affect plant growth and developments in multiple aspects.

ACKNOWLEDGMENTS

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Keywords: Plant gravity sensing, Ca^{2+} signaling, Space farm, high CO_2

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Influences of microgravity on the crosstalk between muscle and bone

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Sarcopenia is characterized by progressive and systemic decrease in skeletal muscle mass and function. Recent numerous clinical evidence indicate that the decreases in muscle mass, strength and function are related to decreased bone mineral density (BMD) and an increase of fracture risk, indicating that sarcopenia is linked to osteoporosis. It has been recently noted that there are some interactions between skeletal muscle and bone. Genetic, endocrine and mechanical factors affect both muscle and bone. Local and remote interactions are included in muscle/bone relationships. Local mechanical stimulation and paracrine factors are included in the local interactions. As remote interaction, we can raise humoral factors, neuronal regulation and blood supply. Numerous humoral factors produced from muscle or bone tissues affect each other. Muscle-derived factors, myokines, include myostatin, insulin-like growth factor (IGF)-I, fibroblast growth factor (FGF)-2, interleukins (ILs), irisin and so on. Bone-derived factors, osteokines, include sclerostin, osteocalcin, prostaglandins and BMPs (Figure 1) [1].

As for osteokines affecting muscle, osteocalcin secreted from bone enhances glucose uptake into muscle fibers and subsequent muscle function through its receptor in mice [2]. Osteocalcin secreted from osteoblasts directly stimulates muscle as well as indirectly exerts positive effects on muscle partly through enhanced androgen secretion from testis and enhanced insulin secretion from pancreas in mice. On the other hand, sclerostin secreted from osteocytes suppresses bone formation by inhibiting canonical Wnt-β-catenin signaling. A recent study showed that anti-sclerostin antibody improves muscle wasting and a decrease in muscle function through an inhibition of TGF-β-mediated NFκB pathway and p38 mitogen-activated kinase (MAPK) in breast cancer model mice [3]. These findings suggest that sclerostin secreted from osteocytes might suppress muscle as well as inhibit bone formation. There have been several evidence suggesting roles of sclerostin in mechanical stress- or gravity change-induced muscle and bone changes.

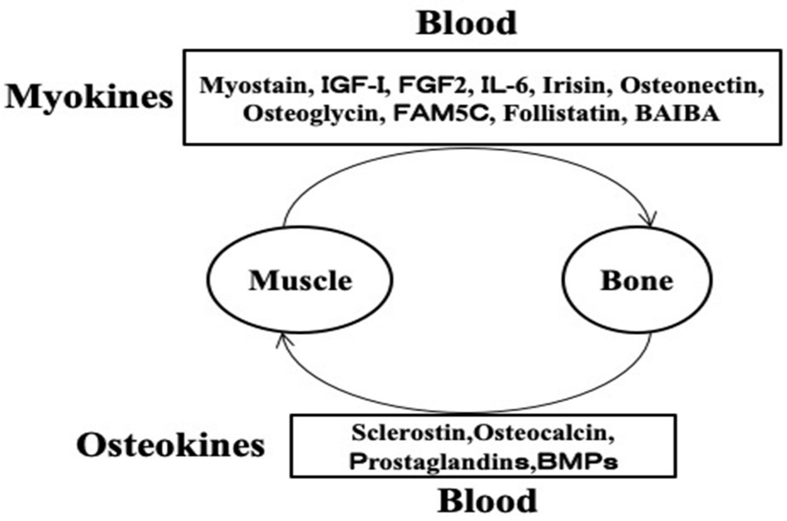

FIGURE 1: Linkage between muscle and bone. Humoral factors secreted from skeletal muscle and bone influence bone and muscle, respectively, in physiological and pathophysiological states.

Myostatin, expressed mainly in skeletal muscle tissues, is a potent suppressor of skeletal muscle mass. It binds the activin receptor type IIB, then phosphorylates Smad2/3, TGF-β-specific Smads. Previous study indicated that myostatin enhances osteoclast formation in a mouse model of rheumatoid arthritis [4]. In that study, myostatin deficiency caused an improvement of arthritis and less bone destruction. Myostatin might function as a humoral factor affecting the distant organs as well as directly affecting muscle in the pathological state. Myostatin is an important myokine that negatively regulates bone. Microgravity induces dysregulation of cardiovascular system, muscle atrophy and bone loss in astronauts. Microgravity-induced bone loss has been considered to be due to enhanced bone resorption and decreased calcium absorption. However, the precise mechanisms by which the gravity changes affect muscle and bone have remained unknown. The previous studies suggest that baroreceptor and vestibular–sympathetic reflexes are involved in the control of arterial blood pressure during hypergravity in rats. Moreover, gravity changes induce plastic alteration of the vestibular function, such as vestibular-sympathetic reflex. Moreover, labyrinthectomy or vestibular lesion reduces BMD through the sympathetic nerve system in rodents. Taken together, we hypothesized that vestibular system might play some roles in the changes of muscle and bone induced by gravity changes. We therefore investigated roles of vestibular system in the effects of hypergravity on muscle and bone using vestibular-lesioned mice. Mice 2 weeks after bilateral vestibular lesions were kept in 1 or 2 *g* environment using the centrifuge machine for 2 or 8 weeks [5]. Hypergravity with 2 *g* for 2 weeks enhanced osteoblast differentiation partly through the vestibular system in mice, although hypergravity with 2 *g* or vestibular lesion did not affect the expression of myogenic genes in the soleus muscle of mice. The vestibular system might contribute to adaptive responses in bone tissues by affecting osteoblast differentiation during gravity change in mice. However, hypergravity with 2 *g* did not affect muscle mass and bone mass in mice in this study. Therefore, higher gravity might be necessary for the induction of muscle and bone mass change, especially for muscle change. We therefore performed similar experiments using hypergravity with 3 *g* for 4 weeks in mice [6]. Vestibular lesion attenuated the increases in tibial and soleus muscle masses induced by hypergravity. Moreover, vestibular lesion attenuated the hypergravity-induced increases in cross-sectional area of myofibers and the expressions of MyoD, Myf6 and myogenin, muscle differentiation genes, in the soleus muscle. Vestibular lesion attenuated the increase in trabecular bone mineral content (BMC) induced by hypergravity. The previous evidence suggest that gravity changes affect sympathetic outflow in rats, and vestibular system links to sympathetic nerve system physiologically. Our data showed that proplanolol, a sympathetic β blocker, attenuated the increases in tibial muscle mass, muscle size and MyoD expression induced by hypergravity in the soleus muscle, indicating that hypergravity enhances muscle mass through the sympathetic system. Taken together, our data indicated that hypergravity affects muscle and bone through vestibular signals and subsequent sympathetic outflow in mice.

Next, we extracted FK506 binding protein (FKBP5) as a gene whose expression was enhanced by hypergravity through vestibular system in the comprehensive DNA microarray analysis of the soleus muscle of mice [7]. Stable FKBP5 overexpression enhanced the expression of myosin heavy chain and muscle protein synthesis mTOR pathway in mouse myoblastic C2C12 cells. Moreover, stable FKBP5 overexpression suppressed the expression of muscle protein degradation-related genes, such as atrogin-1 and Murf-1, in mouse myoblastic cells. Taken together, our data suggest that FKBP5 induced by hypergravity through vestibular system enhances muscle mass in anti-gravity muscles of mice.

Next, we examined the effects of hypergravity and vestibular lesion on the expresions of humoral factors linking muscle to bone in the soleus muscle of mice [8]. We revealed that vestibular lesion attenuated the expression of follistatin enhanced by hypergravity in the soleus muscle, suggesting the possibility that follistatin is involved in the influences of hypergravity on muscle and bone through the vestibular system in mice. Then, we examined the effects of simulated microgravity on the expression of follistatin in mouse myoblastic C2C12 cells using the clinostat device. Microgravity decreased follistatin expression in C2C12 cells, suggesting that gravity change regulates follistain expression in muscle. Follistatin is expressed in all tissues, and previous evidence indicated that follistatin increases muscle mass and regeneration after injury presumably directly antagonizing activin and/or myostatin-induced phosphorylation of Smad2/3. Our experiments showed that follistatin increased the phosphorylation of Akt and p70 S6K in C2C12 cells. Moreover, vestibular lesion antagonized follistatin level as well as the phosphorylation of Akt and S6K enhanced by hypergraivity in the soleus muscle of mice. These data suggest that follistatin is involved in hypergravity-enhanced muscle mass by directly affecting muscle cells. Vestibular lesion seemed to attenuate serum follistatin levels enhanced by hypergravity in the soleus muscle, although hypergravity and vestibular lesion did not affect the expression of follistatin in liver and tibia. This data suggest that the change of circulating follistatin level by hypergravity is due to skeletal muscle-derived one. Follistatin might affect bone as a myokine in hypergravity. Our data showed that follistatin attenuated osteoclast formation enhanced by myostatin in the presence of receptor activator of nuclear factor kB ligand (RANKL) in mouse monocytic Raw264.7 cells, suggesting that follistatin exerts bone anabolic action through the suppression of osteoclast differentiation by antagonizing myostatin action. Moreover, serum follistatin levels were positively related to trabecular BMC, but not cortical, in the tibia of mice in the simple regression analyses of the samples of mice used the experiments. Taken together, our data indicated that hypergravity increases follistain expression in skeletal muscle partly through the vestibular system in mice. Follistain might play some roles through both the direct action on muscle and the actions on bone as a circulating myokine in the regulation of muscle and bone in response to gravity change through vestibular signal.

Exercise enhances the expression of irisin in muscle through PGC-1α. It is well known that irisin induces a browning response of white adipose tissues. Irisin may be related to a reduction in insulin resistance and cardiovascular disease incidence in clinical studies. As for the effects of irisin on bone in mice, irisin enhances osteoblast differentiation through canonical Wnt-β-catenin and

MAPK pathways, although irisin suppresses osteoclast formation by affecting RANKL/NFATc1 pathways. These effects lead to the increases in bone mass and strength in vivo. We next examined the effects of mechanical unloading on the myokines linking muscle to bone using hindlimb unloading and sciatic neurectomized mice [9]. Our data showed that mechanical unloading reduced muscle weight and trabecular BMD in mice. Mechanical unloading decreased irisin expression in muscle. Irisin dose-dependently suppressed osteoclast formation from mouse bone marrow cells in the presence of macrophage-colony stimulating factor and RANKL. Moreover, irisin reduced RANKL expression in mouse osteoblasts. In addition, the simple regression analysis using mouse samples showed that irisin expression in the soleus muscle was positively and negatively related to trabecular BMD and RANKL expression in the tibia of mice, respectively. In summary, our data with the other experiments indicated that mechanical unloading and immobilization reduces irisin secretion in the skeletal muscle partly through decreased bone morphogenetic protein and mTOR signals in muscles, then leads to osteopenia. Our data suggested that irisin might be involved in unloading-induced osteoporosis in mice.

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Keywords: Gravity, Muscle, Bone, Vestibular system, Myokine

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Study of human gait characteristics under different low-gravity conditions

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INTRODUCTION

Future space exploration missions are being planned for both The Moon and Mars, and stress the need for a better understanding of the effects of low-gravity on human physiology [1-3]. Various studies are currently being performed in our laboratory in the context of the Doshisha Space-DREAM Project which promotes space exploration research. The objective of this study was to perform detailed low-gravity gait analyses on the AlterG anti-gravity treadmill which we have already used in the past to study joint angles and electromyogram activities [4, 5].

METHODS

The AlterG treadmill features an enclosure that surrounds the subject's lower body and the treadmill itself. The enclosure is sealed around the subject's waist, and by regulating the pressure inside of it hypogravity can be generated. The presence of that enclosure prevents the use of optical motion capture systems, so we have designed our own wearable measurement device in order to perform low-gravity analyses on this treadmill [5, 6]. This device consists in a pair of instrumented sandals and of five sensor units (with compass, acceleration, and gyro sensors in each unit). The shoes have forceplates implemented in their soles to measure ground reaction forces and the sensors measure the movement. We performed two rounds of experiments using these devices together. The first focused only on the ground reaction forces and joint angles (measured with the wearable device), as well as lower-leg muscle activity (measured with surface EMG sensors). Five subjects performed walking trials on the treadmill at 3.5 km/h and under five different gravity conditions: 100%, 80%, 60%, 40%, and 20% of body weight (BW). The second round of experiments was meant to perform a more thorough analysis and look at additional parameters through inverse dynamics simulations. The method of this experiment was similar to the previous one, with five other subjects, but the gravity conditions were chosen to focus more on the low-gravities (100% as a reference, and then 65%, 50%, 35%, and 20%). The EMG sensors were also used in this experiment. The data obtained through the measurement device was adapted to create simplified models of the subjects' gaits and then uploaded to the SIMM software to run simulations and study joint angles, joint moments, muscle activations, and muscle forces. One of the main focuses of this second experiment was to compare the surface EMGs and the muscle activations of SIMM for the soleus and gastrocnemius muscles, as they both express muscle activity.

RESULTS

The results of the first rounds of experiments showed various changes in the joint angles with the reduction in BW: an increase in the plantar flexion peak, a decrease in the knee flexion peak, and an overall decrease of the hip movement range. The swing phase also appeared to progressively lengthen, which is in accord with literature. Overall, significant changes in joint angle characteristics appeared between 60% and 40% of BW. The results of the second rounds of experiments showed the progressive evolution of each parameter with the decrease in BW. The results obtained for joint angles confirmed the ones obtained in the previous experiment, with the same trends appearing, and the joint moments appeared to decrease almost linearly. The muscle activations tend to saturate for the highest BW values (100% and 80%) while the surface EMGs do not and have a different shape (especially for the soleus muscle). The two signals also seem to have very different scales, with the muscle activations going up to 1 while the EMGs do not go above 0.35. Despite their differences, both expressions of muscle activity displayed a common trend: where the gastrocnemius and soleus activity usually only presented a single peak of activation under Earth gravity, the shape of the muscle activity signals evolved at lower gravities and displayed a second peak of activity earlier in the gait cycle. More precisely, it seems that a local minimum in muscle activity appeared between 10% and 35% of the gait cycle when reducing the gravity below 50% of BW, thus giving the impression that the signal presented two peaks of activity, before and after that local minimum.

DISCUSSION

The results obtained for the joint angles and the length of the swing phase are in accord with literature, and confirm that the wearable device is suitable to study low-gravity gait. The big differences observed between the two expressions of muscle activation seem to come from their respective definitions. On one hand, the surface EMGs measure through the skin the electric signal that activates the muscle; on the other hand SIMM's muscle activations seem to correspond more to the movement of the muscle itself. Both of these parameters are normalized, but it is likely that their normalization methods are different, which explains the difference in their scales. The change in the pattern of muscle activity is likely connected to the natural change in gait of choice that comes with low gravity. Indeed, literature shows that under gravity conditions such as the ones of The Moon and Mars, walking is not necessarily the optimal gait of choice anymore (several gaits competes on Mars, and skipping is overall the most efficient one on The Moon) [7-10]. In this experiments, the subjects had to use the walking gait for all BW conditions; therefore, their body adapted to the changing gravity by adapting the walking gait and the muscle activation pattern. The phase of the gait during which these changes were significant was between 10% and 35% of the gait cycle, which corresponds to the initial and medium stances of the single support phase of gait. The soleus and gastrocnemius muscles being strongly related to the ankle joint, we have looked into the kinematics outputs that were obtained for this joint between 10% and 35% of the gait cycle. During this phase, under normal conditions, the ankle angle remains almost constant; however, for the lowest gravity conditions, the results show that this angle progressively decreases. Meanwhile, the ankle moment decreases in an almost linear fashion under all conditions. These observations confirm that the ankle joint kinematics are directly affected by the change in the muscle activation pattern. Therefore contraptions affecting the ankle joint could play an important role in counteracting these changes caused by the low-gravity environment.

CONCLUSION

The study of two different expressions of muscle activity showed a change in the muscle activation pattern at the lower gravity conditions. This change comes from the reaction of the human body trying to adapt to a new gravity environment in which walking is not the optimal gait anymore. Countermeasure exercises should take this phenomenon into consideration and target the single support phase where that gap in muscle activity appears.

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Keywords: Low-gravity environment, kinematics, Muscle activation, Wearable Device, Inverse dynamic analysis

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Changes in the mice bone tissue elements content under hypergravitation

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INTRODUCTION

A negative calcium balance was revealed during the examination of astronauts at the initial period of regular space flights. As the methods of intravital densitometry of bones were introduced into practice, it was specified to what extent this finding reflects the degree of demineralization of the skeleton [1], [2]. The revealed changes moved specialists in the field of osteology to study the mechanisms of the osteopenia occurrence in zero gravity. A biomechanical hypothesis, which was based on a shortage of mechanical load, and metabolic hypothesis, associated with the redistribution of fluid in the cranial direction, were proposed [3], [4].

To confirm the hypotheses explaining the revealed changes, as well as to develop countermeasures, experiments with animals are being and were conducted in real and simulated microgravity conditions. The model of orthostatic hypokinesia in the case, was used for a long time, then it became more preferable to use anti-orthostatic hypodynamia - the “hanging” model [5].

The Institute of Biomedical Problems in the 80s years of last century suggested that the stopping of action of increased weight on the body (30 days, 1.1g and 2.0g) can be considered as a model of weightlessness (RRW - relative reduced weight) [5]. It is also concluded that intermittent centrifugation can be used as a means to create artificial gravity in space flight [6], [7].

At the Crimean State Medical University named after S.I. Georgievsky, the histomorphometric parameters and indicators of the mechanical-plastic properties of rat bones with more significant (9g) overloads were being studied in 2009-2011. The dependence of the mechanical-plastic properties and the state of the mineral component on the age of the experimental animals and on the multiplicity of hypergravity effects was determined [8].

MATERIALS AND METHODS

The experiment was conducted at the Jean Monnet University (Saint-Etienne, France). The objects of our osteological study were the calvaria bones of mice. Animals of the "2g" group were exposed to a 30-day rotation in a centrifuge, which allowed maintaining a constant level of hypergravity (2g). The centrifuge is a carousel with a radius of 1.4 m; eight aluminum capsules with a volume of 173 liters hang on its periphery. Three cages with 4 mice can be placed in each capsule. Biomaterial collection in group 2g was conducted immediately after centrifugation.

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

Animals of both groups were kept in cages, four mice in each cage, at a temperature of 22°C with a controlled light cycle (12 hours of light / 12 hours of darkness). All capsules had a video surveillance system to monitor both food and water supplies.

The control group mice were kept in vivarium for 30 days.

The procedures for the care and killing of the animals were in accordance with the European Community Standards on the care and use of laboratory animals (Ministère de l'Agriculture, France, Authorization 04827). All animal experiments were approved by the local Animal Care Committee.

To determine the content of elements (Ca, Mg, P, Zn) in the calvaria bones, a laser-spark emission method was used, similar to the X-ray fluorescence control method, but with a higher sensitivity, and the laser-induced breakdown spectroscopy (LIBS) of the elemental composition developed in FSBI "SPA Typhoon" (Obninsk) allowing implementation of the method. To study the elemental composition of biological samples using LIBS, the measurement technique is specially adapted. The results obtained are statistically processed using variance analysis.

RESULTS AND DISCUSSION

A decrease in the percentage content of all determined elements in the calvaria bones at the 30-day hypergravity was found for the first time. Similar losses were observed for the elements included in the composition of hydroxyapatite (Ca (Fig. 1), P (Fig. 2)) (12% and 11%, respectively). Losses of Mg and Zn were more significant and similar (20% each).

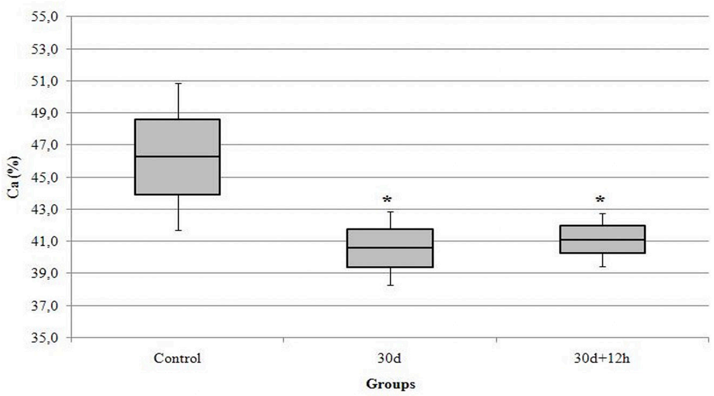

FIGURE 1: Changes in calcium in hypergravity 2g.

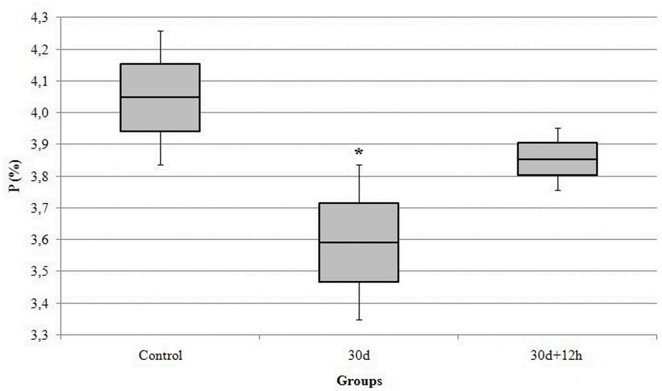

FIGURE 2: Changes in phosphorus in hypergravity 2g.

Readaptation within 12 hours revealed interesting features: Mg recovered during this time by 7.2% (Fig. 3), a relatively rapid recovery was also observed in P (6.4%) (Fig. 2). Zn (3.3%) recovered twice as slowly (Fig. 4). The rate of Ca recovery was the lowest (1.2%) (Fig. 1).

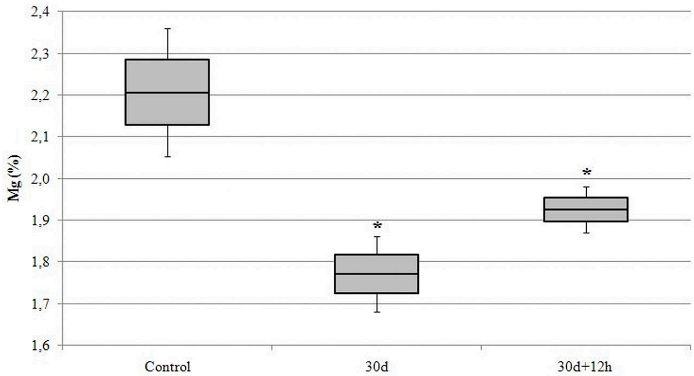

FIGURE 3: Changes in magnesium in hypergravity 2g.

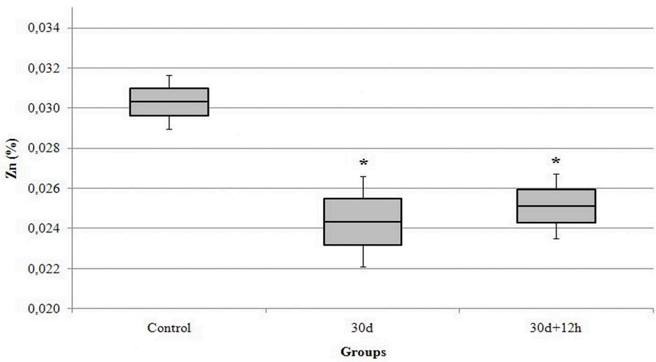

FIGURE 4: Changes in zink in hypergravity 2g.

Another interesting feature was observed while analyzing the individual indicators in animals. In the control group, we observed a noticeable dispersion in the Ca content in the calvaria bones, as is usually the case in natural populations, whereas in mice exposed to hypergravity, the indices were very close in value.

CONCLUSION

According to the results of this experiment, it can be assumed that the reason for the loss of the mineral component in the calvaria bones was the fluid redistribution in the body under the influence of 2.0 g acceleration.

But to confirm this working hypothesis, we consider it necessary to conduct further research to study the content of elements in other regions of the skeleton.

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Keywords: Hypergravity, mice, bone, elemental composition

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

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INTRODUCTION

The current stage of manned cosmonautics is characterized by the transition from orbital space flights to preparation for space missions outside the Earth's orbit into deep space. The nearest target of such missions is the Moon. In view of this, the primary importance should be given to advanced research on the risk factors to human health and performance which will take place in the Moon exploration. The lunar gravity equal to 1/6 of Earth's gravity (hypogravity) is one of these medical risk factors.

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

MATERIAL AND METHODS

Thirty two healthy male volunteers aged 20-36 years took part in the study. Informed consent was obtained from each subject. The study was performed in accordance with the ethics standards of the Helsinki Declaration for Human Experimentation. Study protocol was approved by Ethics Committee of the FRCC of FMBA of Russia.

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 +9.6° angle (HUBR) with a supporting load on the musculoskeletal system equal to 1/6 of the body weight [1].

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), 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 (bed rest, BR) (10 subjects) and head-down bed rest (HDBR) at -6° angle (6 subjects).

The spiroergometry system "MetaLyzer 3B" ("Cortex Biophysik") was used for registration of spirometry and pulmonary gas exchange parameters.

During spirometry tests the subjects firstly performed three slow vital capacity (VC) maneuvers and three maximum expiratory flow volume maneuvers (the highest of them was chosen for analysis), then - one maximum voluntary ventilation (MVV) maneuver. Vital capacity (VC), inspiratory reserve volume (IRV), forced vital capacity (FVC), forced expiratory flow (PEF) and maximum voluntary ventilation (MVV) were measured via volume in 1 second (FEV_1) of FVC, forced expiratory flow at 25-75% of FVC (FEF_{25-75}), peak expiratory flow (PEF) and maximum voluntary ventilation (MVV) were measured via spirometry.

Lung ventilation and gas-exchange measurements were done under calm breathing for seven minutes. Tidal volumes (V_T), respiratory rate (R_f), minute ventilation (V_E), oxygen consumption (VO_2) and production of carbon dioxide (VCO_2) were recorded.

The data were analyzed by Wilcoxon signed-rank and Mann-Whitney U tests using SPSS. Changes were considered significant at $p<0.05$.

RESULTS

By the 7th hour of HUBR VC decreased by about 6% ($p<0.01$) (Fig.1A). Further, the value of VC tended to recover by 7th day of the study. The opposite trend was observed in changes of IRV (Fig.1B). On the 1st day of HUBR a 16% increase in IRV ($p<0.05$) followed by its decrease by the 7th day.

Similarly to the changes in VC a 7% decrease in FVC occurred by the 7th hour of HUBR. Further, an increase in FVC is observed, but the changes did not exceed the baseline level (Fig.1C).

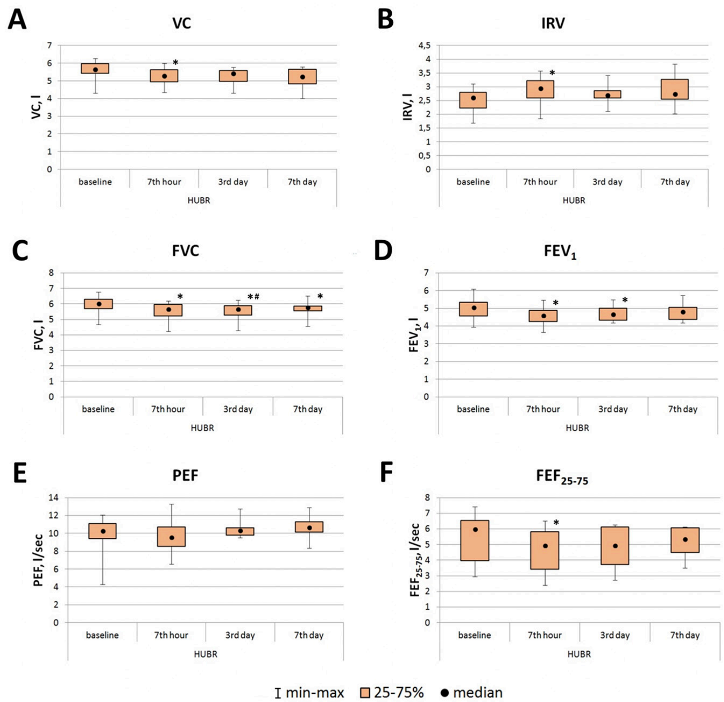

FIGURE 1: Effects of 7-day head-up bed rest (HUBR) on VC – vital capacity (**A**), IRV – inspiratory reserve volume (**B**), FVC – forced vital capacity (**C**), FEV_1 – forced expiratory volume in 1 second of FVC (**D**), PEF – peak expiratory flow (**E**), FEF_{25-75} – forced expiratory flow at 25-75% of FVC (**F**). * - as compared to the baseline ($p<0.05$), # - as compared to 7^{th} hour ($p<0.05$).

On the 1^{st} day a significant decrease in FEV_1 was marked (~8%) (Fig.1D). At the same time, the dynamics of the FEV_1/FVC remained without significant changes.

In 7 hours of HUBR a significant decrease ($p<0.05$) followed by an increase in forsed expiratory flow at 25-75% of FVC (FEF_{25-75}) (~12%) was found (Fig.1F).

PEF (Fig.1E) and MVV had a decreasing trend by the 7^{th} hour of HUBR with a further recovery.

V_E had a decreasing trend in the early period of exposure to HUBR with subsequent recovery to 7^{th} day. However, a significant decrease in the V_T

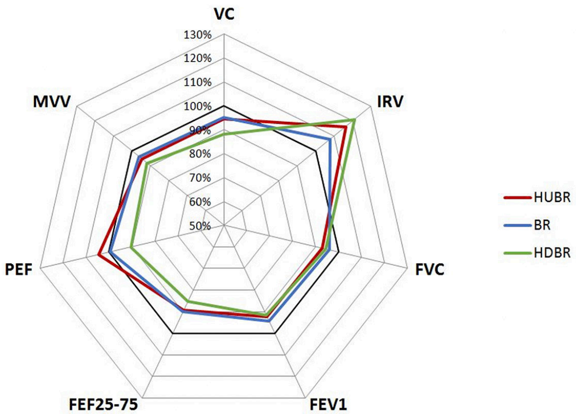

FIGURE 2: Percent changes in values of spirometry parameters after 7 hours of exposure to head-up bed rest (HUBR), supine position (BR) and head-down bed rest (HDBR). VC – vital capacity, IRV – inspiratory reserve volume, FVC – forced vital capacity, FEV_1 – forced expiratory volume in 1 second of FVC, FEF_{25-75} – forced expiratory flow at 25-75% of FVC, PEF – peak expiratory flow, MVV – maximum voluntary ventilation. Red lines –HUBR; blue lines – BR; green lines – HDBR.

($p<0.05$) was marked on the 1st day of HUBR. Then V_T tended to recover but did not exceed the baseline level by the 7th day. Significant changes in R_f, VO_2 and VCO_2 were not obtained.
Uni-directional early changes in respiratory parameters were observed in BR and HDBR (Fig.2). Changes in most of them were less pronounced under BR, and more pronounced in HDBR than in HUBR. However, the differences did not reach statistical significance.

Compared to background measurements it was found a ~4% decrease of VC ($p<0.05$) in BR and a ~10% decrease of VC ($p<0.05$) in HDBR. A significant increase in IRV during HDBR by ~30% was also noted. In BR there was an increasing trend of IRV value, but its changes did not reach statistical significance.

There was a significant decrease in FVC and the magnitude of these changes was approximately ~5% in HDBR and ~5% in BR. The FEV_1 decreased by ~5% in BR and by ~8% in HDBR.

Significant changes in PEF, MVV and FEF_{25-75}, V_T and V_E were not observed. But there was a decreasing trend for values of these parameters. Significant changes in VO_2, VCO_2 and, accordingly, R_f were not found.

DISCUSSION

The data obtained do not contradict the results of previous published studies devoted to the effects of lunar gravity and posture on lung function [2,3].

Reduction in V_T, VC, FVC and FEV_1 during HUBR can probably be attributed to an early increase in intrathoracic blood volume resulted from headward shift of body fluids, changes in position of diaphragm and abdominal organs as well as chest configuration [4]. These early changes in the structure of pulmonary volumes, the elastic characteristics of the lungs and the biomechanics of respiration are also manifested by a decrease in such parameters as FEF_{25-75}, PEF, MVV on the 1st day of HUBR. But the unchanged ratio of FEV_1/FVC indicates the preservation of normal bronchial patency.

Similar changes in respiratory parameters are observed under HDBR and BR by the 7th hour of exposure, as they have similar causes. But slight differences between physiological effects of HUBR and HDBR or BR can be traced. This inequality in the respiratory changes is most likely associated with the posture differences. Thus, more pronounced changes of most respiratory parameters in HDBR can be explained by a large redistribution of fluids in the cranial direction at tilt angle of -6° than +9.6°. But we cannot exclude the fact that in the comparison of BR and HDBR effects the number of subjects in these groups was smaller compared to HUBR. This fact could affect the calculated average values in the groups.

The increase in intrathoracic blood volume at the beginning of hypokinesia is replaced by its decrease due to a reduction in blood volume. This fact is associated with the gradual recovery of all volumetric parameters of the respiratory system after the 1st day to the 7th day of in HUBR.

The values of VCO_2, VO_2 and R_f remained unchanged, probably, due to the absence of significant metabolic changes in subjects during exposure to HUBR, HDBR and BR.

CONCLUSION

During HUBR as a physiological model of lunar gravity the moderate decrease in the majority of the main spirometric parameters was noted. The most noticeable changes were found on the first day of exposure to HUBR. These changes were transient and tended to baseline level to the end of the 7-day study.

Early physiological responses are similar to responses in 7 hours of horizontal position and head-down bed rest.

As a whole, it can de concluded that exposure of subjects to 7-day simulated lunar gravity does not lead to significant disturbance of function of the respiratory system.

ACKNOWLEDGMENTS

The authors wish to express their gratitude and deep appreciation to the medical staff of the Federal Biomedical Agency of Russia for their invaluable assistance in the organization of the experiment, as well as to the volunteers for their active participation in the study.

Keywords: Respiratory system, spirometry, gas exchange, simulated lunar gravity

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

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INTRODUCTION

Backpain phenomenon is regularly observed in cosmonauts during the first days of space flight (SF) and early post flight period (Wing P.C. et al., 1991). On Earth, the etiology of back pain syndrome includes an increase of gravitational loading on the spine and can be classified as mechanical, non-mechanical, and visceral back pain (Lawrence et al., 1992). Traditionally, the changes observed in weightlessness are associated with axial unloading of the vertebral column.

Gravitational unloading and support withdrawal in a complex with a decrease of motor activity leads to a decline of activity of skeletal muscles. At the same time, such changes are typically observed in the muscles involved in posture maintaining, which include a number of muscles of the lower extremities and part of the back muscles (Kozlovskaya et al., 2007; Kertsman et al., 2012). Exposure to weightlessness is also followed by height increase. Both phenomena can be accurately reproduced under simulated microgravity conditions - Dry Immersion (DI) (Tomilovskaya et al., 2019) and antiorthostatic bedrest (Baum K. et al., 1999). The aim of the study was to investigate the changes of characteristics of back and trunk muscle changes under conditions of simulated microgravity.

MATERIAL AND METHODS

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

RESULTS

Data analysis revealed the decrease of back extensors muscles 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 (CSA) of m. quadratus lumborum decreased to the level of 86,68 ± 13,32% from baseline values (F(2, 9)=8,748; P=0,0078) (figure 1). The analogous changes were obtained in m. multifidus - decrease to 88,21±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±9,01% and 91,99±8,01% from the baseline values.

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 (figure 2). Neck kyphosis was flattered by 6.6±3.29 degrees, thoracic - by 6.0±2.58 degrees and lumbar lordosis was flattered by 6.6±3.29 degrees.

Almost all participants of DI reported the back pain of different intensity. In most of the cases back pain had similar characteristics as discomfortable feelings, wide spreaded in lumbar area, out of any acute symptomes, similar to

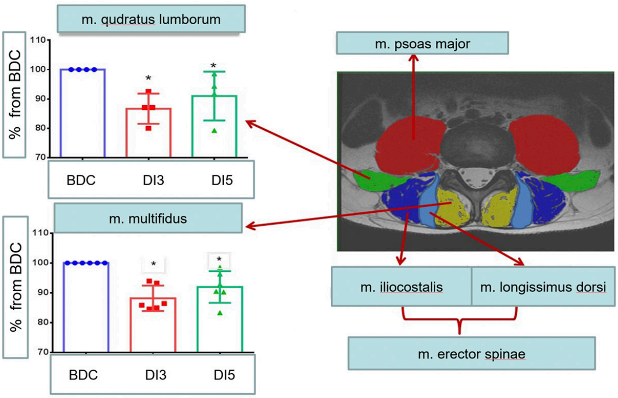

FIGURE 1: MRI studies: changes in muscle cross-sectional area of the dry residue in m. quadratus lumborum and m.multifidus on the 3rd and 5th days of Dry Immersion exposure.
X axis: BDC and days in Dry Immersion exposure,
Y axis : percantage of muscle cross-sectional changes.
* - significant differences from background values.

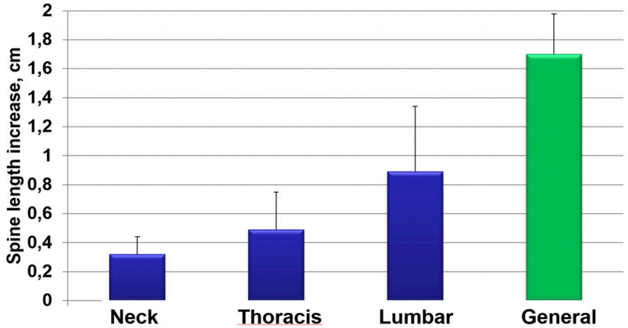

FIGURE 2: MRI data of changes in the length of the spinal column, the average values for different departments of spinal column after 5 days of Dry Immersion exposure.

"muscle kind" of pain. The intensity of pain, reaching 9-10 points in a number of subjects, was characterized by high variability. In average, the intensity of back pain in the first two days fluctuated around 5 points, on the third day of DI it was about 4 points on a 10-point subjective scale. On the 4th and 5th days of DI, only one subjectreported about back pain of low intensity.

At the same time, correlation analysis revealed a high positive relationship between the increase in body height and length of the spine (r = 0.98). However, Pearson coefficient for the relationship of changes in height/length of the spine with the intensity of pain syndrome was low (r = 0.23/r = 0.48, respectively). The relationship between changes in the transverse stiffness of the back muscles and the intensity of pain was detected only for one measurement point (r = -0.99) - at the level of the lumbar spine (T12-L1). At the same time a high direct correlation of changes in the height and transverse stiffness of the back muscles was revealed, amounting to r = -0.98 in the upper lumbar region (table 1). Changes of the cross-sectional area of all the studied muscles showed a high correlation with changes in the length of the spinal column (r = -0.97) and height (r = -0.9); significant relationship was identified between pain intensity and changes in CSA of m. quadratus lumborum (r = -0.78) and m. multifidus (r = -0.84). The high positive Pearson coefficient corresponded to the relationship between reduce of CSA of m.erector spinae and decreasing of its muscle tone (r=-0.97).

Table 1: Correlation between body height, spinal length, back pain intensity and decrease of cross-sectional area of back muscles in DI conditions.

	Body height	Spinal length	Back pain intensity	↓CSA general	↓CSA m.quadratus lumborum	↓CSA m.erector spinae	↓CSA m.psoasmajor	↓CSA m.multifidus
Body height								
Spinal length	0,98							
Back pain intensity	0,23	0,48						
↓CSA general	-0,90	-0,97	-0,67					
↓CSA m.quadratus lumborum	-0,83	-0,92	-0,78	0,99				
↓CSA m.erector spinae	-0,99	-1,00	-0,44	0,96	0,91			
↓CSA m.psoas major	-0,94	-0,99	-0,60	0,99	0,97	0,98		
↓CSA m.multifidus	-0,77	-0,88	-0,84	0,97	0,99	0,86	0,94	

DISCUSSION

The studies have revealed the significant decrease of muscle tone of back extensor muscles under conditions of simulated microgravity. At the same time the height increase and back pain phenomenon were registered. Also the changes in muscle transverse stiffness were observed during the first 3 days of exposure to Dry Immersion. Interestingly, that back pain phenomenon was observed only during the 1-3 days of Dry Immersion as well.

The high values of back pain intensity found in our 5-days DI study are also confirmed by the results of recent experiments of French researchers under 3-day DI conditions: according to their data, 92% of the subjects had a phenomenon of pain in the lumbar back with an average severity of 3.75±2.4 points in the first two days of exposure (Treffel et al., 2017).

Changes in muscle tone and severity of back pain did not show a direct correlation, although there was a significant correlation between body height changes and severity of pain, as well as between height changes and transverse stiffness of the back muscles. Probably, the muscle tone and the phenomenon of back pain are determined by a large number of intermediate factors.

At the same time, some of the authors ignores the fact that the lengthening of the spine, associated with a decrease in mechanical stress is provided not only by the axial weight load, but also by the tonic activity of the back muscles (Hutchinson et al., 1995; Kershner & Binhammer, 2004; Sayson & Hargens, 2008).

The analysis of the obtained data revealed a significant decrease in the cross-sectional area of the muscles located around the spine during the support withdrawal. The most pronounced changes in muscle CSA on MRI scanning was observed in our study at the level of L4/L5 that noticed a significant reduce of muscle CSA already on day 3 of DI in mm.quadratus lumborum, multifidus and erector spinae.

Previous studies describe a decrease in the tonic activity of postural muscles with subsequent development of signs of atrophy in them (LeBlanc et al.,2000).This observation suggests that the observed MRI changes in the cross-sectional area of the spinal extensors can be interpreted as an early signs of the process of atrophy.

CONCLUSION

It can be suggested that the original cause of back pain phenomenon in a complex of spinal changes under microgravity conditions can be due to axial unloading and atonia of back extensor muscles.

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Keywords: Back pain, dry immersion, posture muscles, muscle tone

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

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INTRODUCTION

Studies carried out in zero gravity and under the model of microgravity simulations pointed out a wide range of changes of the muscular periphery (atony, atrophy), force-velocity properties loss [1-3] and also mineral density loss [4] that, apparently is a result of the long-term hypodynamia and lack of axial loading, as well as postural regulation [5, 6] and locomotor coordination [7-10] caused, in part, by interactive changes between vestibular and muscular afferentation [11]. The purpose of this study was to obtain quantitative data which show alterations in the biomechanical characteristics of arbitrary walking after long-term space flights (SF).

MATERIAL AND METHODS

We have chosen arbitrary walking as the object of our study due to the locomotor disorders observed after space flight. It is a motor stereotypical process that when walking the multitude elements of the musculoskeletal system and nerve centers interact with each other [12]. It is important to note that walking has a cycle structure [13] due to mechanical stimulation of the soles support zones [14].

The work was carried out as a part of the joint Russian-U.S. investigation known as the “Field Test” in which 5 Russian cosmonauts (age 50,1 $\pm$ 7,4 yr and weighing approximately 89,3 $\pm$ 7,1 kg) lived aboard the International Space Station for 168,($\pm$20,5 days). Crewmembers performed the 5 meter (providing from 6 to 8 steps for analysis) arbitrary walking test 60 and 30 days before SF, during the first hour after landing and then on the 4th and the 12th days after SF.

The Motion Open Go (Motion GmbH, München, Germany) shoe insole sensor was used for data recording and place in standardized athletic shoes. The sensor system provided 13 capacitive pressure sensors, a temperature sensor, a tri-axial accelerometer, and a data storage chip per insole. Pressure sensors covered 52% of the insole area. Data was wirelessly sampled at 50 Hz and connected by USB antenna to a computer where Moticon's Beaker 5 software (Moticon GmbH, München, Germany) was used to initiate and stop data recordings (Figure 1). MATLAB© scripts were used for calculating the results of ground reaction forces (GRF) mean values (for an objective assessment, the data of the mean GRF values was calculated in % of the cosmonaut's Earth weight values), temporal structure of the gait phases (± mean values in ms with their standard deviation). The principles for

FIGURE 1: The Moticon OpenGo instrumented insoles (Moticon GmbH, München, Germany) and their sensor lay out (USB antenna not shown). Images showing the OpenGo instrumented insole front (A), the back with battery compartment (B) and the sensor lay out (C).

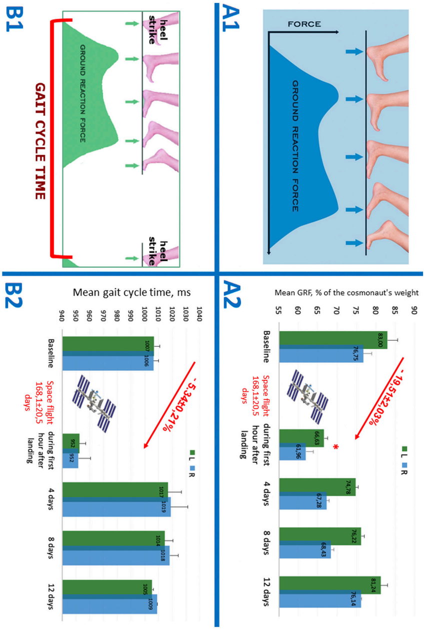

FIGURE 2: The dynamics of changes in the mean ground reaction forces (GFR) values and the mean gait cycle time value while walking after long-term space flight:
A1 - the principles for calculating the GFR values;
A2 - the dynamics of changes in the mean GFR values after long-term space flight, on the ordinate axis: mean GRF, % of the cosmonaut's weight, on the abscissa axis - the session of the experiment;
B1 - the principles for calculating the gait cycle time value;
B2 - the dynamics of changes in the mean gait cycle time values after long-term space flight, on the ordinate axis: mean gait cycle time, ms, on the abscissa axis - the session of the experiment.
*- significant difference compared to baseline values (p <0.05).

calculating the estimated characteristics are shown in the figures 2A1, 2B1, 3A1, 3B1, 4C1. To identify significant differences between the values of different sessions of the experiment, we used the nonparametric Wilcoxon signed-rank test. The difference was considered to be significant with p less than 0,05. When calculating the significant difference between different sessions of the experiment, the data from the left and right insole were not separated.

RESULTS

The first estimated characteristic is the mean GRF values while walking (Figure 2A1). During the first hour after the landing, this parameter was 19.51, ±2,03% lower than the Cosmonaut's baseline values and the difference is significant. On the 4th and the 8th days after the SF the mean GRF values were gradually increasing and became approximately equal to the baseline values on the 12th day after returning from SF (Figure 2A2).

The gait cycle is the time from the start of heel strike phase to the next heel strike (Figure 2B1). During the first hour after the SF, this parameter was about 5.34, ±0,21% lower than baseline values. The baseline values were gradually reached on 12th day after the SF (Figure 2B2).

The stance phase time while walking is the time from the start of "heel strike phase" to the end of the "toe off phase" (Figure 3A1). During the first hour after the landing, the mean stance phase time values were 2.95, ±0,86% higher than baseline values. The baseline values gradually have been reached on 12th day after the SF (Figure 3A2).

The next estimated characteristic is the single support time. It is the time from the end of "heel strike phase" to the start of the "toe off phase" (Figure 3B1). During the first hour after the landing, this parameter was 11.24, ±1.23% lower than baseline values and the significant difference has been reached (Figure 3B2). Than the baseline values have been reached on 4th day after the SF.

Finally, the mean double support time, when two soles are touching the support surface (one foot is the "heel strike phase" and the other foot is the "toe off phase", Figure 3C1). During the first hour after the end of long-term SF the mean double support time values were 10.32 ±0.76% higher than baseline values and the significant difference has been reached. This parameter was closer to the baseline values on 12th day after the space flight (Figure 3C2).

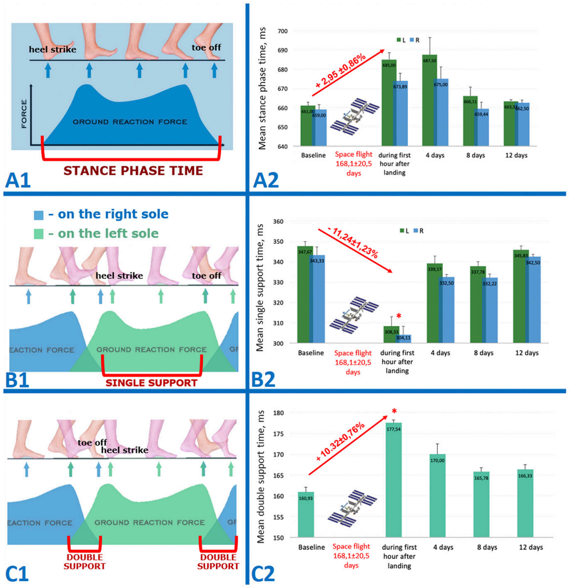

FIGURE 3: The dynamics of changes in the temporal structure of the gait phases values while walking after long-term space flight:

A1 - the principles for calculating the stance phase time value;

A2 - the dynamics of changes in the mean stance phase time value after long-term space flight, on the ordinate axis: mean stance phase time, ms, on the abscissa axis - the session of the experiment;

B1 - the principles for calculating the single support time value;

B2 - the dynamics of changes in the mean single support time values after long-term space flight, on the ordinate axis: mean single support time, ms, on the abscissa axis - the session of the experiment.

C1 - the principles for calculating the double support time value;

C2 - the dynamics of changes in the mean double support time values after long-term space flight, on the ordinate axis: mean double support time, ms, on the abscissa axis - the session of the experiment.

*- significant difference compared to baseline values ($p < 0.05$).

DISCUSSION AND CONCLUSION

The most significant alterations in the characteristics of arbitrary walking can be observed within the first hour after landing. Lowered values of the mean GRF values during the first hour after landing reflect a decrease in muscle forces on the soles from the support surface, and is perhaps the result of muscular system weakening due to muscle disuse during SF [15], locomotor coordination disorder and other functional disorders. At the same time, the decrease in gait cycle time suggests an increase of the step frequency by reducing phase limb advancement (between the two support phases). Additionally, the slight increase in the mean stance phase time values due to a sharp rise in the mean double support phase values and a sharp decline in the single support phase value was noted during the first-hour after the end of SF (Figure 4).

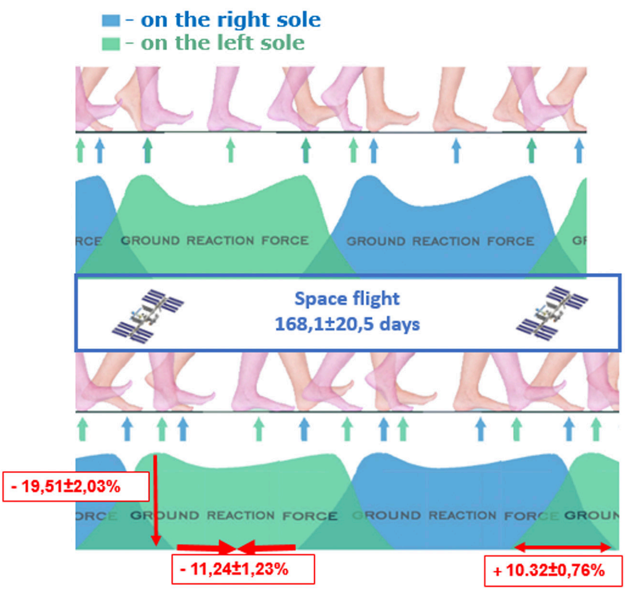

FIGURE 4: The comparison scheme between ground reaction forces value graph before space flight and after long-term space flight during the first hour after the landing.

It can be assumed, that the mentioned effects of long-term SF lead to an increase of density and timing of the contact of the soles with the support surface, which in turn increases the stability of the astronaut's gait, since perhaps the proprioceptive signals entering the central nervous system from the Vater-Pacini corpuscles in the sole carry information about the interaction of the center of mass with respect to the support surface [16].

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Keywords: spaceflight, walking, microgravity, GRF, physiological changes

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Natural astaxanthin as a novel nutritional supplement for spaceflight

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Natural astaxanthin is a carotenoid found in a variety of organisms, among which the freshwater alga *Haematococcus pluvialis* produces the highest astaxanthin content found in nature. While astaxanthin has numerous clinically proven benefits for human health, the only significant source of natural astaxanthin in the human diet comes from wild salmon. The limited intake of wild salmon in a typical diet makes astaxanthin derived from *H. pluvialis* an effective and sustainable source for dietary supplementation of astaxanthin.

Natural astaxanthin possesses extraordinary antioxidant powers and is the strongest quencher of free radicals and singlet oxygen among all known natural antioxidants [1].

Unlike other carotenoids or vitamins, astaxanthin's unique molecular structure allows this amphiphilic molecule, approximately 30 Å in length, to partition into, and fully span the cell- and mitochondrial membranes co-axially (Fig. 1). Matching lipid bilayer architecture allows astaxanthin to stabilize and protect against reactive oxygen species (ROS) on both sides of the membrane[2]. Astaxanthin has sufficient hydrophobic character to cross the blood/brain- and blood/retinal barrier, and 60+ clinical studies have demonstrated the ability of astaxanthin to exert a variety of health benefits in earthbound individuals, including anti-inflammatory effects, immune function support, and suppression and prevention of muscle loss. These features make astaxanthin a promising candidate for nutritional support of physiological acclimation during spaceflight.

During spaceflight, the absence of gravitational loading is responsible for a loss in muscle mass and strength, with a 20% reduction in muscle mass reported after 2 weeks of spaceflight, and 30% loss on 3–6 month missions[3]. Current exercise interventions are insufficient to fully prevent muscle atrophy, and additional nutritional interventions are an active area of research.

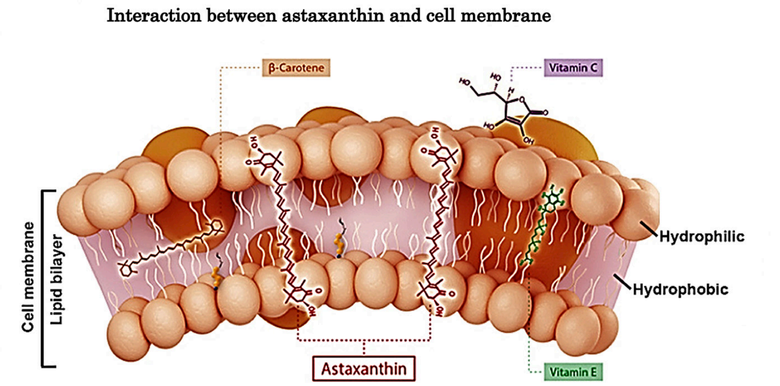

FIGURE 1: Astaxanthin's unique structure allows the molecule to embed itself into The lipid bilayer and span the entire width of the membrane structure. With its hydrophilic ionone rings it protects the membrane from free radicals whereas the hydrophobic polyene chain dissipates the energy from reactive oxygen species and provides structural stability to cell and mitochondrial membranes.

Astaxanthin supports muscle integrity under conditions of physiological stress associated with both exercise and disuse. Numerous animal studies have shown that astaxanthin prevents atrophy of muscle tissue and the capillary system responsible for muscle oxygenation and nourishment.[4] In young soccer players undergoing prolonged training, astaxanthin prevented the accumulation of the muscle injury marker creatine kinase. Astaxanthin also suppressed exercise-induced chronic inflammation, as indicated by low levels of C-reactive protein after heavy exercise.[5,6]

A recent study[7] demonstrated that astaxanthin supplementation boosts the effect of exercise in the elderly with age-related muscle atrophy. The combination of astaxanthin and moderate exercise led to an increase in maximum voluntary contraction by 14.4%, an increase in *tibialis anterior* cross-sectional area by 2.7%, and an 11.6% increase in specific force, whereas exercise alone showed no significant improvements.

Both functional training studies in young soccer players and in the elderly demonstrated the ability of astaxanthin to boost the effects of exercise training, and suggest that dietary supplementation with astaxanthin may also boost the effects of standard endurance and resistance training routines of astronauts in spaceflight[8].

Data collected on board the International Space Station confirmed that extended spaceflight compromises the human immune system, resulting in hypersensitivities, autoimmune and allergic reactions, susceptibility to infectious disease, and reactivation of latent viruses. Immune cells are particularly sensitive to oxidative stress, responding with reduced natural killer cell function, diminished monocyte function or dysregulated T cell signaling.

Immunoglobulins are the first line of defense against pathogens, and secretory immunoglobulin A (sIgA), stands guard in mucous membranes of the oral cavity, gut, and lungs. sIgA secretion into saliva is inhibited by conditions of physiological stress that lead to oxidative imbalance, such as physical inactivity and immobilization, or physical over-exertion. For example, ultra-marathon runners experienced a 10% reduction in salivary sIgA following a long-distance race, and 25% of those runners consequently developed upper respiratory tract infections (URTIs) after the race[9].

Since URTIs are known to commonly plague astronauts, it is important to establish a regular regimen of moderate exercise to maintain muscle mass in conjunction with dietary antioxidant supplementation that can effectively modulate oxidative stress and reduce the incidents of URTI under the unique conditions experienced by astronauts in spaceflight[10].

In a 2015 study, it was shown that supplementation with natural astaxanthin had a positive effect on salivary IgA secretion.[6] After 90 days of taking 4 mg/day astaxanthin, only the astaxanthin group showed significant improvements in sIgA secretion and general antioxidant status, whereas the placebo group showed increased levels of oxidative stress and signs of inflammation.

Nuclear Factor-κB plays a key role in inflammatory signaling. NF-κB controls the transcription of inflammation-specific gene products, and is a major player in regulating the immune response to infections.

Astaxanthin down-regulates the inflammatory response by inhibiting activation of NF-κB and its translocation into the nucleus, where it would otherwise bind to specific DNA-sequences and initiate the expression of inflammation-associated genes.

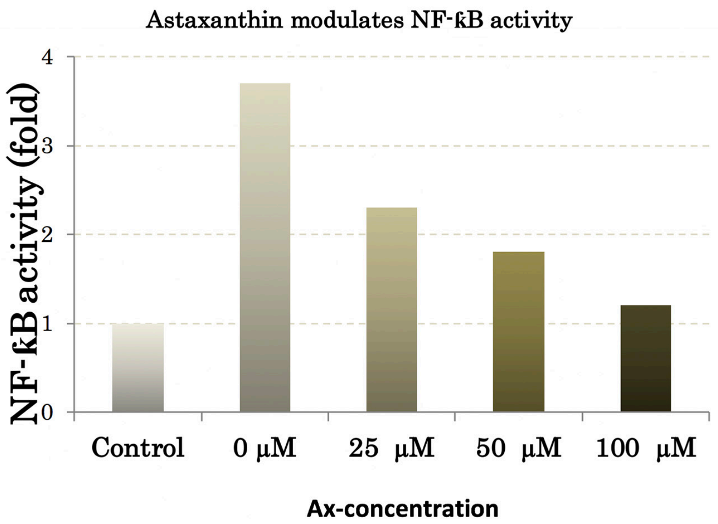

FIGURE 2: Astaxanthin inhibits the activation of NF-κB in cells under oxidative stress induces by lipopolysaccharides (LPS) in a dose-dependent manner.

By inhibiting NF-κB (Fig. 2), astaxanthin prevents the expression of important pro-inflammatory cytokines such as TNF-α, IL-1β or NO_x as marker of oxidative stress(Fig. 3)[11].

The classic features of inflammation are the symptomatic manifestations of complex tissue responses, like swelling, redness, heat, etc., in which matrix metalloproteinases (MMPs) are also involved. Upregulated MMP-expression has been observed in almost every human disease in which inflammation occurs. Here too, astaxanthin has an attenuating effect and the mechanism is very likely to be a direct interaction between astaxanthin and MMP-13 in the form of hydrophobic interactions of the astaxanthin molecule with amino acids at the active center of MMP-13 (Fig. 4)[12].

Space radiation and UV radiation are major health threats during spaceflight and previous studies, finding that natural astaxanthin can protect the skin against harmful UV radiation, suggest similar protective effects on the skin of astronauts when in space.

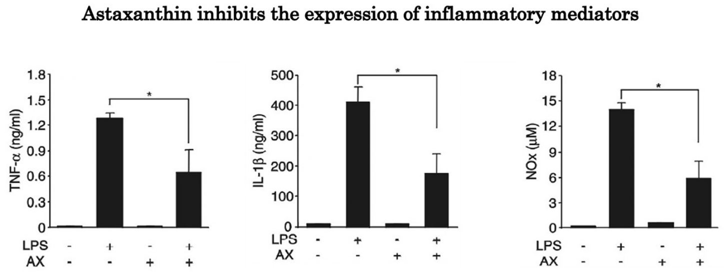

FIGURE 3: Astaxanthin decreased plasma levels of TNF-α, IL-1β and NOx. After pretreatment with astaxanthin, mice were injected with LPS to induce oxidative damage and inflammation.

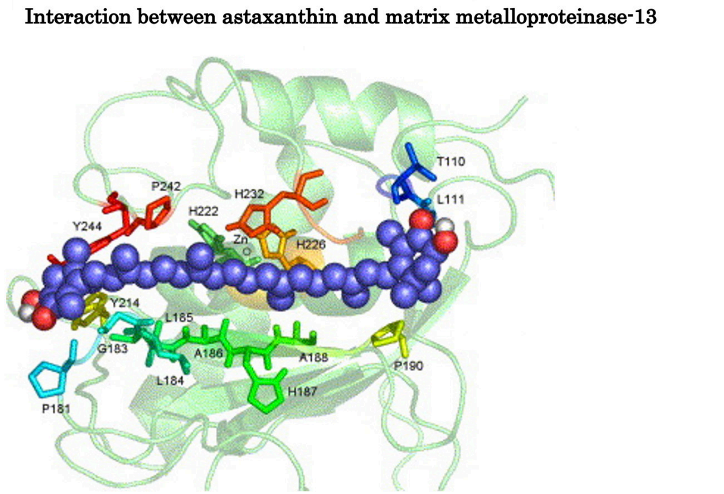

FIGURE 4: Molecular modeling of a non-covalent binding of astaxanthin to matrix metalloproteinase-13 (MMP-13). Astaxanthin was predicted to bind to alanine (A), leucine (L), tyrosine (Y), proline (P) and histidine (H) residues in close proximity to the catalytic center of MMP-13.

Several clinical studies have shown how astaxanthin, taken orally and as a topical product, prevents the dehydration of the outer layers of the skin and improves the corneocyte status or inhibits the degradation of the collagen layer by suppressing MMP activities and enhancing collagen production by fibroblasts[13].

In the epidermis, extended illumination with UVB light can induce a series of skin conditions ranging from mild sunburn to serious inflammation. A recent study gave clear evidence that pre-incubation with astaxanthin protects human keratinocytes from the damaging effects of UVB light. Astaxanthin inhibited the release of the pro-inflammatory cytokines IL-1α, IL-6 and IL-8 and TNF-α which are known to stimulate the expression of inflammation-associated genes like cylooxygenase 2, phospholipase A or inducible nitric oxide synthase[14]. UV light and space radiation generate large quantities of free radicals which can cause a variety of skin conditions. Antioxidants, which are capable of quenching free radicals, may represent an effective strategy for mitigating the physiological response to UV and cosmic radiation exposure during space-flight. The capacity for free radical quenching varies across antioxidants, as has been demonstrated in a human fibroblast model. Survival of human fibroblasts was measured after exposure to singlet oxygen in the presence or absence of pre-treatment using various antioxidants. All but two of the antioxidants tested adversely affected cell survival. Astaxanthin was the only antioxidant to produce a cell survival rate comparable to that of the control cells that had not been exposed to singlet oxygen[13].

In more than 120 clinical studies the diverse health benefits of natural astaxanthin have been documented. Its strong antioxidant and anti-inflammatory properties, together with its ability to partition into cell and mitochondrial membranes, explain its remarkable effect on cell metabolism and energy production. Astaxanthin's ameliorating effect on inflammation and immune health has been the focus of several publications, clearly showing astaxanthin's value as a nutritional supplement[15, 16].

Future research examining natural astaxanthin as a nutritional intervention for space travelers may be a natural extension of its already clinically proven earthbound health benefits.

Keywords: natural astaxanthin, antioxidant, anti-inflammatory, immunoglobulin A, muscle atrophy

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

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INTRODUCTION

Results of previous studies have shown that hypogravitational motor syndrome is characterized by changes in all components of the motor system [1; 2]. According to the data of IBMP RAS researches most of motor effects of microgravity are fully reproduced on Earth under conditions of Dry Immersion (DI), which seems to be one of the most adequate ground simulation model of weightlessness [3; 4]. The alterations in afferent systems' activity in particular a decrease of volume of proprioceptive afferentation activating motor neurons of skeletal muscles are considered to be one of the main reasons for changes in motor system following exposure to weightlessness. Electromyostimulation (EMS) can be used as a countermeasure mean to maintain contractile activity of skeletal muscles and additional proprioceptive afferentation.

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).

MATERIAL AND METHODS

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 (Figure 1); in the second one (group "Immersion+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

FIGURE 1: Dry Immersion – ground-based model of microgravity.

FIGURE 2: "Stimul-01 NCH" electrostimulation system developed by the IMBP RAS.

use of "Stimul-01 NCH" device (Figure 2). Electrical stimuli were applied to all the stimulated muscles simultaneously; stimulation session lasted for 1 s and was followed by rest period of 2 s. Stimulator generated bipolar 1 ms impulses with the 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. (Figure 3). H-reflex was elicited by single

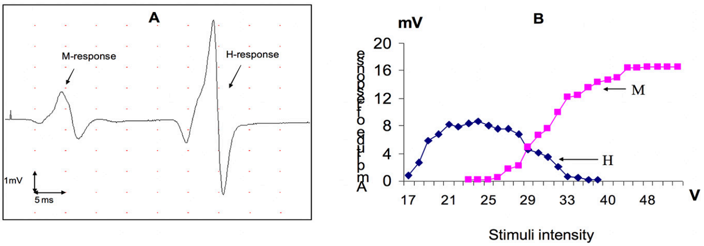

FIGURE 3: H-reflex. The native curve of M- and H-response (A). H-reflex recruitment curve (B).

1ms electrical pulses applied to tibial nerve. Stimuli were applied monopolarly: the active electrode was placed in the popliteal space and the reference electrode was located on the patella. Intervals between stimuli were not less than 10 s. Evoked responses of the studied muscles were recorded bipolarly with the surface electrodes of 5 mm in diameter located above the myogaster center 20 mm apart. Stimuli of increasing intensity was applied for obtaining the H-reflex recruitment curve: from minimal strength of stimulation when minimal reflex response appears to maximal – with the peak muscle response (Figure 3). Reflex threshold and maximal peak-to-peak amplitudes of reflex and muscle responses in the H-reflex recruitment curves of shin extensors were evaluated (Figure 3).

Reflex responses testing was performed before immersion, on the 3d and 5th days of DI and on the 2nd day after DI completion. The Wilcoxon non-parametric criteria was used for statistical data analysis.

RESULTS AND DISCUSSION

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 (Figure 4A) and by 28% ($p<0.05$) – in m. gastrocnemius lat. (Figure 4B). 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 decreased, however significant changes were observed only on the day 5 of DI (29% decrease, $p<0.05$). After DI completion in both groups a tendency to recovery of H-reflex threshold in the studied muscles was observed.

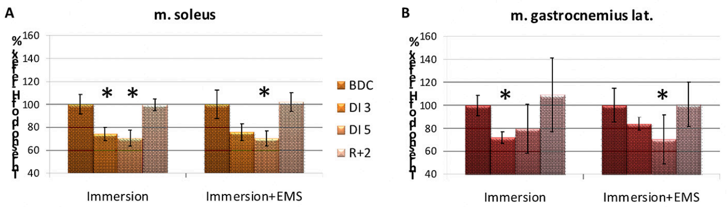

FIGURE 4: The threshold of H-reflex in m. soleus (**A**) and in m. gastrocnemius lat. (**B**) BDC – base data collection, DI3 – 3d day of DI, DI5 – 5th day of DI, R+2 – 2d day after DI.
* – significant changes in comparison to BDC, p<0,05.

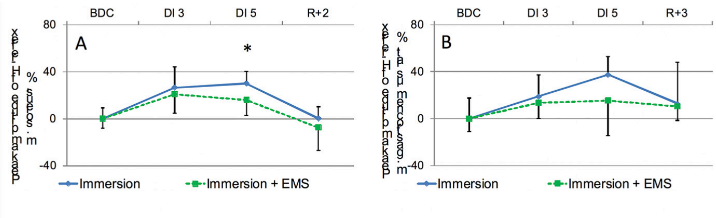

FIGURE 5: Maximal amplitude of H-reflex in m. soleus (**A**) and in m. gastrocnemius lat. (**B**), %. BDC – base data collection, DI3 – 3d day of DI, DI5 – 5th day of DI, R+2 – 2d day after DI.
* – significant changes in comparison to BDC, p<0,05.

Amplitudes of H-reflex responses in "Immersion" group significantly increased by the 5th day of DI: in m.soleus – by 30% ($p<0.05$) (Figure 5A), in m. gastrocnemius lat. – by 37% (Figure 5B), suggesting an increase in excitability of the m. soleus motoneuron pool. So the results show once again that hyperreflexia develops in hypogravity, as has been observed in other studies [5; 6; 7].

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; no significant changes of reflex amplitude were registered in this group (Figure 5).

As a result of the numerous studies it was established that the low-frequency electromyostimulation led to the increase of the muscle work capacity, fatigue resistance, elevated oxidative enzyme activities and the other structural and metabolic markers and steady fast-to-slow fiber type transformation. The similar data were obtained during the studies of the healthy volunteers.

The low-frequency stimulation exerted the beneficial effects on the atrophied disused muscle [8].

CONCLUSIONS

The data obtained confirms the supposition that the excitability of moto-neurons of calf extensor muscles increases due to reflex changes under conditions of support unloading. The rise of absolute amplitudes of reflex responses in immersion reflects the changes in the state of motoneuron pool. During DI in the group without EMS, the excitability of motor neurons of the extensor muscles of the legs increases due to reflex (central) and muscle changes.

Additional proprioceptive stimulation with the use of low-frequency EMS training leads to lessen of this effect, i.e. helps to maintain the level of moto-neurons excitability and contractile activity of muscles extensors shin.

ACKNOWLEDGMENTS

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Keywords: Dry immersion, low frequency electromyostimulation, H-reflex

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

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INTRODUCTION

Currently, more and more studies in space biology and medicine are devoted to modelling physiological changes in various body systems in terrestrial conditions, and under lower gravitation levels [1, 2] to better understand changes in humans which occur during space flights to celestial bodies (Moon, Mars).

Previous studies of the musculoskeletal system following zero gravity exposure have shown changes throughout all parts of the motor system [3, 4, 5, 6]. Studies performed in real and simulated microgravity environments showed a wide range of muscle changes and sensory input changes, including changes in supporting, muscular, and vestibular inputs [7, 8, 9]. These changes adversely affected motor control performance [10, 11] and disrupted postural regulation and locomotion [12, 13]. It is reasonable to assume that, in reduced gravitational environments like the lunar surface, cosmonauts experience similar changes in musculoskeletal function.

Studying the human musculoskeletal system is not an easy task. First of all, given the complexity of human locomotion, one has to consider both internal and external factors. External biomechanical locomotion parameters include joint angles, angular velocities, and angular accelerations. They also include locomotion effort, amplitude, and speed of both individual body parts and the entire human body [14]. Internal locomotion parameters include muscle activity to ensure the performance of a specific movement (EMG-activity) and the work through which these movements manifest (EMG-cost).

MATERIAL AND METHODS

The paper analyzes the results obtained in studies of the state of the musculoskeletal system 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 space flights (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 of leg joints, EMG-activity of m. tibialis anterior, m. soleus and m. gastrocnemius medialis, double step length.

Head-down and head-up bed rest. 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 steps/minute was performed on a treadmill.

Locomotion in conditions of reduced weight load on the musculoskeletal system. 48 subjects participated in the study: 39 males and 9 females. Purpose of our work was to study the human locomotion strategy and energy costs under normal walking and walking under reduced weight loading. Subjects performed locomotor tests on the treadmill, which included walking at a pace of 90 steps/minute under various conditions of weight load on the musculoskeletal system: normal walking, without external body weight support; 38% body weight support is specific for a human walking on the Martian surface; 17% body weight support is specific for a human walking on the lunar surface.

RESULTS

Long-term space flights. The cosmonauts were divided into two groups: Group-A and Group-B. In Group-A, the cosmonauts trained on the treadmill in interval mode, which includes the use of alternating high-intensity running and walking. In Group-B, the basis consisted of long intervals of running with a constant or gradually speed increasing.

EMG-cost of locomotion SF increased in Group-A in the flexor m. tibialis anterior, in Group-B in the extensors m. soleus and m. gastrocnemius medialis. Analysis of changes in EMG-cost of gravity-dependent extensor m. soleus did not reveal significant changes in Group-A. In Group-B, a significant increase of EMG-cost was up to the tenth day after SF (Fig. 1, A). In the hip joint after SF, no significant changes in angles have been identified. In the knee joint, an increase in flexion was detected in all phases of the double step. In the ankle joint in Group-A, a significant decrease in the values of the angles during first peak phase was detected on the third day after SF. In Group-B in the ankle joint, during first peak phase, the amplitude of the angles increased, and during the push-back phase it significantly decreased (Fig. 1, B). It should be noted that the increase in flexion in the joints during the walking was maintained until the tenth day after SF. As an example, the results of the analysis of angles in the knee joint in Group-B on the third, seventh and tenth day after SF (Fig. 1, B, pink area). The length of the double step after SF decreased to the maximum in both groups on the third day after SF. By the tenth day after SF, the length of the double step was restored and this happened faster in Group-A (Fig. 1, C, green area).

Head-down and head-up bed rest. After hypokinesia in HDBR-group, angles in all joints significantly decreased during push-back phase. In HUBR-group, the angles during push-back phase decreased slightly in the hip and knee joints. In the ankle joint – significantly compared with baseline. Along with a change in the joint angles, decrease in the angular velocity in the joints was also detected. This is indicated by a decrease in the area of phase trajectories after hypokinesia in all joints (Fig. 2, A). The increase of EMG-activity after hypokinesia observed in both groups, with more pronounced changes in HDBR-group (Fig. 2, B). EMG-activity of m. tibialis anterior increased during the first peak phase and swing phase. The maximum increase in EMG-activity of m. soleus after hypokinesia detected during the push back phase in both groups. EMG-activity of m. gastrocnemius medialis in HDBR-group during the push back phase has increased, in HUBR-group did not change.

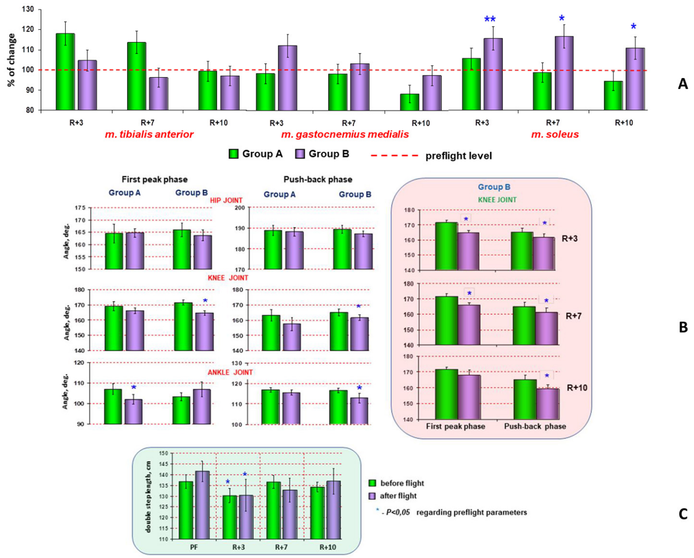

FIGURE 1: – EMG-cost of walking in the double step cycle (**A**), the angles in the joints of the feet (**B**) and the length of the double step (**C**) after long-term space flight.

After hypokinesia EMG-activity of m. vastus lateralis and m. rectus femoris increased during the first peak phase and during the push-back phase in both groups (Fig. 2, B, pink area).

Locomotion in conditions of reduced weight load on the musculoskeletal system. During the walking 38% and walking 17% the EMG-activity m. tibialis anterior in the male- and female-group changed during the first peak phase and during the swing phase. The EMG-activity m. soleus and m. gastrocnemius medialis changes in the push back phase only. During the first peak phase, walking with 100% of body weight the main load is exerted by the extensors of the knee joint m. vastus lateralis and m. rectus femoris. With decrease in the weight load, there is a decrease in the EMG-activity of these muscles. Figure 3 shows typical phase trajectories which characterize hip (A), knee (B), and ankle (C) joint movements. Similar phase trajectories were met in 11 out of 15 subjects (group No 1). According to results obtained, all phase trajectories, except those of the ankle joint, have a reduction of phase

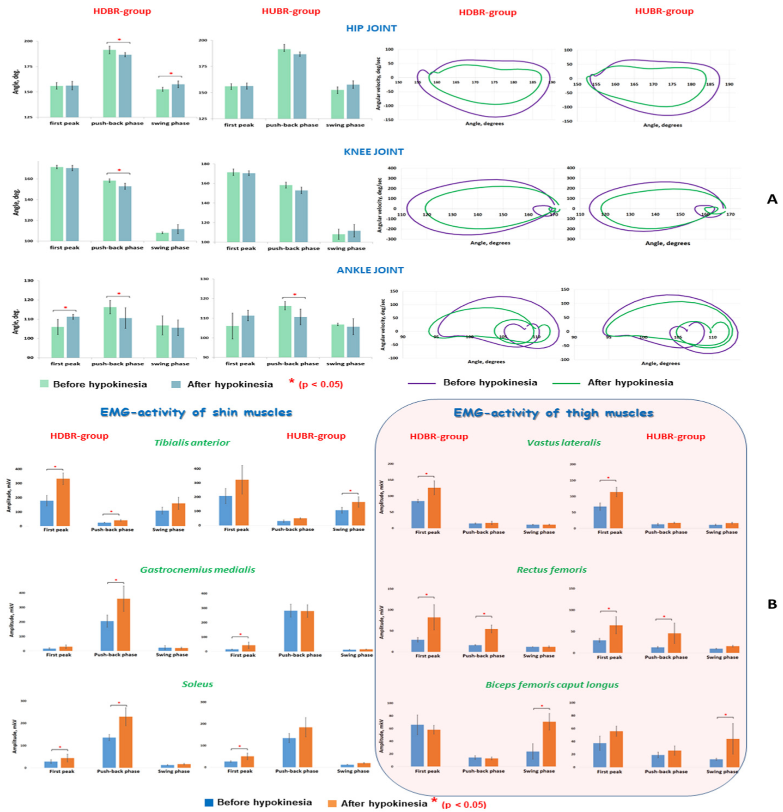

FIGURE 2: The angles in the joints of the feet (**A**) and EMG-activity of shin and thigh muscles (**B**) during walking after head-down bed rest and head-up bed rest.

trajectory area simultaneously to the reduction of weight loading. To compensate these changes, motions to the ankle joint are redistributed. As a result, phase trajectories for the ankle joint increase in area with the decrease of weight loading. The remaining four subjects (group No 2) showed a marked displacement of phase trajectories in angular coordinates during maximal reduction of weight loading (up to 17% body weight support). In them, we observed a shift in the phase trajectories in the direction of reducing the

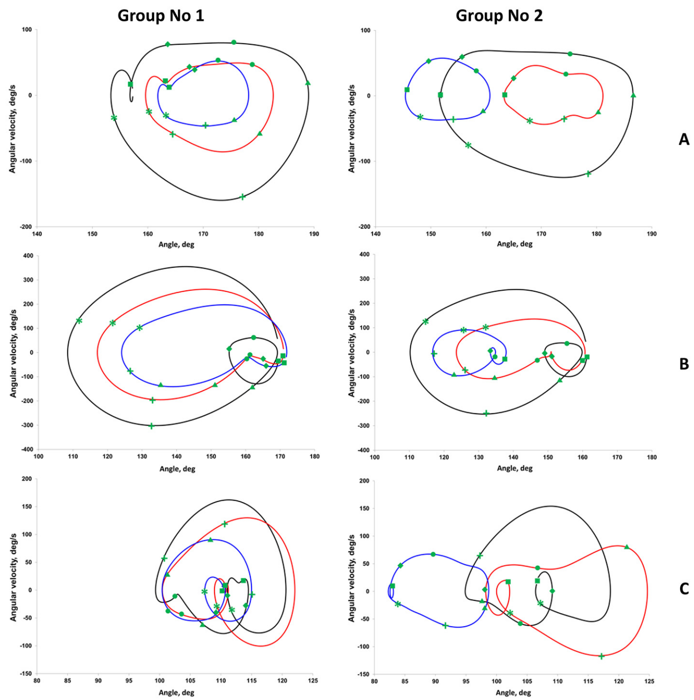

FIGURE 3: Normalized phase trajectories of the hip (**A**), knee (**B**) and ankle (**C**) joints during walking under various weight loadings on the musculoskeletal system. Black line – walking in 100% body weight support; red line – 38%; blue line – 17%.
Symbols for phases and moments of the double step:

■ -- Initial contact
◆ -- Foot flat (opposite toe off)
● -- "Vertical" moment in the phase of the middle support
▲ -- Heel off (beginning of push back phase)
+ -- Toe-off (repulsion is almost completed, push back phase)
✱ -- Feet adjacent
■ -- Initial contact (of the next step cycle)

angles of the joint while reducing the support for body weight up to 17%. Such locomotion strategy can be explained by the fact that these subjects completely rely on the body weight support system and take semi-bent posture.

In addition to the analysis of phase trajectories, we made an analysis of energy costs. The kinetic energy of rotational motion, which was associated with joint flexion and extension, was calculated as:

$$E_i^{\pm} = \frac{1}{2}\sum_k (J_i + m\ r^2) * (\varpi_{ik}^{\pm})^2 \quad (1)$$

where, J_i equals inertia moment in *the i*-link segment, *m* equals link weight, *r* is the distance from the mass link centre to the point of suspension, and *k=1* weight load of 100%, *k=2* weight load of 38%, *k=3* weight load of 17%. For defining the kinetic energy of flexion and extension in *i*-link, angular velocities were divided into positive - $\varpi_{ik}^{\pm}$ (extension) and negative - ϖ_{ik}^{-} (flexion) ones. The ratio of kinetic energy of extension to kinetic energy of flexion for joint was calculated using the formula:

$$R_i^{+/-}(k) = 100\% * E_i^{+}(k)/E_i^{-}(k) \quad (2)$$

Significant changes are seen in the hip joint (table 1). Walking under 100% body weight, flexion kinetic energy dominates over extension kinetic energy by 90%. If weight loading on the musculoskeletal system decreases up to 17%, difference between flexion and extension kinetic energies becomes less and is only 9%. In the ankle joint one can see an opposite situation. Ratio of flexion and extension energy becomes equal in values when musculoskeletal system has less loading. Body weight support of subject till 38% is enough to have flexion and extension energies close in values.

Table 1: The ratio of extension kinetic energy to flexion kinetic energy for different joints

Ratio, %	Hip joint	Knee joint	Ankle joint
k=1; 100% body weight	190±22	89±7	47±11
k=2; 38% body weight	143±28	74±7	97±29
k=3; 17% body weight	109±29	77±15	99±23

CONCLUSIONS

1. In long-term space flights, interval locomotor physical training, such as alternation of intense running and walking, prevents an increase in the physiological costs of locomotion and provides more efficient maintenance of the performance of the neuromuscular system after the flight.

2. Cosmonauts who used locomotor training in interval mode during space flights demonstrate smaller changes in the double step length and other biomechanical characteristics of walking after flight.

3. The intensity of locomotor training performed in interval mode is the leading factor in preventing adverse effects of microgravity.

4. Staying in conditions of antiorthostatic hypokinesia accompanied by the development of significant changes in biomechanical characteristics of walking.

5. Significant changes in angles joints are the probably the result of reducing the contractile properties of the leg muscles and increases "physiological cost" of walking.

6. Simulation of lunar gravity conditions by method of orthostatic hypokinesia did not lead to significant changes in the biomechanical parameters of walking.

7. The EMG-activity of the leg muscles decreases (4-65%) compared to normal walking.

8. The locomotion strategy changes during walking when weight loading at the musculoskeletal system becomes less.

9. The first one, dominating in all subjects, lies in less angle variations and angular velocities in hip and knee joints under reduced weight loading. Such strategy ensures posture stability while walking.

10. The second strategy is characterized with slightly bending type of walking. Such walking is less stable and less effective while reducing the weight load on the musculoskeletal system.

11. In the hip joint while walking under 100% body weight, extension energy dominates. With the reduction of body weight up to 17%, the ratio between extension and flexion energies comes to balance.

12. The obtained results allow us to say with confidence that video analysis of movements in combination with EMG-activity registration is a very informative method that allows assessing the functional state of the human musculoskeletal system not only under conditions of gravitational unloading, but also in other areas (for example, sports exercises, rehabilitation).

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Keywords: Long-term space flights, musculoskeletal system, body weight support, video analysis, joint angles, phase trajectories, the energy cost of walking

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Lung surfactant system in C57BL/6 mice after long-term space flight onboard Bion-M1 biosatellite and international space station

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INTRODUCTION

Lung surfactant (LS) is one of the important components in respiratory system. The main property of LS is a decrease of surface tension at the air-liquid interface and, thus, prevent the expiratory collapse of alveolar units. Along with this, LS affects the oxygen transfer through alveolar-blood barrier, water exchange and lung innate immunity cells. The impact of space flight upon LS properties is unknown. We have shown earlier (Bryndina et al., 2013) that, in the early period of antiorthostatic suspension (AOS), surfactant function is activated. It leads to the decrease of surface tension of bronchoalveolar lavage fluid (BALF). On the other hand, long-term AOS declines lung surface-active properties due to lysophospholipids accumulation. In BION-M1 mission, we did not find a significant disturbance of LS function in space-flown mice (Bryndina et al., 2014).

The aim of the present work was to compare the impact of 30-day space flight onboard the BION-M1 biosatellite and 37-day flight in ISS (Rodent Research program, RR-1) on pulmonary surfactant of C57Bl/6 mice.

MATERIALS AND METHODS

The experiments were conducted in C57Bl6 (n=20) and in C57Bl6/J mice (n=20) from the BION-M1 and RR-1 missions, respectively. In both projects, 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 staying in conditions similar to those in flight), and 4 - space flight (SF). SF mice from BION-M1 project were dissected in IBMP 12 h after landing.

In the RR-1 project, rats were euthanized and frozen directly onboard the ISS. All procedures with RR-1 SF mice were performed as described (I.V.Ogneva et al., 2018). The lungs were isolated by the scientists from the Ames Research Center of NASA from the frozen carcasses delivered to Earth. The biomaterials were kindly provided to us by IBMP. In the BION-M1 project, there was a possibility to investigate both bronchoalveolar lavage fluid (BALF) and lung tissue. We analysed the composition, functional properties of pulmonary surfactant and expression of some enzymes regulating phospholipids metabolism in lung. Surfactant properties were estimated by measuring the BALF surface tension according to Wilhelmi method. In the RR-1 project, only lung tissue samples were studied. TLC was used to assay the amount of total phospholipids (PL) and their fractions in lung tissue or BALF. The fractions were phosphatidylcholine (PC), lysophosphatidylcholine (LPL) and phosphatidylethanolamine (PEA). The expression of the key enzymes of PL metabolism was assayed by IFA and RT-PCR. Statistical analysis was performed using SPSS 6.0 program. To estimate the significant differencies between groups, Cruskall-Wallis test and Mann-Whitney U test were used. The differences considered significant at $p<0.05$.

RESULTS

No significant differences in surfactant activity were found in BION-M1 project between all groups of mice (Fig. 1). Total PL in BALF increased, although the tissue expression of choline phosphate cytidilyltransferase (CCT), the main enzyme regulating phosphatidylcholine biosynthesis, was unchanged. It should be taken into account a hypergravity during landing and 12-hour period between landing and dissection (early recovery period).

In lung tissue of SF mice from RR-1 project, the increased amount of total PL was found. Interestingly, CCT expression and PC level did not change (Fig. 2). In the phospholipid spectrum of lung tissue, the increase of LPL, PEA and LPL / PC rate was detected. We did not find the elevated expression of phospholipase A2 and phosphatidylethanolamine transferase, which could lead to LPL and PEA upregulation. Nevertheless, it does not exclude the changes in the activity of those enzymes. Note that all detected changes were significant only in SF or GC groups in comparison with VC or BC experiments. No differences were shown between GC and SF groups. It means that the same factor could affect surfactant PL spectrum in habitat equipment both in space and on Earth. PCR analysis detected 12% decrease in beta

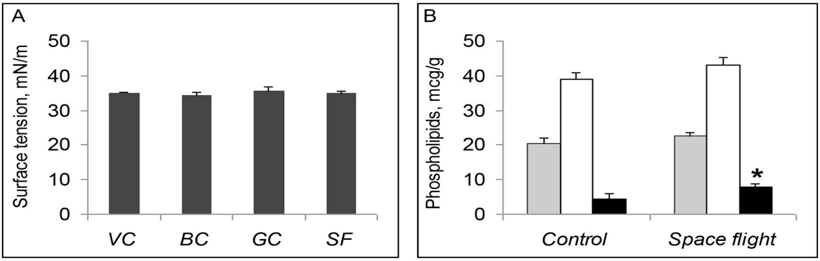

FIGURE 1: (**A**) - Surface activity (surface tension of bronchoalveolar lavage fluid, BALF) in BION-M1 mice; (**B**) – Phospholipids in BALF: lysophospholipids (LPL, gray bar), phosphatidylcholine (PC, open bar), and phosphatidylethanolamine (PEA, black bar); VC - vivarium control, BC - basal control, GC - ground control, SF - space flight. Data are presented as M±SEM, * p<0.05 is statistically significant difference in comparison with VC.

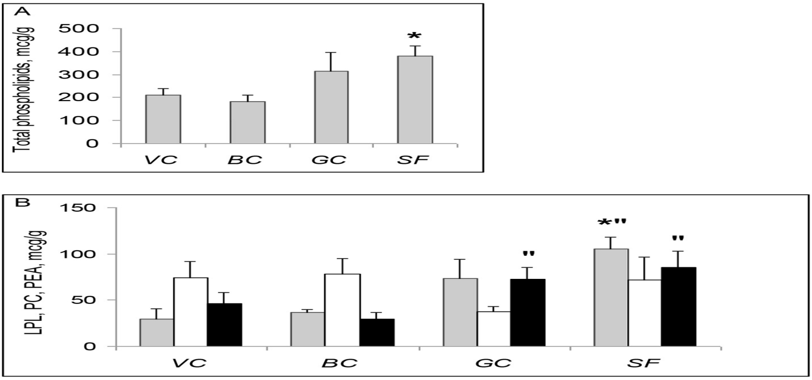

FIGURE 2: Phospholipids (PL) in lung tissue of mice (RR-1 program). (**A**) – total PL, (**B**) - lysophospholipids (LPL, gray bar), phosphatidylcholine (PC, open bar) and phosphatidylethanolamine (PEA, black bar); VC - vivarium control, BC - basal control, GC - ground control, SF - space flight. Data are presented as M±SEM; * p<0.05 is statistically significant difference in comparison with VC, "p<0.05 – in comparison with BC.

2-adrenoreceptors expression in lung tissue of RR-1 SF rats. However, the similar changes were also found in BC and GC groups. It suggests that this effect was not associated with space flight only, but depended on a habitat conditions in BC, GC and SF experiments.

The obtained results evidence that, in long-term space flight, LS is prone to alterations, but probably the influence of the microgravity upon surfactant system is not substantial. Although, the negative consequences of LPL accumulation in lung cannot be excluded. To know the precise effect, the detection of surfactant activity in BALF obtained in space is needed.

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Keywords: space flight, lung surfactant, phospholipids

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Inhibitor of acid sphingomyelinase clomipramine partly prevents atrophic changes in disused skeletal muscle

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INTRODUCTION

Sphingolipids are a class of molecules playing a pivotal structural and signaling role in a wide range of intracellular processes: cell viability and apoptosis, cell circle arrest and senescence, insulin signaling and membrane raft formation, etc. Some effects of exogenous or endogenous ceramide (Cer), the backbone molecule of sphingolipids (see for review Nikolova-Karakashian, Reid, 2011), resemble the changes occurring in disused skeletal muscle, including fatigue, power loss, declined protein synthesis and enhanced protein degradation.

We have demonstrated previously (Bryndina et al., 2014, 2018a) the increased ceramide generation in postural skeletal muscle (m. soleus) of mice and rats subjected to short-term (6-12 h) or more prolonged (4-30 days) hind-limb unloading (HU). Disuse led to the acid sphingomyelinase (aSMase) upregulation in murine and rat soleus muscle (Bryndina et al., 2017, 2018a). Clomipramine, belonging to the family of functional inhibitors of aSMase (FIASMA), attenuated a number of effects caused by disuse, including lipid rafts disruption and formation of ceramide enriched membrane domains (Bryndina et al., 2018a, b). Clomipramine is a drug belonging to the functional inhibitors of aSMase (FIASMA) family.

The role of sphingolipids in the development of disuse muscle atrophy is not entirely studied. Interestingly, the inhibitor of Cer *de novo* synthesis, myriocin, did not prevent muscle fiber atrophy during mechanical unloading (Salaun et al., 2016). So the authors concluded that this pathway is not involved in disuse muscle atrophy.

No prior study had explored the contribution of sphingomyelinase hydrolysis (another pathway of Cer formation) to disuse muscle atrophy. So the objective of the present work was to estimate the ability of clomipramine to prevent atrophic changes in rat soleus muscle subjected to 14-day unloading.

MATERIALS AND METHODS

Male Wistar rats (m=220-250g, n=12) were suspended by tail for two weeks according to the Ilyin-Novikov method modified by Morey-Holton. The 1st group of animals (n=6) was subjected to hindlimb unloading (HU) with clomipramine treatment (Anafranil, Novartis Pharma, intramuscularly in a dose of 1.25 mg/g every other day), the second group of suspended rats (n=6) was treated with vehicle - 0.9% saline solution. Intact, freely moving rats were used as a vivarium control (n=6). After the end of HU rats were anaesthetized with telazol (Zoetis). Soleus muscles were removed, weighed and immediately frozen in liquid nitrogen. Before study, the samples were stored at -80°C. For morphological and fluorescent assay, the muscles were cut into longitudinal and transverse sections (14 μm thick) using cryostat microtome (Shandon Cryotom E). For morphological analysis, sections were stained with hematoxylin-eosin and studied with Microoptix MX 300 microscope using Image J program. Cer distribution in soleus muscle fibers was estimated by staining with anti-Cer antibodies (mouse, 1: 300, Abcam), anti-mouse biotinylated antibodies (goat IgG, 1: 200, Abcam) and streptavidin-FITC conjugate (1:100, Abcam). The images were studied with the Nikon Eclipse E200 microscope combined with the Canon PowerShot 600 photo attachment using ImagePro Plus 6.0 and ImagePro Insight programs (Media Cybernetics, USA). The semiquantitative analysis of Cer amount was performed by the detection of fluorescence intensity of the test sections on a standard area (0.1mm2). For both morphological and immunofluorescent studies, each 5th section was taken and 10 measurements of intensity were made (at least 110-150 measurements per animal).

Statistical analysis was performed using SPSS 6.0 program. Depending on the distribution, one-way ANOVA with post hoc t test or Mann-Whitney U test were used, the differences between groups considered significant at $p<0.05$.

RESULTS

Hindlimb unloading led to the typical atrophic alterations in soleus muscle with parallel increase in Cer immunofluorescence throughout the muscle

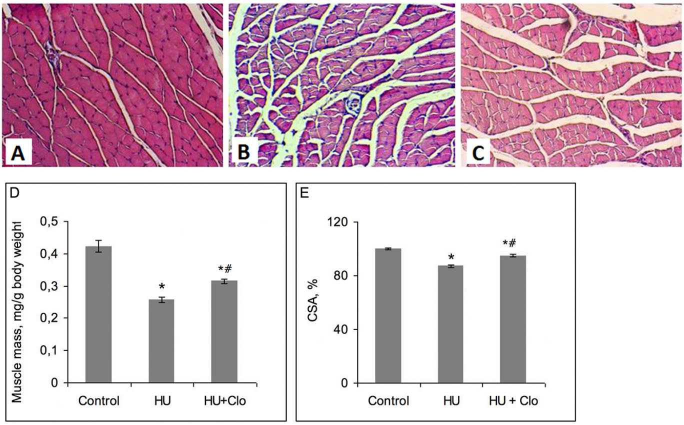

FIGURE 1: Atrophy of soleus muscle in hindlimb unloaded rats is partly prevented by clomipramine treatment. A, B, C – light microscope images of soleus muscle fibers stained with hematoxilin-eosin, (**A**) – muscle of freely moving rat (control), (**B**) – muscle of hindlimb unloaded rat (HU), and (**C**) - muscle of unloaded and clomipramine treated rat (HU+Clo), (**D**) – changes in muscle mass, (**E**) – changes in muscle cross-sectional area (CSA). Data are given as M±SEM, *P<0.05 is statistically significant difference compared with control value, and #p<0.05 - between non-treated and clomipramine treated groups.

fiber (Fig.1, 2). We observed a decline in muscle mass by 35% in comparison with the control freely moving rats (p<0.05) (Fig. 1). Also, a decrease of cross-sectional area (CSA) of muscle fibers, a morphological sign of atrophy, was found (from 100±0.8% to 87±0.9%, p<0.05). Another finding was the change in Cer distribution. Immunofluorescent analysis demonstrated a substantial intensity of Cer staining especially in the plasma membrane of the fibers (Fig. 2). Cer formed a kind of membrane clusters of medium and large sizes (Cer-enriched membrane domains), as it was shown previously for short-term disuse (Bryndina et al., 2018b). Clomipramine treatment during HU decreased the intensity of Cer accumulation and Cer domains formation. Atrophic changes in disused soleus muscle was partly prevented: muscle mass and CSA were 15% and 9% (p<0.05) increased, respectively, in hindlimb suspended with Clo treatment group in comparison with animals administered with vehicle.

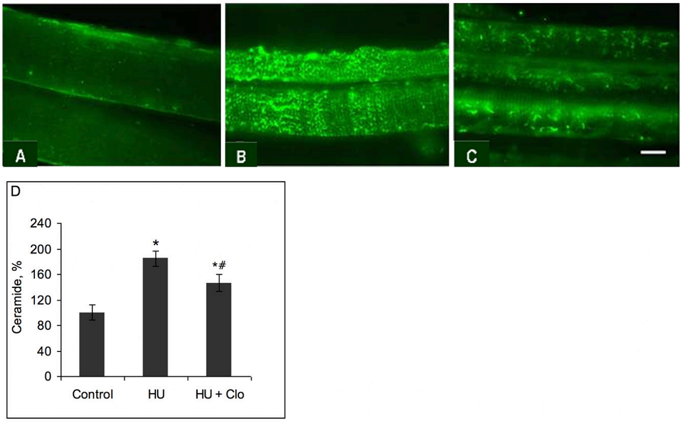

FIGURE 2: Hindlimb unloading increases the immunofluorescent labeling of ceramide in soleus muscle fibers. Clomipramine partly prevents this effect. A, B, C - fluorescent images of soleus muscle fibers stained with anti-Cer antibodies, (**A**) – muscle of freely moving rat (control), (**B**) – muscle of hindlimb unloaded rat (HU), and (**C**) - muscle of unloaded and clomipramine treated rat (HU+Clo); (**D**) – the histogram reflecting ceramide immunofluorescent labeling changes in soleus muscle of the control, HU and HU+Clo rats. Data are given as M±SEM, *P<0.05 is statistically significant difference compared with control value, and #p<0.05 - between non-treated and clomipramine treated groups.

CONCLUSION

Thus, inhibition of sphingomyelinase hydrolysis with clomipramine treatment decreases Cer formation in soleus muscle subjected to 14-day unloading and partly prevents the development of muscle atrophy. Whereas the inhibition of the de novo Cer synthesis by myriocin does not prevent disuse atrophy of soleus muscle (Salaun et al., 2016), clomipramine effect suggests that another pathway of Cer formation, sphingomyelinase hydrolysis, could be involved. Note that the drug can partly prevent lipid rafts disassembly in short-term disuse (Bryndina et al, 2018a). Taking into account our present and earlier data, we suppose that SMase hydrolysis and the membrane Cer accumulation resides in different stages of muscle inactivity. It is probably one of the mechanisms triggering the initial steps of muscle adaptation to unloading and supporting the atrophic process in long-term disuse.

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Keywords: muscle atrophy, disuse, sphingolipids, ceramide

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