11:30 Katherine Warthen – "Quantification of intracranial tissue, fluids, and ocular structural changes due to long-duration spaceflight"

Quantification of Intracranial Tissue, Fluids, and Ocular Structural Changes Due to Long-Duration Spaceflight

K.W. Warthen[1], S. Sater[2], G. Conley Natividad[3], L. Kramer[4], K. Hasan[5], B.R. Macias[6], S. Laurie[7], B.A. Martin[8]
[1]Alcyone Therapeutics; (KWarthen@alcyonetx.com), [2]Alcyone Therapeutics; (SSater@alcyonetx.com), [3]Alcyone Therapeutics; (GNatividad@alcyonetx.com), [4]UT Health (Larry.A.Kramer@uth.tmc.edu), [5]UT Health (Khader.M.Hasan@uth.tmc.edu), [6]NASA (Brandon.R.Macias@nasa.gov), [7]KBR (Steven.Laurie@nasa.gov), and [8]Alcyone Therapeutics; (Bryn@alcyonetx.com)

INTRODUCTION

Long duration spaceflight (LDSF) astronauts experience prolonged reduced gravitational pull on the body, which has been documented by post-flight MRI to result in a combination of structural changes to the brain and eye in some astronauts including: a) increased ventricular cerebrospinal fluid (CSF) volume[2], b) flattening of the posterior optic globe[3] and c) optic disc edema and choroidal engorgement[4]. These structural changes are believed to potentially underlie symptoms related to spaceflight-associated neuro-ocular syndrome (SANS). It is unclear how these changes relate to one another, if at all, and what metric, or combination of metrics, might best serve as a diagnostic biomarker for SANS and potential SANS countermeasures. Here, we apply methods to quantify structural changes in the intracranial compartment and eye in a cohort of LDSF astronauts and analyze their interrelationships.

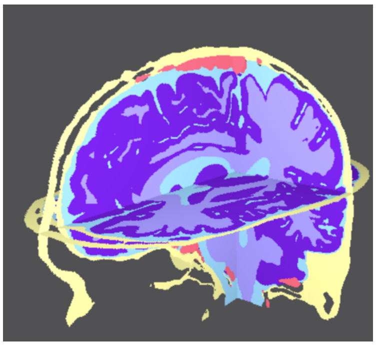


Figure 1. Example intracranial compartment segmentation, including skull (beige),blood (pink), CSF (blue), gray matter (dark purple), and white matter (light purple).

METHODS

MRI scans were collected from LDSF astronauts (n=13) pre- and R+3 days post-spaceflight. For reliability and comparison, control cohort (n=10) underwent MRI collection at a baseline timepoint and at 12-months. Brain volume analyses were performed in subject space, where "post" images are registered to "pre" images with skull segmentation only with a rigid body registration (SPM[5]). Each brain image was cropped at the McRae line at the foramen magnum and segmented into the individual tissue components with Charm[6]. An example segmentation is shown in Figure 1. Volume was calculated with FSL[7]. Optic globe segmentation was performed in an identical manner to Sater et al.[3] (Figure 2) with globe deformation averaged for left and right eye, and optic nerve and nerve sheath cross-sectional area 3mm posterior to globe were segmented according to Rohr et al.[8]. These methods were utilized to quantify posterior globe volume displacement (flattening), optic nerve sheath cross-sectional area, gray matter volume, white matter volume, total intracranial blood volume, and total intracranial CSF volume. Results were tabulated for each case and computed to determine change over LDSF. P-values were calculated using two-sided Wilcoxon signed-rank test with significance threshold $\alpha = 0.05$. A linear regression between changes in variables was performed to quantify Pearson correlation coefficient (r) slope direction, regression significance, and visualized in a correlation matrix.

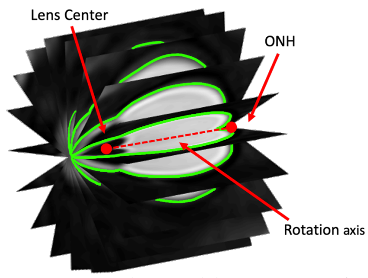


Figure 2. Example optic globe segmentation for quantification of posterior globe volume displacement (globe flattening) (modified from[1]).

RESULTS

Mean values for intracranial fluids (blood and CSF) and tissues (white and gray matter) (Table 1) were within the range of those previously reported in the literature, giving confidence that the applied methodology was robust. White and gray matter volume increased statistically after LDSF, but intracranial CSF volume decreased significantly (Table 1). Intracranial blood volume (arterial and venous), optic nerve and nerve sheath change after LDSF was not significant. The correlation matrix results (Figure 3), showed relationship of multiple variables, with strong relations for the obvious – such as pre versus post flight metrics for the same variable. A strong inverse relation of change in CSF volume and brain tissue volume was found. Optic globe volume displacement had a weak, but statistically significant, inverse relation with intracranial blood volume change.

Table 1. Summary of intracranial tissue, fluid, and ocular changes detected over LDSF.

Parameter	Preflight mean	Postflight mean	Change	Wilcoxon Rank Sum P-value
Blood Volume (cm3)	41.97	41.53	-0.44	0.17
White Matter Volume (cm3)	570.91	576.67	5.77	0.033
Gray Matter Volume (cm3)	745.99	753.22	7.23	0.0046
CSF Volume (cm3)	227.35	222.45	-4.89	0.04
Globe Deformation (mm3)	na	na	8.35	0.0024
Optic Nerve Area (mm2)	10.43	9.86	-0.57	0.81
Optic Nerve Sheath Area (mm2)	25.80	27.46	1.65	0.63

DISCUSSION

The findings indicate a balance of fluids and tissues within the intracranial compartment, akin to that predicted by Monro-Kellie hypothesis, and that LDSF may impact this balance. Both white and gray matter volume increased over LDSF (Figure 4) and corresponded to a concomitant decrease in intracranial fluid volume (Figure 5). The slope of intracranial tissue versus fluid volume changes was less than unity (Figure 5), potentially suggesting that some of the increase in fluid volume in the brain may not be detectable, for example residing within the interstitial region (altered brain water content). Interestingly, the data indicated that there was a significant correlation with change in intracranial blood volume and optic globe deformation, but not ONS cross-sectional area. This may indicate a more direct role of the vascular system impacting the eye. In the control cohort, analyzed at a 12-month follow-up, nearly zero change was detected in all parameters measured, indicating a likelihood that effect size in the LDSF cohort was attributed to spaceflight. This is a limited cohort of only 13 astronauts, thus statistical power is limited and must be confirmed for all metrics in an expanded data set.

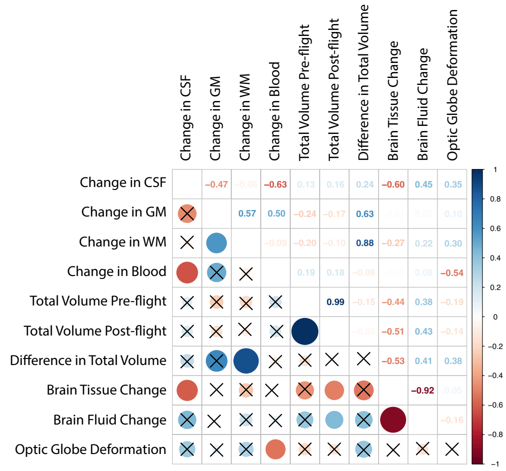


Figure 3. Statistical findings for comparisons across variables. Upper quadrant values represent Pearson's r while lower quadrant values include a visual representation of the correlation strength, with X indicating p>0.05.

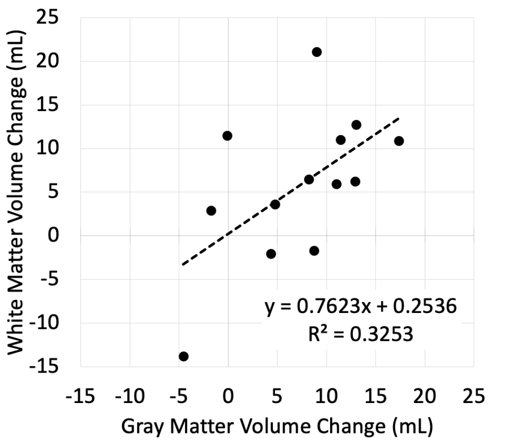


Figure 4. Intracranial white and gray matter volume change are positively correlated, indicating potential that both components of the brain tissue are altered after LDSF.

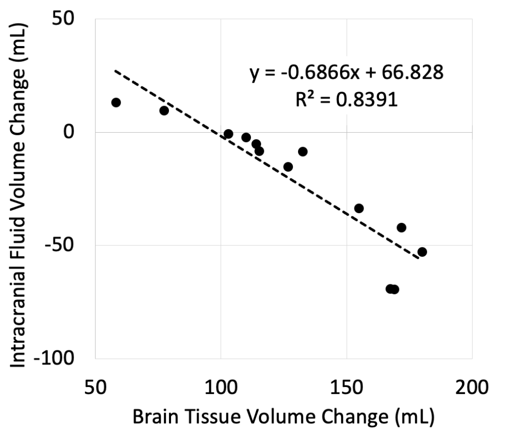


Figure 5. Change in intracranial fluid volume (blood and CSF) is inversely related to change in brain tissue volume (WM and GM), indicating that increase in brain tissue volume after LDSF is compensated by a decrease in intracranial CSF.

REFERENCES

1 Sater, S. H. *et al.* MRI-based quantification of posterior ocular globe flattening during 60 days of strict 6 degrees head-down tilt bed rest with and without daily centrifugation. *J Appl Physiol (1985)*, doi:10.1152/japplphysiol.00082.2022 (2022).

2 Kramer, L. A. *et al.* Intracranial Effects of Microgravity: A Prospective Longitudinal MRI Study. *Radiology* **295**, 640-648, doi:10.1148/radiol.2020191413 (2020).

3 Sater, S. H. *et al.* Automated MRI-based quantification of posterior ocular globe flattening and recovery after long-duration spaceflight. *Eye (Lond)* **35**, 1869-1878, doi:10.1038/s41433-021-01408-1 (2021).

4 Laurie, S. S. *et al.* Optic Disc Edema and Choroidal Engorgement in Astronauts During Spaceflight and Individuals Exposed to Bed Rest. *JAMA Ophthalmol* **138**, 165-172, doi:10.1001/jamaophthalmol.2019.5261 (2020).

5 Ashburner, J. Computational anatomy with the SPM software. *Magn Reson Imaging* **27**, 1163-1174, doi:10.1016/j.mri.2009.01.006 (2009).

6 Puonti, O. *et al.* Accurate and robust whole-head segmentation from magnetic resonance images for individualized head modeling. *Neuroimage* **219**, 117044, doi:10.1016/j.neuroimage.2020.117044 (2020).

7 Smith, S. M. *et al.* Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* **23 Suppl 1**, S208-219, doi:10.1016/j.neuroimage.2004.07.051 (2004).

8 Rohr, J. J. *et al.* Quantitative magnetic resonance image assessment of the optic nerve and surrounding sheath after spaceflight. *NPJ Microgravity* **6**, 30, doi:10.1038/s41526-020-00119-3 (2020).

11:45 Danniella Hurt – "Cephalad fluid shifts associated with neuroprotective alterations in cerebral perfusion and haemostasis independent of systemic oxygenation status"

Cephalad fluid shifts associated with neuroprotective alterations in cerebral perfusion and haemostasis independent of systemic oxygenation status

D. Hurt[1*], B.S. Stacey[1], D. Lanéelle[1,2,3], L. Fall[1], R. Sixtus[4], C.J. Marley[1], A. Haq[1], T. Owens[1], L. Yandle[1], C. Puhl[5], H. Normand[3] and D.M. Bailey[1]

[1]Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Glamorgan, UK (danniella.hurt@southwales.ac.uk; benjamin.stacey@southwales.ac.uk; chris.marley@southwales.ac.uk; lewis.fall@southwales.ac.uk; adnan.haq@sotuhwales.ac.uk; tom.owens@southwales.ac.uk; leon.yandle@southwales.ac.uk; damian.bailey@southwales.ac.uk;); [2]Service de Médecine Vasculaire, Centre Hospitalo-Universitaire, Caen (damien.laneelle@unicaen.fr; herve.normand@unicaen.fr); [3]Normandie University, UNICAEN, INSERM, COMETE, GIP CYCERON, Caen, France; [4]School of Biosciences, University of Cardiff, Glamorgan, UK (sixtusr@cardiff.ac.uk); [5] elespazio Belgium S.R.L. for the European Space Agency, Noordwijk, Netherlands (Christopher.Puhl@ext.esa.int)

BACKGROUND

Gravity-dependent shifts in central blood volume (CBV) induced by the microgravity of orbital spaceflight pose unique physiological challenges for the astronaut brain. Interest continues to focus on the complex pathophysiology underlying a constellation of debilitating neurological, ophthalmological and neurovestibular symptoms known collectively as the spaceflight-associated neuro-ocular syndrome (SANS, formerly the visual impairment/intracranial pressure syndrome). Recent attention has focused on gravity-induced redistribution of fluids toward the head and associated haemostatic consequences associated with altered regional cerebral perfusion (Marshall-Goebel et al., 2019). Changes in posture in terrestrial analogues (stand to head-down tilt) allows for the opportunity to induce a large gravity-dependent shift in CBV to better phenotype underlying mechanisms. Furthermore, the future of human space exploration will require extended extravehicular activities and consequent exposure to low levels of oxygen (hypoxia) that has been associated with blood brain-barrier disruption subject to cerebral hyperperfusion and activated coagulation (Bailey et al., 2009; Bailey et al., 2020).

AIM

To determine to what extent postural changes independently, or in conjunction with inspiratory hypoxia, impacts clot microstructure and implications for regional cerebral perfusion.

METHODOLOGY

Ten healthy males aged 30 (mean) ± 9 (SD) years old were examined in two different postural positions (standing and 180° head-down tilt) for 10 min each, under conditions of normoxia (FIO_2 = 20.93 %) and hypoxia (FIO_2 = 12 %, randomised, single-blind, counterbalanced design with 60 min inter-trial washout). Anterior (internal carotid artery, ICAQ) and posterior (vertebral artery, VAQ) blood flow was assessed using duplex ultrasound. Global cerebral blood (gCBF) was calculated as (ICAQ + VAQ) × 2. Shear rate (SR) was calculated as 4 × peak envelope velocity/ arterial diameter. Cerebrovascular conductance index (CVCi) was calculated as Q /mean arterial blood pressure. Cephalic venous blood was obtained without stasis for haemorheological assessment of the fractal dimension (df), a novel biomarker of insipient clot microstructure.

RESULTS

Head-down tilt was generally associated with a selective elevation in ICACVCi due primarily to the increase in ICAQ that was not apparent in the posterior circulation (unchanged VAQ/CVCi). Despite global hypoxic cerebral vasodilation (elevated gCBF), this did not affect the regional responses to postural tilt. Hypoxia increased df with a general and consistent reduction observed during head-down tilt.

Table 1. Cerebral haemodynamics and haematological responses

Inspirate:	Normoxia (21% O_2)		Normoxia (12% O_2)	
Position:	Head-up	Head-down	Head-up	Head-down
ICA_Q (mL/min)	141 ± 54	182 ± 45	170 ± 40	206 ± 68
Position (P = 0.070)				
ICA_{SR} (/s)	253 ± 50	238 ± 78	311 ± 117	257 ± 71
ICA_{CVCi} (mL/min/mmHg)	1.54 ± 0.78	2.00 ± 0.64	1.83 ± 0.61	2.32 ± 0.62
Position (P = 0.048)				
VA_Q (mL/min)	74 ± 17	78 ± 29	90 ± 30	87 ± 23
VA_{SR} (/s)	221 ± 53	227 ± 35	245 ± 62	237 ± 74
VA_{CVCi} (mL/min/mmHg)	0.78 ± 0.21	0.86 ± 0.34	0.94 ± 0.32	1.01 ± 0.31
g_{CBF} (mL/min)	431 ± 122	520 ± 102	520 ± 110	586 ± 163
Inspirate (P = 0.022)				
g_{SR} (/s)	948 ± 186	930 ± 194	1113 ± 336	989 ± 248
g_{CVCi} (mL/min/mmHg)	4.66 ± 1.89	5.72 ± 1.57	5.55 ± 1.6	6.65 ± 1.6
Inspirate (P = 0.025)				
d_f (AU)	1.94 ± 0.13	1.79 ± 0.09*	1.85 ± 0.11	1.79 ± 0.08*
Inspirate (P = 0.035); Inspirate × Position (P = 0.029)				

*different (P < 0.05) between positions as a function of inspirate

DISCUSSION

The present study has identified several important findings. First, head-down tilt was associated with a selective elevation in regional perfusion to the anterior and not posterior circulation, a response that was not further compounded following superimposition of additional (hypoxia-mediated) vasodilation. Given that the posterior circulation is more prone to hyperperfusion-mediated autoregulatory breakthrough and consequent structural damage to the neurovascular unit (Bailey et al., 2020), this 'constrained' perfusion may prove a neuroprotective response. Second, that the novel biomarker df was consistently lower implies a collective reduction in incipient clot viscoelastic strength, polymerisation and crosslinking during head-down tilt, that may equally prove a compensatory antagonistic neuroprotective response.

CONCLUSIONS

These findings collectively demonstrate constrained perfusion to the posterior cerebral circulation and consistent reduction in activated coagulation independent of systemic oxygenation status during head-down tilt. These responses may provide some degree of neuroptection against the marked CBV-induced cephalad fluid shifts that can cause structural-functional damage to the neurovascular unit.

REFERENCES

Bailey DM, et al. (2009). Emerging concepts in acute mountain sickness and high-altitude cerebral edema: from the molecular to the morphological. Cell Mol Life Sci 66, 3583-3594.

Bailey DM, et al. (2020). Gravitational transitions increase posterior cerebral perfusion and systemic oxidative-nitrosative stress: implications for neurovascular unit integrity. Neuroscience 441, 142-160.

Marshall-Goebel K, et al. (2019). Assessment of Jugular Venous Blood Flow Stasis and Thrombosis During Spaceflight. JAMA Netw Open 2, e1915011

12:00 Constance Badali – "SpaceBike – Preliminary data on cycling neuromechanics in weightlessness"

SpaceBike – Preliminary Data on Cycling Neuromechanics in Weightlessness

Constance Badalì[1], Petra Wollseiffen[1], and Stefan Schneider[1]
[1]German Sport University Cologne, Cologne, Germany, Institute for Movement and Neurosciences
(c.badali@dshs-koeln.de, p.wollseiffen@dshs-koeln.de, schneider@dshs-koeln.de)

INTRODUCTION

Regarding the plans of national and international space agencies with longer stays in weightlessness, it is essential to investigate the influence of weightlessness on neuromuscular coordination. This underlies human locomotion, as one of the most important movements and a fundamental ability for human life on Earth. Walking, running and jumping are considered as natural forms of movement that are important for locomotion and everyday activities (Kramer et al., 2012). These forms of movement are based on well-established neural communication sequences called engrams. Engrams are stable over time, but can be influenced by external factors. Similarly, cycling movements are based on engrams. The pedalling movement is a repeatable two-dimensional form of movement that serves well to study neuronal activation under altered environmental conditions such as weightlessness. It has already been shown that brain cortical activity during cycling can be recorded and localised with an electroencephalogram (EEG) and combined with electromyography (EMG) (Schneider et al., 2013). EEG and EMG activity showed comparatively stable oscillations over different intensities during cycling and had a strong correlation at higher intensities.

The data presented here are preliminary data from one parabolic flight campaign. Generally, the SpaceBike project aims to deepen the knowledge of the relationship between mechanical components of muscle physiology and their neuromuscular control in weightlessness. It is hypothesized, that the engrams underlying cycling, change or lose their stability. The results will be of importance for the development of appropriate countermeasures for astronauts during long-term space flights.

METHODS

Data were measured from two participants during a parabolic flight campaign in Bordeaux, France in September 2022. A parabolic flight is a suitable analogue to simulate weightlessness. One parabola consists of four phases that begin at 1G, enter hypergravity at 1.8G and peak at 22 seconds of weightlessness before ending at 1.8G and 1G again. During this manoeuvre, participants cycled on a bicycle ergometer at an individual cadence in three intensities (1W/2W/3W/per kg body weight) at 1G and 0G. Cortical and muscular activity were measured simultaneously with a 64-electrode EEG cap (actiCap-64Ch, Brain Products GmbH, Munich, Germany) and 14 wireless EMG sensors (Trigno Avanti Sensors, Delsys, Greater Manchester, UK) attached to the legs in accordance with SENIAM standards. The data was normalized to one pedal cycle, so that it is clearly recognizable at what time of a pedal cycle which muscle group is active.

RESULTS

Muscle activity increased with increasing intensity from 1 to 3 watt/kg body weight, regardless of the gravitational condition. Especially at higher cycling intensities, the activity of the muscle groups responsible for actively pulling and pushing the pedals is higher in weightlessness than in normal gravity (s. Figure 1 a,b).

Current cortical density showed a difference depending on the surrounding gravity condition (s. Figure 1c), as weightlessness resulted in higher cortical activity and revealed an oscillation pattern that became more concise at higher cycling intensities.

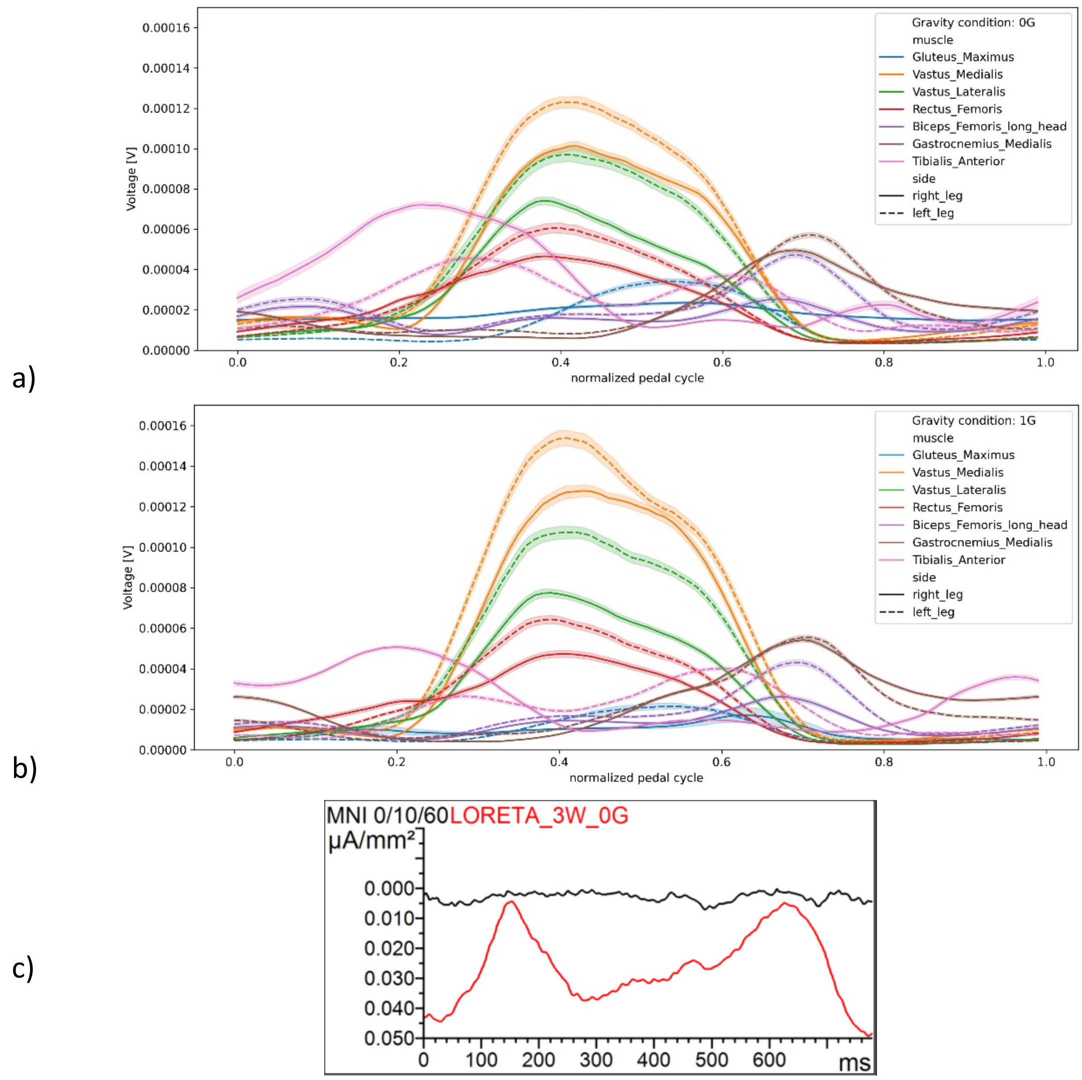


Figure 1: a,b) Muscle activity of 14 muscles (7 muscles per side) of the lower limb in 3W/kg body weight in 0G and 1G condition normalised to one pedal cycle. c) Cortical current density oscillations in 0G and 1G across the pedalling cycling of 680 ms at a sphere of 10 mm around Montreal Neurophysiological Institute (MNI) coordinate (x/y/z) 0/10/60. Black represents the intensity of 3W in 1G and red represents the intensity of 3W in 0G.

DISCUSSION

The presented data clearly show an effect of reduced gravity on muscular and neuronal activity. Furthermore, both parameters seem to be influenced by the different cycling intensities since higher cortical as well as muscular activity at 3 watts compared to the lower intensities could be observed (s. Figure 1). The higher muscular activity most probably indicates an increased recruitment of alpha motor neurons to maintain the cycling movement in an unfamiliar environment such as weightlessness. Further data will be collected during upcoming parabolic flight campaigns in order to identify possible existing patterns and to find neurophysiological explanations for these observations.

REFERENCES

Kramer, A., et al., *Leg stiffness can be maintained during reactive hopping despite modified acceleration conditions.* J Biomech, 2012. **45**(10): p. 1816-22.

Schneider, S., et al., *Cortical current density oscillations in the motor cortex are correlated with muscular activity during pedaling exercise.* Neuroscience, 2013. **228**: p. 309-14.

12:15 Laurent Opsomer – “Movement direction drift in astronauts performing point-to-point arm movements without visual feedback”

Movement direction drift in astronauts performing point-to-point arm movements without visual feedback

L. Opsomer[1], J-L. Thonnard[2], P. Lefèvre[3] and J. McIntyre[4].

[1]Institute of Neuroscience and ICTEAM institute, Université catholique de Louvain, Brussels, Belgium (laurent.opsomer@uclouvain.be).
[2]Institute of Neuroscience and ICTEAM institute, Université catholique de Louvain, Brussels, Belgium.
[3]Institute of Neuroscience and ICTEAM institute, Université catholique de Louvain, Brussels, Belgium.
[4]CNRS, Paris, France; Tecnalia, Basque Research and Technology Alliance, San Sebastian, Spain; Ikerbasque Science Foundation, Bilbao, Spain (joe.mcintyre@parisdescartes.fr).

BACKGROUND

Gravity provides a ubiquitous reference axis defining up and down. On Earth, humans can easily estimate the orientation of their body using gravitational cues conveyed by vestibular, proprioceptive and tactile inputs. In the absence of gravitational cues, spatial orientation is much more dependent on vision, and the “up” and “down” directions become ambiguous. Astronauts working onboard the International Space Station (ISS) can often feel disoriented and experience illusory sensations of self-motion (Lackner and Graybiel, 1981; Kornilova, 1997). Previous studies have also shown that gravity participates in defining a reference frame at the motor planning stage to anticipate the effects of gravity and optimize movements as a function of gravitational forces (McIntyre et al., 2001; Le Séac’h and McIntyre, 2007; Gaveau et al., 2016). How visual, proprioceptive and gravitational cues interact in defining this reference frame is not totally clear. In particular, the impact of microgravity on spatial orientation at the level of arm motor control has been largely unexplored. The present work was designed to fill this gap.

RESULTS

We investigated the kinematics and the accuracy of point-to-point arm movements performed by astronauts (n=11) on the ground and onboard the ISS at multiple time points of a long-duration space-flight (>5 months). To study the interaction between visual, gravitational and egocentric cues on movement kinematics and accuracy, these movements were performed with eyes open or closed, in seated or supine posture, and parallel or perpendicular to the longitudinal body axis.

In agreement with past studies, movements showed direction-dependent kinematics on the ground, with velocity profiles showing asymmetrical peaks along the gravity axis and more symmetrical peaks along the horizontal axis. The dependence of arm kinematics on movement direction was substantially reduced onboard the ISS.

Our data additionally revealed a striking effect of gravity on movement accuracy and stability in the absence of visual feedback (eyes closed). On the ground, when the head was aligned with gravity (seated posture), movements remained quite accurate with eyes closed, and the trajectory was stable over time (Fig. 1). In contrast, when the head was not aligned with gravity (supine posture), movement direction drifted substantially over time, becoming more and more tilted in the plane of motion (para-sagittal plane) relative to the target axis. Onboard the ISS, where gravitational cues are absent, this phenomenon was observed in both the seated and the supine postures. The drift remained significant during the entire space flight.

CONCLUSION

Altogether, these results provide strong support to the hypothesis that gravity is used by the nervous system to implement a reference frame for motor control. They additionally show that gravity can serve as an anchor preventing movement drift in the absence of vision.

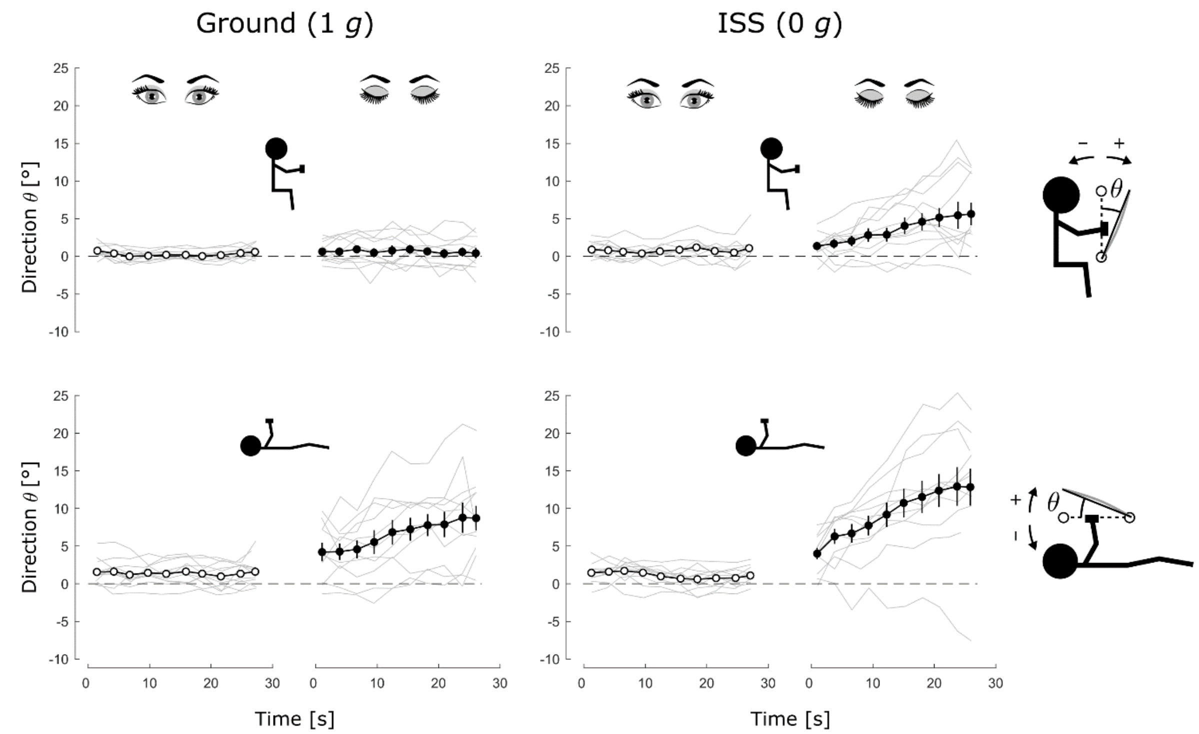


Figure 2 - Movement direction (w.r.t. target axis) during point-to-point arm movements performed by astronauts with eyes open (white circles) and with eyes closed (black circles), in seated (top) and supine (bottom) postures, on the ground (left) and onboard the International Space Station (left). Movement direction θ was computed in the para-sagittal plane as the angle between a line connecting the start and end point of each movement, and a line connecting the targets (here parallel to the longitudinal body axis).

REFERENCES

Lackner, J. R., and Graybiel, A. (1981). *Exp. Brain Res.* 44, 312–316.

Kornilova, L. (1997). *J. Vestib. Res. Equilib. Orientat.* 7, 429–439.

McIntyre, J., Zago, M., Berthoz, A., and Lacquaniti, F. (2001). *Nat. Neurosci.* 4, 693–694.

Le Séac'h, A. B., and McIntyre, J. (2007). *Neurosci. Lett.* 423, 211–215.

Gaveau, J., Berret, B., Angelaki, D. E., and Papaxanthis, C. (2016). *Elife* 5, 1–17.

Tatiana Kostrominova – "P2YI and P2Y2 receptors differ in their role in the regulation of signaling pathways during unloading-induced rat soleus muscle atrophy"

P2Y1 and P2Y2 Receptors Differ in Their Role in the Regulation of Signaling Pathways During Unloading-induced Rat Soleus Muscle Atrophy

T.Y. Kostrominova (tkostrom@iun.edu)[1], K. Zaripova (katsu.no.himitsu@gmail.com)[2], S. P. Belova (swetbell@mail.ru)[2], K. Sharlo (sharlokris@gmail.com)[2], B.S. Shenkman (bshenkman@mail.ru) [2], and T. L. Nemirovskaya (nemirovskaya@bk.ru) [2]

[1] Department of Anatomy, Cell Biology and Physiology, Indiana University School of Medicine-Northwest, Gary, IN, USA.
[2] Myology Laboratory, Institute of Biomedical Problems, RAS, Moscow, Russia.

INTRODUCITON

Increased accumulation of ATP in skeletal muscle during early stages of unloading is well described [1] [2] [3]. Pannexin (PANX) channels transport ATP from the cytoplasm into extracellular space [4]. ATP-dependent signaling pathway purinergic P2Y2 receptor/ PLC/ IP3 regulates release of calcium from the endoplasmic reticulum [5]. P2Y2 receptors activate the release of IP3, then IP3 binds membrane IP3 receptors present on the surface of the nuclei and sarcoplasmic reticulum [6] [7] .

HYPOTHESES

Current study tested a hypothesis that purinergic receptors P2Y1 and P2Y2 regulate gene expression in soleus muscle during unloading. ATP is exiting cells via PANX channels, interacts with P2Y1/2 receptors and this results in activation of markers of protein catabolism and in decreased protein synthesis. The hypothesis was tested using inhibitors of purinergic receptors.

METHODS

Thirty-two male Wistar rats were randomly assigned into one of four groups (8 rats in group): non-treated control (C), 3 days of unloading/hindlimb suspension with placebo (HS), 3 days of unloading treated with P2Y1 receptors inhibitor MRS2179 (HSM), and 3 days of unloading treated with P2Y2 receptors inhibitor AR-C 118925XX (HSA).

RESULTS

This study for the first time showed that after three days of soleus muscle unloading: 1) blocking of P2Y1 or P2Y2 receptors prevents the accumulation of ATP, the increase of IP3 receptors content and the decrease of p-GSK-3 beta, attenuates the diminishment of protein syntheses rate but had no effect on the mTORC1-dependent signaling markers p-4E-BP and p-eEF2; 2) blocking of P2Y1 receptors prevents unloading-induced upregulation of p-p38MAPK and attenuates the increase of MuRF1 mRNA expression; 3) blocking of P2Y2 receptors prevents muscle atrophy and unloading-related decrease of p-ERK1/2, diminishes the increase in mRNA expression of MAFbx, ubiquitin and IL6R, prevents the decrease of p-AMPK and the increase of p-p70S6K.

CONCLUSIONS

It can be suggested that preservation of muscle mass during unloading after blocking of P2Y2 receptors is primarily associated with the inhibition of catabolic processes and with energy homeostasis rather than with changes in the anabolic signaling. Blocking of P2Y2 receptors had no significant effect on the unloading-induced decline of muscle mass.

The study was supported by Russian Science Foundation (RSF grant 21-15-00228)

REFERENCES

1. Gupta, R.C., K.E. Misulis, and W.D. Dettbarn. Neurochem Res, 1989. **14**(7): p. 647-55.

2. Ohira, Y., et al. Acta Astronaut, 1994. **33**: p. 113-7.

3. Zaripova, K.A., et al. Int J Mol Sci, 2021. **22**(19).

4. Dahl, G.. Philos Trans R Soc Lond B Biol Sci, 2015. **370**(1672).

5. Liu, B., et al.. Cell Calcium, 2018. **76**: p. 62-71.

6. May, C., et al.. Biochem Pharmacol, 2006. **71**(10): p. 1497-509.

7. Casas, M., S. Buvinic, and E. Jaimovich. Exerc Sport Sci Rev, 2014. **42**(3): p. 110-6.

Bing Han – “Modulation of macrophage pryptosis by acute or chronic stress - an in vitro approach”

Modulation of macrophage pyroptosis by acute or chronic stress – an *in vitro* approach

Bing Han, Alexander Choukér and Dominique Moser,
Laboratory of Translational Research ‘Stress & Immunity’, Department of Anesthesiology, LMU Hospital, Ludwig-Maximilians-University, Munich, Germany.

E-mail: *Bing Han* Bing.Han@med-uni-muenchen.de; *Alexander Choukér* Alexander.Chouker@med-uni-muenchen.de; *Dominique Moser* Dominique.Moser@med-uni-muenchen.de

INTRODUCTION

During space flight, astronauts are exposed to various conditions, which altogether cause physical or psychological stress and can influence immune system homeostasis. Thereby induced stress occurs both in an acute way (e.g., during EVAs) and in a chronic way (e.g., circadian disruption) and results in the release of different stress hormones (Smith SM, et al. 2012). Among them, the glucocorticoid cortisol plays a key role in suppressing immune responses (Feuerecker M, et al. 2013), which represent one of the reasons for increased susceptibility towards pathogen infections in space (Mehta SK, et al. 2014).

Macrophages are the first-line effector innate immune cells against pathogen infection. To sense and fight against infection, macrophages exert the TLR- and (NLRP3-) inflammasome-sensing, caspase-1-executing dependent programmed cell death named pyroptosis (Hersh D, et al. 1999). Thereby macrophages are lysed and pro-inflammatory cytokines IL-1β and IL-18 are released in the extracellular space and inflammatory cascades to prevent spread of infection (Kepp O, et al. 2010). The impact of cortisol on pyroptosis in macrophages was not sufficiently addressed by now.

METHODS

THP-1 macrophage-like cells were used to investigate the effects of incrementing concentrations of the synthetic glucocorticoid hydrocortisone (HC), the pharmaceutical term for cortisol, on induction and execution of pyroptosis. For this, cells were pre-treated either with 0.1 μg/ml HC to mimic basal human HC levels or with 0.2 and 0.4 μg/ml to mimic moderate and high stress levels. Pyroptosis was induced by LPS (lipopolysaccharide, 5 ng/ml) and ATP (adenosine triphosphate, 5 mM). Acute stress was induced by an increase in HC concentrations from basal level (0.1 μg/ml) to stress level (0.2 or 0.4 μg/ml) after pyroptosis induction. Chronic stress conditions were simulated with constantly moderate (0.2 μg/ml) or high (0.4 μg/ml) stress HC levels as during pre-treatment. Cell pellets and cell culture supernatant were collected for analysis of downstream signaling pathways, protein expression, and cell death.

RESULTS

Results derived from these investigations have shown, that acute stress conditions did not change LPS- and ATP-mediated cell lysis compared to physiological control, however simulated chronic stress dose-dependently attenuated cell lysis. Interestingly, after acute stress conditions, NLRP3 inflammasome activation as well as extracellular IL-1β levels were dose-dependently augmented. However, pro-inflammatory signaling pathway (esp. NF-κB) activation and IL-1β mRNA levels were unaltered under these conditions. Chronic stress suppressed the NF-κB signaling pathway, protein expression and their activation.

CONCLUSION

This study described that the effects of HC on pyroptosis in macrophages as well as on their release of the pro-inflammatory cytokine IL-1β are differently affected in dependence of an acute or chronic increase of HC concentrations. Generally, chronic stress condition inhibited, whereas acute stress condition sensitized macrophage pyroptosis. The results add to the understanding of stress hormones on innate immune responses, especially pathogen sensing and clearance during spaceflight. Further investigations of pyroptosis under stress hormone exposure in (simulated) microgravity, in or/and post-flight will support the elucidation of the impact of weightlessness and stress in space on macrophage functions.

REFERENCES

Smith SM, Heer M, Wang Z, Huntoon CL, Zwart SR. *J Clin Endocrinol Metab.* 2012 Jan;97(1):270-8.

Feuerecker M, Mayer W, Kaufmann I, Gruber M, Muckenthaler F, Yi B, Salam AP, Briegel J, Schelling G, Thiel M, Choukèr A. *Clin Exp Immunol*. 2013 May;172(2):290-9.

Mehta SK, Laudenslager ML, Stowe RP, Crucian BE, Sams CF, Pierson DL. *Brain Behav Immun.* 2014 Oct;41:210-7.

Hersh, D., Monack, D. M., Smith, M. R., Ghori, N., Falkow, S., & Zychlinsky, A. *Proc Natl Acad Sci U S A*. 1999 96(5), 2396-2401.

Kepp, O., Galluzzi, L., Zitvogel, L., & Kroemer, G. (2010). *Eur J Immunol*, 40(3), 627-630.

Ageing and Altered Gravity; a Cellular Perspective

Sharon van Rijthoven[1] and Jack J.W.A. van Loon[2,3]

[1]Delft University of Technology (sharon.v.rijthoven@live.nl)
[2]Department of Oral and Maxillofacial Surgery/Pathology, Amsterdam Movement Sciences & Amsterdam Bone Center (ABC), Amsterdam UMC location Vrije Universiteit Amsterdam & Academic Center for Dentistry Amsterdam (ACTA), Gustav Mahlerlaan 3004, 1081 LA, Amsterdam, The Netherlands.
[3]European Space Agency (ESA), European Space Research and Technology Centre (ESTEC), TEC-MMG, Keplerlaan 1, 2201 AZ Noordwijk, The Netherlands (j.vanloon@amsterdamumc.nl).

Astronauts and the elderly experience similar ageing symptoms. Osteoporosis, muscle atrophy, and a weakened immune system among other things. Although quite some review papers compare the signs of the higher-level systems in the body, like tissue and organs, relatively few analyses have been done on a cellular level. This literature study investigates the relationship between ageing and altered gravity from a cellular perspective. First, an extensive list was constructed consisting of 209 variables described in literature (López-Otín C. et al, 2013; Schmauck-Medina T. et al, 2022; Bajpai A. et al, 2021; Phillip J. M. et al, 2015; Starodubtseva M., 2011) which alter during ageing. All observations were categorised in one of the eleven themes: DNA & epigenetics, Mitochondria, Nucleus, Immune system, Protein& degradation, Cell cycle, Cytoskeleton, Extracellular Matrix (ECM), Cell mechanics and Cell signalling (see Figure 1). Each characteristic was evaluated for gravity-related research and compared to the changes reported in studies regarding ageing. Upon completion of this study, the similarities and differences between ageing and altered gravity from a cellular point of view will be presented along with the identified gaps in knowledge. The outcome could form the basis for future in-flight and ground-based studies.

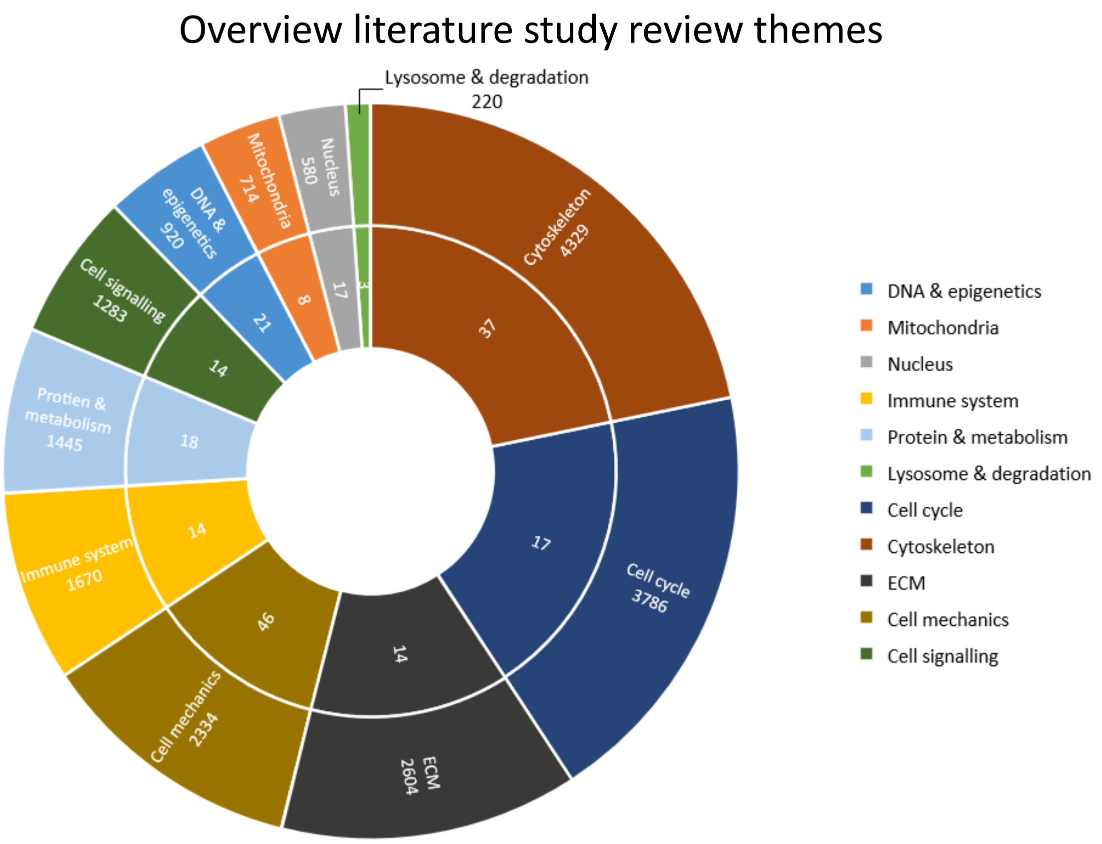


Figure 1. Overview of the themes identified for this literature study. The inner circle shows the number of variables which alter during ageing per theme. The outer circle displays the themes and their relative size based on the number of references in gravity-related research on that theme in either PubMed of Web Of Science. ECM: Extracellular Matrix

REFERENCES

López-Otín C., Blasco M. A., Partridge L., Serrano M., Kroemer G., 2013, Cell, 153, 1194–1217.

Schmauck-Medina T., Molière A., Lautrup S., Zhang J., Chlopicki S., Madsen H. B., Cao S., Soendenbroe C., Mansell E., Vestergaard M. B., Li Z., Shiloh Y., Opresko P. L., Egly J. M., Kirkwood T., Verdin E., Bohr V. A., Cox L. S., Stevnsner T., Rasmussen L. J., Fang E. F.,2022, Aging (Albany NY), 14, 6829–6839.

Bajpai A., Li R., Chen W.,2021, Annals of the New York Academy of Sciences , 1491, 3–24.

Phillip J. M., Aifuwa I., Walston J., Wirtz D., 2015, Annual Review of Biomedical Engineering, 17, 113-141.

Starodubtseva M., 2011, Ageing Research Reviews, 10, 16-25.

Debora Angeloi – “Microgravity and space radiation inhibit autophagy in human capillary endothelial cells”

Microgravity And Space Radiation Inhibit Autophagy In Human Capillary Endothelial Cells

D. Angeloni[1], I. Barravecchia[2], A. Cometa[1], M. Forcato[3], S. Bicciato[3]
1 Institute of Biorobotics, Scuola Superiore Sant'Anna, Via G. Moruzzi, 1, 56124, Pisa, Italy, angeloni@santannapisa.it; 2 Michigan Medicine, University of Michigan, Ann Harbour, MI, USA; 3 Center for Genome Research, Department of Life Science, University of Modena and Reggio Emilia, Modena, Italy

Microgravity and space radiation (SR) are two highly influential factors affecting humans in space flight (SF). Many health problems reported by astronauts derive from endothelial dysfunction and impaired homeostasis. Here, we describe the adaptive response of human, capillary endothelial cells to SF. Reference samples on the ground and at 1g onboard permitted discrimination between the contribution of microgravity and SR within the combined responses to SF. Cell softening and reduced motility occurred in SF cells, with a loss of actin stress fibers and a broader distribution of microtubules and intermediate filaments within the cytoplasm than in control cells. Furthermore, in space the number of primary cilia per cell increased and DNA repair mechanisms were found to be activated. Transcriptomics revealed the opposing effects of microgravity from SR for specific molecular pathways: SR, unlike microgravity, stimulated pathways for endothelial activation, such as hypoxia and inflammation, DNA repair and apoptosis, inhibiting autophagic flux and promoting an aged-like phenotype. Conversely, microgravity, unlike SR, activated pathways for metabolism and a pro-proliferative phenotype. Therefore, we suggest microgravity and SR should be considered separately to tailor effective countermeasures to protect astronauts' health [1].

Further insights into microgravity adaptation of endothelial cells have come from the genome methylation profile analysis of space flown cells compared to ground controls.

REFERENCES

Ivana Barravecchia et al. *Cellular and Molecular Life Sciences*. 2021 Dec 22;79(1):28. doi: 10.1007/s00018-021-04025-z.

Natassi Navasiolava – "Chronic increase in renin-angiotensinaldosterone activity at steady state of microgravity: why and by which mechanisms?"

Chronic increase in renin-angiotensin-aldosterone activity at steady state of microgravity: why and by which mechanisms?

N. Navasiolava[1], A. Robin[1], C. Gharib[2], and M.-A. Custaud[1]

[1]Univ Angers, CHU Angers, CRC, Inserm, CNRS, MITOVASC, SFR ICAT, 49000 Angers, France

[2]Physiopathologie et Génétique du Neurone et du Muscle, Université Claude Bernard Lyon 1, Lyon, France

Nastassia.Navasiolava@chu-angers.fr

The renin-angiotensin-aldosterone axis plays a major role in regulating sodium and potassium balance as well as arterial blood pressure. When exposed to actual or simulated microgravity, the immediate response of the renin-angiotensin-aldosterone system (RAAS) is a suppression of plasma renin activity and plasma aldosterone due to central hypovolemia (Leach Huntoon C.S. et al, 1998). However, in a chronic and stable state of microgravity, an increase in RAAS activity is often reported in the literature (Drummer C. et al, 2001; Sigaudo D. et al, 1998; Custaud M.A. et al, 2005), although the reasons and physiological role of this chronic increase are not entirely clear.

We will present data from several dry immersion (DI) studies (both published and unpublished) as well as some published and unpublished data from our team's HDBR studies, suggesting an increase in renin and aldosterone at steady state during simulated microgravity exposure. We discuss possible reasons for the activation of this sodium-retaining endocrine system, including:

- Reduction in intravascular volume, which could be detected by stretch receptors of the aortic arch/carotid sinuses or by low-pressure baroreceptors of the vascular system,
- Change in oral sodium supply, although efforts are made to keep it the same throughout the protocols - at Pre, During, and Post phases,
- Reduction in renal perfusion leading to excessive RAAS activation, or changes in glomerulo-tubular balance,
- Activation of sodium-retaining mechanisms or a change in regulation with a decrease in urinary sodium-to-potassium ratio. Sodium retention in osmotically inactive form is also suggested, but the location and reason for this retention are unclear.

The increased RAAS activity may indicate an increased physiological cost of maintaining a positive water balance. Furthermore, it could contribute to microgravity-induced vascular alterations, including increased intima-media thickness.

REFERENCES

Leach Huntoon C.S., Grigoriev A.I., Natochin Yu.V., 1998, Science and Technology Series, a Supplement to Advances in the Astronautical sciences, Volume 94.

Drummer C., Norsk P., Heer M., 2001, Am J Kidney Dis, 38, 684-690.

Sigaudo D., Fortrat J.O., Allevard A.M., et al., 1998, Am J Physiol, 274, 1875-1884.

Custaud M.A., Belin de Chantemèle E., Blanc S., et al, 2005, Can J Physiol Pharmacol, 83, 1147-1153.

Roman Zhedyaev – “Direct comparison of tilt test and lower body negative pressure effects on human hemodynamics and baroreflex regulation after dry immersion”

Direct Comparison Of Tilt Test And Lower Body Negative Pressure Effects On Human Hemodynamics And Baroreflex Regulation After Dry Immersion

R. Yu. Zhedyaev[1], O. S. Tarasova[2], E. S. Tomilovskaya[3], Yu. S. Semenov[4], O. L. Vinogradova[5], A. S. Borovik[6]
[1] Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia, zhedyaev-r@mail.ru
[2] Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia, ost.msu@gmail.com
[3] Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia, finegold@yandex.ru
[4] Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia, semenov.yury@gmail.com
[5] Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia, microgravity@mail.ru
[6] Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia, asbor@mail.ru

INTRODUCTION

Head-up tilt test (HUT) and its spaceflight equivalent lower body negative pressure test (LBNP) are widely used in space medicine as cardiovascular system (CVS) stress tests. Both tests cause blood redistribution towards lower body but differ in cardiovascular outcomes due to differences in regulatory mechanisms involved (Kitano A. et al., 2005 and Tanaka, K., et al., 2009). Dry immersion (DI) as model of gravitational unloading impairs CVS regulation (Eckberg D.L., 2003 and De Abreu S. et al., 2017), which may alter these outcome differences.

The analysis of low frequency (LF) heart rate (HR) and mean arterial pressure (MAP) waves including α-coefficient estimation (Pagani M., et al., 1988) is widely used to assess the state of CVS baroreflex control. Thus, we decided to use such analysis during short-term (3 min) HUT and LBNP in the same subjects before and after DI. Exposure durations were set to 3 min, to address short-term hemodynamic responses mediated mostly by the autonomic nervous system and to minimize the possibility of pre-syncope manifestations after DI.

AIM

To compare the responses of MAP, HR and their LF spectral characteristics in 3-min HUT and LBNP before and after 7-day DI.

METHODS

Nine healthy men (age 30.8±4.8 yrs., BMI 22.9±2.3 kg/m2) were exposed to 7-day DI. HUT (65°, 3 min) and LBNP (Chibis suit, -35 mm Hg, 3 min) were performed before and on the first day after DI. ECG (NVX52, MCS, Russia), blood pressure and stroke volume (SV) (Finometer, Finapres Medical Systems, the Netherlands) were continuously recorded; HR and MAP were calculated for every cardiac cycle. Amplitudes of HR (aHR) and MAP (aMAP) waves in LF (0.06-0.13 Hz) band were calculated using continuous wavelet transform. Each test was repeated 5 times alternating with 3-min rest. SV, MAP and HR were averaged for the last 30 sec while aHR, and aMAP were averaged for the last 70 sec of test and rest intervals in all repetitions. LF α-coefficient was calculated as aHR/aMAP.

Percent differences between parameters in rest state and HUT/LBNP state are reported as reactions to test. Two-factor ANOVA with Sidak's multiple comparisons test was used to examine influence of DI and Test factors on hemodynamic parameter reactions.

RESULTS

In both SV and HR the only source of variation was DI factor ($p=0.001$). SV reduction was similar in both HUT and LBNP pre-DI, and was more pronounced after DI (Fig.1). HR increased in similar fashion: HUT and LBNP reactions did not differ pre-DI and were more pronounced after DI. MAP reaction was influenced by both DI ($p=0.04$) and Test ($p<0.0001$); DI decreased the difference in MAP reactions between the tests ($p=0.001$ for interaction of two factors). MAP remained the same in HUT pre-DI and decreased post-DI, while it decreased in LBNP both pre- and post-DI.

LF aHR didn't change in HUT and LBNP neither pre- nor post-DI (Table 1). LF aMAP increased similarly in both tests only after DI. LF α-coefficient did not change in HUT and LBNP performed pre-DI. Post-DI, α-coefficient decreased in HUT ($p = 0.02$) but not in LBNP.

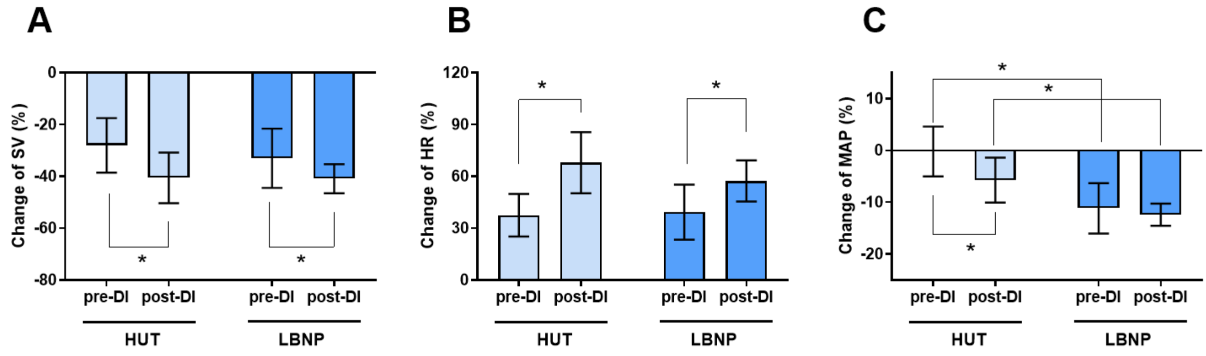


Fig.1 Reactions of SV (A), HR (B) and MAP (C) to HUT and LBNP performed pre- and post-dry immersion. * - p<0.05 (Sidak's multiple comparisons test).

Table 1. Amplitudes of LF HR and MAP oscillations and α-coefficient during HUT and LBNP before and after dry immersion. * - p<0.05 between rest and HUT/LBNP state (Sidak's multiple comparisons test).

	Pre dry immersion				Post dry immersion			
	HUT		LBNP		HUT		LBNP	
	Rest	Test	Rest	Test	Rest	Test	Rest	Test
aHR, bpm	1.32±0.64	1.48±0.29	1.43±0.68	1.41±0.37	1.2±0.44	1.23±0.43	1.14±0.32	1.38±0.42
aMAP, mmHg	1.17±0.37	1.67±0.46	1.18±0.31	1.55±0.57	1.10±0.25	**1.95±0.57 ***	1.02±0.23	**1.79±0.56 ***
α, bpm/mmHg	1.1± 0.39	0.93±0.22	1.2±0.46	0.97±0.31	1.1±0.33	**0.65±0.24 ***	1.12±0.27	0.83±0.31

CONCLUSION

Before DI MAP decreases during LBNP but not in HUT. DI influence dimmed this contrast: it didn't change MAP reaction to LBNP but was associated with MAP decrease in HUT in similar fashion to LBNP. Effect of DI on LF α was also evident only in HUT but not in LBNP. These observations suggest the DI-induced changes in CVS regulation mechanisms which involved mostly during orthostasis, such as vestibulosympathetic reflex (Tanaka, K. et al., 2009) or myogenic constriction of lower limb vasculature (Rodionov I.M. et al., 1999). It seems that short-term HUT may be more sensitive test then LBNP to assess unloading-induced CVS deconditioning. The results of this study may help to interpret spaceflight LBNP and pre-/postflight HUT data.

FUNDING

Cardiovascular studies were performed under the State Program of Basic Research (64.1) and a grant from the Russian Foundation for Basic Research (20-015-00536).

REFERENCES

Kitano A. et al., 2005, Journal of Applied Physiology, 98(6), 2081-2086.
Tanaka K. et al., 2009, Autonomic Neuroscience, 148(1), 90-96.
Eckberg D.L., 2003, Acta Physiologica Scandinavica, 177, 299–311.
De Abreu S. et al., 2017, Frontiers in physiology, 8, 799.
Pagani M. et al., 1988, Hypertension, 12(6), 600-610.
Rodionov I.M. et al., 1999, Environmental Medicine, 43(1), 1-9.

Artur Fedianin – "The effect of various spinal cord stimulation and support afferentation on the condition of the muscles of the rat's lower leg during simulated gravitational unloading"

The effect of various spinal cord stimulation and support afferentation on the condition of the muscles of the rat's lower leg during simulated gravitational unloading

A. O. Fedianin[1,2] , M. E. Baltin[1,2], T. N. Zaytceva[2], S. O. Bikeeva[2], T. V. Baltina[2] and A. A. Eremeev[2]
[1] Volga Region State University of Physical Culture, Sport and Tourism (e-mail: info@sportacadem.ru)
[2] Kazan Federal University (e-mail: public.mail@kpfu.ru)

Model experiments were carried out on laboratory male rats weighing 200 g. in strict accordance with accepted bioethical norms. The animals were randomly divided into the following experimental groups: "HU" – animals with simulated gravitational unloading of the hind limbs; "HU+MS" - animals with simulated gravitational unloading of the hind limbs combined with magnetic stimulation of the spinal cord; "HU+ES" – animals with simulated gravitational unloading of the hind limbs combined with electrical stimulation spinal cord; "HU + SU" - animals with simulated gravitational unloading of the hind limbs, combined with the action of axial load and the reaction force of the support.

Simulation of gravitational unloading was carried out by the generally accepted method of anti-orthostatic hanging of a rat by the tail for 7, 14 and 35 days. Magnetic and electrical stimulation of the spinal cord, combined with HU, was performed daily for 90 minutes in series of 10 minutes with an interval of 10 minutes. Animals of the HU+SU group was placed on a solid horizontal surface daily for 90 minutes. After exposure to experimental conditions, the wet and dry weight of the muscle was analyzed. The data of intact animals served as a control.

After 7 days of unloading, the wet weight of soleus muscle in the HU group was 67 ± 8% ($p<0.05$) of the control, dry - 58±12% ($p<0.05$). Spinal cord stimulation did not prevent atrophic changes: wet weight of soleus muscle in group HU+ES was 77±7% ($p<0.05$), in the HU+MS group - 75±10% ($p<0.05$), dry - 70±7% ($p<0.05$) and 71±10% ($p<0.05$), respectively. The presentation of the support limited the severity of atrophic processes, however, the dry weight of soleus muscle in group HU+ SU decreased to 78=11% ($p<0.05$). There were no significant changes in the weight of gastrocnemius muscle and tibialis anterior muscles after 7 days of simulated hypogravity.

After 14 days of exposure to experimental conditions, the results of gastrocnemius muscle and soleus muscle studies corresponded to the data obtained at the previous stage (the wet and dry weight was reduced relative to the control values). The analysis of tibialis anterior muscle weight also revealed the development of atrophic processes in all experimental groups. When exposed to experimental conditions for 35 days, a significant decrease in the wet and dry weight of all the studied muscles was found in all experimental groups.

Thus, a decrease in wet and dry muscle weight indicates the development of atrophic processes initiated by unloading. Spinal cord stimulation and activation of support afferentation (with prolonged exposure to hypogravity) do not prevent atrophic changes.

The research was funded by the Russian Science Foundation (project No. 23-25-10065)

Daniela Santucci – "Neurobehavioural evaluation in mice exposed to acute 2G hypergravity in normothermic or synthetic torpor conditions"

Neurobehvioural evaluation in mice exposed to acute 2g hypergravity in normothermic or synthetic torpor conditions

A Stronati[1], G. Macchioni[1], A. Racca[1], D. Santucci[1], and M. Cerri2

[1]Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy, alessandro.stronati@iss.it, giorgia.macchioni@iss.it, arianna.racca@iss.it, daniela.santucci@iss.it
[2]Department of Biomedical and NeuroMotor Sciences, Alma Mater Studiorum -University of Bologna, Bologna, Italy, matteo.cerri@unibo.it

Individual response to stress and the study of possible new strategies to reduce human suffering in space are relevant for future space missions. Among these, a reduction in the metabolic state has been longely hypothised to possibily mitigate biological and logistical challenges for mammals spaceflight. Aim of the present stydy was to investigate the possibility that hibernation-like condition (a state of reduced metabolic activity followed by a decrease in body temperature in non-hibernating animals) could reduce hypergravity induced stress in mice. The behavioral profile of C57BL6J mice subjected to synthetic torpor and relative controls was monitored before, during and after acute 2g-hypergravity exposure, and frequency and duration of several behavioral items assessed. Beside regular controls, stationary ones were represented by mice in hibernating-like conditions placed close to the centrifuge during the rotation test. Short- and medium-term effects in spontaneous behavior and in emotional and cognitive indexes were evaluated in the successive days. Moreover, NGF, BDNF as well as p-tau levels were evaluated in selected area of the central nervous system to correlate behavioral changes with alterations in central levels of neurotrophins, involved in neuroplasticity phenomena. Short-term changes were observed in several behavioral items, some of them probably correlated with thermoregulatory function, in subjects who undergone synthetic torpor, while subtle but specific differences were evident in their emotional profile. Data will be discussed in the framework of individually-related neurobehavioral responsiveness in the context of hypergravitational exposure and the possibility that synthetic torpor may favorably impact on it through improving neuroplasticity.

Ann-Sofie Schreurs – "Candidate countermeasure to mitigate adverse effects of spaceflight on musculoskeletal, cardiovascular and central nervous systems tissues"

Candidate countermeasure to mitigate adverse effects of spaceflight on musculoskeletal, cardiovascular and central nervous systems tissues

Ann-Sofie Schreurs[1], Sonette N. Steczina[2], Moniece Lowe[3], Linda Rubinstein[4], Candice G. T. Tahimic[5], Josh Alwood[6] and Yasaman Shirazi[7], Ruth K. Globus

[1]Schreurs.annsofie@gmail.com, [2] ssteczin@uw.edu, 3 moniecegothard@gmail.com, [4]Linda.Rubinstein@sheba.health.gov.il, [5]c.tahimic@unf.edu, [6]joshua.s.alwood@nasa.gov, [7]yasaman.shirazi-fard@nasa.gov

The spaceflight environment, including microgravity and ionizing radiation, poses multiple challenges to human physiology homoestasis. Together, these factors contribute to cellular stress, and effects include increased generation of reactive oxygen species (ROS), oxidative and DNA damage, cell cycle arrest and cell senescence. We have previously shown that a purified diet supplemented with dried plum (DP, 25%) conferred full protection of cancellous structure from the rapid bone loss caused by exposure to 2 Gy of ionizing radiation (137Cs) (Schreurs et al. 2016). Furthermore, we have shown the capacity of this countermeasure to prevent damage at the skeletal tissue as well as to the osteoprogenitors from exposure to the more harmful high-LET radiation, such as (56Fe). In addition, the deleterious effects of simulated microgravity alone and in combination with total body irradiation (TBI) on bone structure and strength were prevented (Steczina et al. 2020). While our current and past research has focused on the protective effect of this countermeasure on the skeletal system, data relating to this nutritional countermeasure also revealed a potential systemic effect demonstrated reduced oxidative damage in the serum of mice provided the nutritional countermeasure and exposed to TBI, even when provided 24 hours after radiation exposure. These results supported the investigation of the protective effect of this countermeasure on non-skeletal tissues. Other key tissues of interest, in the context of the damaging effects of spaceflight, are the cardiovascular system and the central nervous system (CNS). Both the cardiovascular system and CNS are linked to degenerative pathologies, resulting in short and long-term risk for astronauts. Thus, in our study, we aimed to examine the combined impact of simulated microgravity and ionizing radiation on mice fed our countermeasure of interest. Adult mice underwent either simulated weightlessness via hindlimb unloading (HU) or exposed TBI (137Cs), or the combination of both HU and TBI. After 14 days of hindlimb-unloading, tissue samples were collected. We performed array-based gene expression analysis of ~160 genes related to cardiovascular disease, neurotoxicity pathways, aging and DNA damage and repair, from bone marrow cells, eyes, hippocampus, and left ventricle of the heart.

Acknowledgement

This work is supported by a NASA HRP HHC Omnibus Grant 15-15Omni2-0039 (AS).

A blood vessel microfluidic chip to study vascular remodeling in space

Kevin Tabury1, Emil Reghnberg2, Bjorn Baselet3, and Sarah Baatout4

1Radiobiology Unit, Belgian Nuclear Research Centre (SCK CEN), Boeretang 200, 2400 Mol, Belgium; Department of Biomedical Engineering, College of Engineering and Computing, University of South Carolina, Columbia, SC 29208, USA, ktabury@sckcen.be, 2Radiobiology Unit, Belgian Nuclear Research Centre (SCK CEN), Boeretang 200, 2400 Mol, Belgium, 3Radiobiology Unit, Belgian Nuclear Research Centre (SCK CEN), Boeretang 200, 2400 Mol, Belgium, 4Radiobiology Unit, Belgian Nuclear Research Centre (SCK CEN), Boeretang 200, 2400 Mol, Belgium

BACKGROUND

Following two decades of human presence on the International Space Station (ISS), the next challenge in deep space exploration is to facilitate human settlement on the Moon and Mars. Exiting the protection of our magnetosphere also lead to increase health risks from prolonged exposure to microgravity and space radiation that could seriously jeopardize space missions (Fogtman, Baatout et al. 2023). Cardiovascular dysfunction (Baran, Marchal et al. 2022) and in particular accelerated cardiac aging is one of the concerns (Rehnberg, Quaghebeur et al. 2023). Aging of the heart causes atherosclerosis, fibrosis, and reduced heart muscles contractility leading ultimately to heart failure. Despite our technological advances, the current molecular and cellular understanding of cardiovascular diseases caused by aging is limited and mainly based on animal models. With an estimated radiation exposure of 0.5-1Sv for a manned mission to Mars (Hughson, Helm et al. 2018), such increased cardiovascular risk becomes extremely relevant. In Space, the risks of cardiovascular diseases are also enhanced by microgravity.

An important component of the cardiovascular system are the blood vessels. Structure-wise, a blood vessel is composed of a series of layers of vascular cells that are arranged one on top of another around the lumen through which blood flows. Endothelial cells line the inside of blood vessels and form a continuous monolayer on top of a very thin layer of extracellular matrix (mainly collagen and elastin). In space, an increase of carotid arterial stiffness has been observed following 6 months of spaceflight which corresponds to one to two decades of aging in the context of the beta-stiffness index (marker of arterial stiffness (Rosenberg, Lane-Cordova et al. 2018)) and carotid distensibility coefficient (Patel 2020). Interestingly, sex differences were observed where women showed greater changes in carotid artery beta-stiffness index after spaceflight, whereas men showed greater changes in pulse wave transit time after spaceflight. However, due to small sample size and the limitation related to human testing, the understanding of the phenomenon remains unsolved.

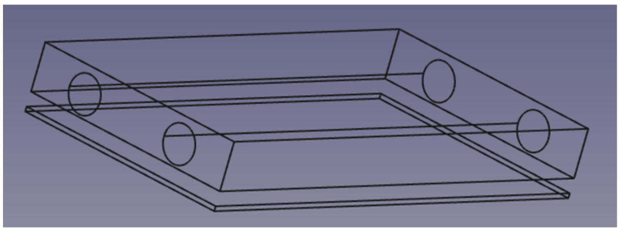
Hence, a deeper understanding of the biological mechanisms of vascular remodeling leading to cardiac aging is one of the key challenges in developing countermeasures. In this context, investigating the interplay between space radiation and microgravity on vascular remodeling becomes crucial.

PURPOSE

The purpose of our study is to generate controlled muscular arteries composed of concentric layers of endothelial cells, smooth muscle cells, fibroblasts and collagen fibers as a model to study vascular remodeling after exposure to space radiation and microgravity.

METHODOLOGY

HiPSCs from a female donor was differentiated into endothelial cells, fibroblasts and smooth muscle cells. The microfluidic chip was generated by pouring PDMS onto a needle holder. This allowed controlled positioning of the first needle (21G). Once cured, the needle was extracted and the PDMS construct was bonded to a thin glass slide. Then, a smaller needle (27G) was introduced into the channel around which a collagen hydrogel containing fibroblasts was polymerized. Finally, concentric layers of smooth muscle cells, a layer of collagen and a layer of endothelial cells was created. To induce adequate endothelial barrier function, a flow rate resulting in a shear stress of 10 dyn/cm2 was used.



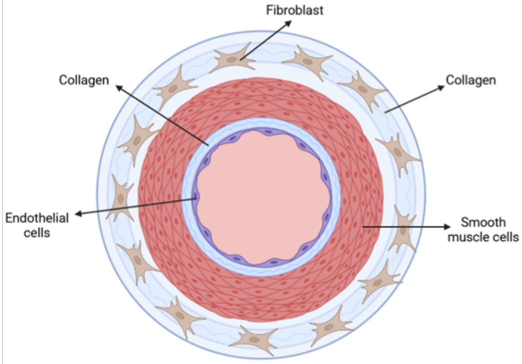


Figure 3: Microfluidic chip (Left) composed of a PDMS structure embedding 2 blood vessels, inlets and outlets are on the side and chip is formed by bonding the PDMS structure to a thin glass slide; Blood vessel composition with the different layers (Right).

Subsequently, the blood vessel chip was exposed to simulated microgravity using a random positioning machine and chronic neutron radiation (total dose of 0.1 Sv) separately or combined.

Subsequent analysis included, immunofluorescence staining (VE-cadherin to visualize the endothelial cell junctions and SM22 to visualize smooth muscle cell morphology) and scanning electron microscopy to characterize the blood vessels. Changes in stiffness were investigated by quantifying collagen IV by western blotting.

OUTLOOK

This study intended to validate our blood vessel microfluidic chip as a platform to study vascular remodeling in space. In particular, to investigate the interplay between space radiation and microgravity. We successfully demonstrated the potential of our platform and intend to also develop additional blood vessel microfluidic chips using multiple hiPSCs (female and male donor) to enable the study of sex differences in vascular remodeling in space.

REFERENCES

-Baran, R., S. Marchal, S. Garcia Campos, E. Rehnberg, K. Tabury, B. Baselet, M. Wehland, D. Grimm and S. Baatout (2022). "The Cardiovascular System in Space: Focus on In Vivo and In Vitro Studies." Biomedicines 10(1): 59.

-Fogtman, A., S. Baatout, B. Baselet, T. Berger, C. E. Hellweg, P. Jiggens, C. La Tessa, L. Narici, P. Nieminen, L. Sabatier, G. Santin, U. Schneider, U. Straube, K. Tabury, W. Tinganelli, L. Walsh and M. Durante (2023). "Towards sustainable human space exploration—priorities for radiation research to quantify and mitigate radiation risks." npj Microgravity 9(1): 8.

-Hughson, R. L., A. Helm and M. Durante (2018). "Heart in space: effect of the extraterrestrial environment on the cardiovascular system." Nat Rev Cardiol 15(3): 167-180.

-Patel, S. (2020). "The effects of microgravity and space radiation on cardiovascular health: From low-Earth orbit and beyond." IJC Heart & Vasculature 30: 100595.

-Rehnberg, E., K. Quaghebeur, B. Baselet, N. Rajan, T. Shazly, L. Moroni, S. Baatout and K. Tabury (2023). "Biomarkers for biosensors to monitor space-induced cardiovascular ageing." Frontiers in Sensors 4.

-Rosenberg, A. J., A. D. Lane-Cordova, S. O. Wee, D. W. White, T. I. M. Hilgenkamp, B. Fernhall and T. Baynard (2018). "Healthy aging and carotid performance: strain measures and β-stiffness index." Hypertension Research 41(9): 748-755.

Influence of prolonged exposure to microgravity on perception of verticality

Stijn Meel[1], Chloë De Laet[1], Catho Schoenmaekers[1], Steven Jillings[1], Floris Wuyts[1]
[1]Lab for Equilibrium Investigations and Aerospace (LEIA), University of Antwer, Antwerp, Belgium

In this study we investigated if there is a change in perception when a person went to space for a period of 6 months and subjected to microgravity. For this study we used 27 cosmonauts from which some were evaluated multiple times so we had a total of 44 test subjects. These cosmonauts were evaluated twice before and three times after the space flight. The test consisted out of a rotation in the clock wise direction and a rotation in the counter clockwise direction where the cosmonauts were asked to keep a joystick 'up right'. Before the rotation, the joystick was calibrated. The cosmonauts were than rotated at a constant angular speed of 254°/s, so we expected a tilt of 45°. On average the cosmonauts indicated a roll of (12 ± 3)°, (14 ± 3)°, (15 ± 3)°, and (16 ± 4)° for respectively the CW and CCW rotation of the BDC1 and BDC2 experiments and (8 ± 3)° and (7 ± 3)° for the CW and CCW rotation of the R+3 experiments.

We found that there was no significant difference between the BDC and R+3 experiments, although there is a decrease of the average roll. We divided the cosmonauts in distinct groups, the group with an increase, a decrease or constant between the BDC and R+3. Now the difference was significantly different, which means that microgravity can have an influence on perception of verticality, but it's different for everyone. We also investigated if having experience would have an influence. We found that both the first-time flyers and the frequent flyers had no difference between their BDC and R+3 experiments, although the frequent flyers did have a larger average and are closer to the expected value of 45°.

Lastly, we investigated if there was a difference between the tilt indicated with the joystick and the tilt they stated verbally. We found that there was a large difference between the two ways of reporting the tilt (subjective data was around two times larger than the joystick data). There was also an increase in the angle between the BDC and R+3 measurements reported verbally, while there was a decrease in the angle indicated with the joystick.

“SPACE DENTISTRY”-AN INVENTION OR A MODERN NECESSITY?

During space missions; space crews undertake complex tasks in remote, hostile, and hazardous environments exacerbated by microgravity and heightened levels of radiation. These conditions subject astronauts to various physiological stresses, both during short-duration and long-duration space trips. Notable effects include bone loss, muscular atrophy, cardiac dysrhythmias, altered spatial orientation, and psychological well-being. There is limited research however regarding the impact of factors such as UV rays, microgravity, and other space-related conditions on the oral cavity.

The oral cavity plays a critical role in numerous physiological processes including taste perception, mastication, solubilization of nutrients, and digestion. Moreover, it contributes to innate and adaptive immunity, respiration, speech, and other vital bodily functions. Alterations in saliva composition and rate of production of saliva can profoundly affect oral tissues and overall health.

Aeronautical dentistry, also known as space dentistry, focuses on studying the pathophysiology of the oral cavity in airborne and extra-terrestrial conditions. Understanding this topic is of paramount importance due to the potential oral health implications in extra-terrestrial environments. The poster will delve into the significance of comprehending the oral health impacts of space travel, addressing dental emergencies that have occurred during space missions, and proposing potential strategies for managing dental crises in space.

By investigating the effects of space-related conditions on the oral cavity, aeronautical dentistry aims to develop effective protocols and treatments to maintain astronauts' oral health during missions. This knowledge is crucial for ensuring the well-being and performance of astronauts in the demanding and challenging environment of space travel.

REFERENCES

1. Bansal P, Nikhil V, Ali S, Mishra P, Bharatiya R, Space and aeronautical dentistry: A review. Indian J Pharm Pharmacol 2018;3(3):59-64
2. Savage DK. A brief history of aerospace dentistry. J Hist Dent. 2002 Jul;50(2):71-5. PMID: 12125697.
3. https://www.colgateoralhealthnetwork.com/wp-content/plugins/pdf-viewer-for-wordpress/web/viewer-shortcode.php?file=https://www.colgateoralhealthnetwork.com/wp-content/uploads/2016/03/Colgate-Space-Dentistry1.pdf&settings=111101011&lang=en-US#page=&zoom=auto.
4. Thara Bhavani M.S et al.2017, Aviation Dentistry: Effect of Microgravity on Teeth and oral Cavity- A Review. Int J Recent Sci Res. 8(6), pp. 17339-17342.
5. Bohra A, Vengal M, Patil N, Udawat V. Maxillofacial aeronautics: An evolving entity. SRM J Res Dent Sci 2016;7:205-7.

NAME OF AUTHOR:

Dr. Victoria Sampson

BDS MFDS RCS Ed PgDip

NAME OF CO-AUTHORS:

1. Dr. Ishita Singhal

   BDS, MDS in Oral Pathology & Microbiology, and Forensic Odontology, ESA Topical Team member

2. Dr. Małgorzata Kulesa- Mrowiecka

   PT, MSc, PhD

   Department of Rehabilitation in Internal Diseases, Institute of Physiotherapy, Faculty of Health Sciences Jagiellonian University Medical College in Krakow, Poland

3. Dr. Edward Kijak; Assistant professor and head in the Department of Prosthetic Dentistry, Wroclaw Medical University
4. Dr. Dirk Neefs
   MSD, ddcare and B-dent, Antwerp, Belgium, ESA Topical Team moderator

Optimizing Scientific Output: Assessing the Benefits and Risks of Analog Astronaut Missions for Human Physiology Research

Margot Winters

TU Delft, Faculty of Aerospace Engineering [MSc]

margotwinters@hotmail.com

Space exploration presents astronauts with unique health challenges that are not encountered on Earth (Aubert et al., [1]). Analog astronaut missions are highly valued for providing controlled and realistic environments that replicate various aspects of space travel, making them an important method for studying human physiological and psychological responses. Furthermore, space analogs are cost-effective and can investigate factors like prolonged isolation, communication delays, disrupted sleep-wake cycles, and psychological stress (Balwant et al., [2]).
A significant distinction can be observed between analog missions conducted in collaboration with space agencies or space medicine institutes and populist analog missions. The former benefit from strong research-scientific connections with other ground or space missions and a long history of lessons learned in the management of analog activities. The latter often lack such connections and may place insufficient emphasis on safety and mission design information (Cinelli, [3]). This makes them prone to a scientific validity below their potential.

This paper, proposed for the 42nd ISGP Meeting 2023, critically reviews the value and advantages of analog astronaut missions for human physiology research. It also addresses the risks and key factors that must be considered to optimize scientific output. The paper draws on the author's experiences during the EMMPOL16 analog astronaut mission (EuroMoonMars POLand) conducted in March 2023 at the Analog Astronaut Training Center in Krakow, Poland.

The future of human physiology research in analogue missions holds great promise, especially as the demand for cost-effective simulated environments grows alongside continued space exploration. Further studies should aim to further develop comprehensive guidelines for the design of analog missions, ensuring resilient and scientifically optimized research that can thrive in space exploration.

Figure 1: Stratospheric balloon flight, Krakow, Poland in March 2023

REFERENCES

[1] Aubert A et al. (2016) Towards human exploration of space: the THESEUS review series on cardiovascular, respiratory, and renal research priorities. Microgravity 2, 16031.

[2] Balwant R et al. (2012). Proposed new approaches for future human space and interplanetary missions. North American Journal of Medical Sciences 4 (11), 548-557.

[3] Cinelli I (2020) Short- and Long-Duration Mission Human Factors Requirements, 2020. In: Seedhouse, E., Shayler, D. (eds) Handbook of Life Support Systems for Spacecraft and Extraterrestrial Habitats.

14:00 Ilya Rukavishnikov – “Comparative Results Of 21-Day Dry Immersion And 21-Day Head Down Tilt Bed Rest”

Comparative Results Of 21-Day Dry Immersion And 21-Day Head Down Tilt Bed Rest

E. S. Tomilovskaya[1], I. V. Rukavishnikov[1], T. A. Shigueva[1], A. A. Saveko[1], M. P. Bekreneva[1], A. V. Shpakov[1], A. A. Puchkova[1], I. I. Ponomarev[1], N. V. Shishkin[1], Yu. D. Zhurina[1], V. V. Kitov[1], Turtikova O.V.[1], B. S. Shenkman[1], and O. I. Orlov[1]
[1]Institute of Biomedical Problems of the Russian Academy of Sciences; finegold@yandex.ru

INTRODUCTION

One of the main factors of space flight is gravitational unloading (Kozlovskaya I.B., 2007). There are several ways to simulate gravitational unloading effects on Earth. The two most used models that reproduce the physiological effects of microgravity are Dry Immersion (DI) and Head Down Tilt Bed Rest (HDBR). Although both models have been widely used for years, there are no complex experiments to compare their 21-day effects. Head down (antiorthostatic) bed rest reproduces physical unloading (skeletal muscles disuse), redistribution of support (weightbearing) loads, axial unloading, and fluid shifts (Watenpaugh D.E., 2016). DI accurately and rapidly reproduces such factors of space flight as lack of support, mechanical and axial unloading, fluid shifts as well as physical inactivity (Navasiolava N.M. et al., 2011; Tomilovskaya E.S. et al., 2019). A test subject wearing a shirt and trunks is placed on the waterproof fabric and immersed into a deep bath up to the neck, in a supine position. The area of the fabric’s surface considerably exceeds the surface area of water. The folds of the waterproof fabric allow the person’s body to be enveloped from all sides freely. The high elasticity properties of the fabric artificially create conditions similar to zero gravity via floatation (Shulzhenko E.B. and Vil-Villiams I.F., 1976).

To directly compare the effects of these two models over the past few years, two large experimental studies have been conducted in the IBMP under conditions of 21-day DI and 21-day -6° HDBR using the same battery of studies.

METHODS

22 healthy volunteers of the same age and physical state took part in the study; 10 of them underwent 21-day DI, and the rest 12 – spent 21 days in HDBR (-6o). The daily routine, diet, and set of experimental studies in the two experiments were almost identical. The time outside the immersion bath and bed rest didn’t exceed 15-20 minutes per day, except for the days with orthostatic tests (3 times per experiment at the end of each week). All participants passed the medical commission at the IBMP of the RAS, and no pathologies excluding their participation in the experiment have been found. Before their final approval for the experiments, the subjects signed the informed consent to their participation in the study. The experiment was held at the IBMP of the RAS on the Dry Immersion Simulation and Training Facility, a component of the Unique Scientific Installations, Medical and Engineering Complex for Testing Innovational Technologies of Space Biomedicine for the Provision of Orbital and Interplanetary Missions, as well as for the Development of Practical Healthcare.

RESULTS

The exposure to DI was accompanied by a significant loss of fluid – on the 1st day of DI, it was 1269±244 ml; in HDBR the subjects lost 2 times less fluid – 765.0 ± 315.3 ml. Further in the course of HDBR fluid loss was also less compared to DI. As was shown in previous studies, DI exposure is characterized by the development of hypogravitational spinal syndrome lasting 3-4 days (Rukavishnikov I.V. et al., 2017; Tomilovskaya E.S. et al., 2019). In 21-day DI the intensity of back pain reached maximum values, i.e., 6–8 points on days 1 and 2 of exposure. Significant differences in this indicator against the baseline data were recorded on DI days 1, 2, and 3. However, unlike shorter DI experiments, back pain continued to be recorded in some individuals even during 6th day of exposure. In 21-day HDBR this discomfort was less pronounced, however, the duration of the syndrome was increased. It indicates the key role of the support afferentation input in the development of this syndrome, which is consistent with a less pronounced increase in body length in HDBR compared to DI. The studies of muscle tone, spinal reflexes, vertical balance, orthostatic tolerance, and other physiological parameters revealed unidirectional changes in both models but these changes were more expressed in DI. Interestingly, the signs of muscle atrophy (decrease of muscle fibers cross-sectional area in m. soleus) were significant only after 21 days of DI exposure.

FUNDING

The study was supported by a grant from the Russian Science Foundation № 19-15-00435, https://rscf.ru/en/project/19-15-00435/.

REFERENCES

Kozlovskaya I.B., 2007, In Modern classical Physiology course, Moscow: Geotar-Media.

Watenpaugh, D.E. (2016) Analogs of microgravity: head-down tilt and water immersion. J Appl Physiol 120:904–914.

Navasiolava N.M., Custaud M.-A., Tomilovskaya E.S. et al., 2011, Eur J Appl Physiol, 111(7), 1235-1261.

Tomilovskaya E.S., Shigueva T.A., Sayenko D.S. et al., 2019, Front Physiol, 10:284.

Shulzhenko E.B., and Vill-Villiams I.F., 1976, Russian Biol and Aerospace med, 9, 82-84.

Rukavishnikov I.V., Amirova L.E., Kukoba T.B. et al., 2017, Hum Physiol, 43(3), 291-300.

14:15 Bjorn Baselet – "Combined biological effects of ionizing radiation, psychological stress and microgravity in space: the hind limb unloading mouse model"

Combined biological effects of ionizing radiation, psychological stress and microgravity in space: the hind limb unloading mouse model

B. Baselet. Author[1], S. Miranda. Author[2], M. Neefs. Author[3], and S. Baatout. Author[4]
[1] Radiobiology Unit, Belgian Nuclear Research Centre SCK CEN, Mol, Belgium (bbaselet@sckcen.be), [2]Faculty of Bioscience Engineering, Department of Biotechnology, Ghent University, Ghent, Belgium and Radiobiology Unit, Belgian Nuclear Research Centre SCK CEN, Mol, Belgium (sfdmira@sckcen.be), [3]Radiobiology Unit, Belgian Nuclear Research Centre SCK CEN, Mol, Belgium (mneefs@sckcen.be), and [4]Radiobiology Unit, Belgian Nuclear Research Centre SCK CEN, Mol, Belgium (sbaatout@sckcen.be).

Space travel comprises a unique and complex stress model composed of physical (i.e. ionizing radiation and microgravity) and psychological stressors (i.e. isolation and confinement and distance from Earth). These extreme conditions can induce specific responses in the human body that will ultimately affect several organ systems. The precise nature of these health effects is not completely understood, and multiple underlying causes might be involved. In view of future interplanetary travel, studies onboard the International Space station will help to answer many critical questions. However, due to financial and technical restrictions of these flight experiments, ground-based analogues are required for researchers to test theories without launching experiments into space.

The Radiobiology Unit of SCK CEN has implemented multiple ground-based in vitro and in vivo experiments using space flight analogues, like the murine hind limb unloading (HLU) model. This ground-based analog model has been historically developed to study mechanisms, responses, and treatments for the adverse consequences of microgravity conditions during spaceflight. Recently, we implemented changes to the model, thereby limiting its invasive nature and allowing for full specified pathogen free housing conditions. In collaboration with (inter)national scientists we are, at current, looking into the effects of a combined exposure to psychological stress, ionizing radiation and microgravity on a multitude of murine organ systems, such as the bones, the muscles, the feces/microbiome and the immune organs. Regular animal experiments are scheduled using this validated model and are opened for tissue sharing.

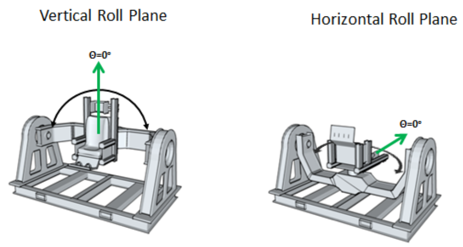
Acknowledgements: This research is financially supported by the ESA/BELSPO/Prodex PRODEX IMPULSE contract (CO-90-11-2801-03) and via the ELGRA Research Prize 2020-21.

14:30 Vivekanand Vimal – "Vibrotactile Feedback as a Countermeasure to Spatial Disorientation"

Vibrotactile Feedback as a Countermeasure to Spatial Disorientation

Vivekanand Pandey Vimal[1], James Lackner[2], and Paul DiZio[3]
[1]Brandeis University (somde@brandeis.edu), [2]Brandeis University (lackner@brandeis.edu), [3]Brandeis University (dizio@brandeis.edu)

Were astronauts to land on the Lunar or Martian surface using manual control of their vehicle they would not have familiar gravitational cues because the gravity would be only 0.16 or 0.38g, respectively, and they could become susceptible to spatial disorientation, potentially causing mission ending crashes. In our earlier studies, we secured blindfolded subjects into a Multi-Axis Rotation System (MARS) device that was programmed to behave like an inverted pendulum (Vimal et al., 2016-2019). Participants were instructed to use an attached joystick to stabilize around the balance point. We created a spaceflight analog condition by having participants dynamically balance in the Horizontal Roll Plane (Figure 1), where they did not tilt relative to the gravitational vertical and therefore could not use gravitational cues to determine their ongoing position and had to rely only on motion cues (Vimal et al., 2017, 2019, 2021). We found 90% of our participants in this condition reported spatial disorientation and all of them showed it in their data (Vimal et al., 2017-2022). Compared to a control condition with gravity dependent position cues (Vertical Roll Plane), all participants showed significant deficits in performance and learning. Could vibrotactile feedback enhance performance in the spaceflight condition?



A Vibrotactile cue group had 4 C-2 vibrotactors placed on each arm from the shoulder to the wrist. The first vibrotactor (near the shoulder) activated when the MARS deviated 1 deg from the balance point, the second at 7 deg, the third at 15 deg and the fourth (near the wrist) at 31 deg. On Day 1 of experimentation, participants balanced in the Vertical Roll Plane with the vibrotactors (i.e. they trained with natural terrestrial gravitational cues augmented by vibrotactors) and on the second day they were placed in the Horizontal Roll Plane with the vibrotactors (i.e. they were tested in the spaceflight analog condition with vibrotactile cue replacement of missing gravitational cues). The Control group experienced the same set of trials however did not have the vibrotactors on either day.

We found that the Vibrotactile group performed significantly better than the Control group in the spaceflight analog condition (Table 1), showing that vibrotactors are a valuable countermeasure to spatial disorientation. However, the Vibrotactile group had minimal improvement across trials on Day 2 in the Horizontal Plane spaceflight analog condition, which was worse overall than the Vertical Roll Plane. When questioned, 90% of the Vibrotactile group participants reported feeling a conflict in the spaceflight analog condition, where their sense of self orientation felt different and less accurate than what the vibrotactors were indicating.

We next studied if people could learn to adopt an orientation dominated by accurate assistive vibrotactile tilt cueing while ignoring misleading vestibular afferent tilt signals. We trained participants on Day 1 to find and stabilize around randomized off-vertical balance points in the Vertical Roll Plane which made them rely on the vibrotactile cues while ignoring the vestibular tilt signals (Vimal et al., 2019). When tested on Day 2 with vibrotactile cues in the spaceflight analog condition which lacked vestibular tilt information, they performed statistically better than the Vibrotactile and Control groups from the first study on Day 2 (Table 1), who had been trained on Day 1 with concordant vibrotactile cues and vestibular afferent tilt signals. Additionally, they showed continued learning and improvements on Day 2. On the final block of Day 2 we deactivated the vibrotactors and while performance worsened it was not statistically worse than the Control group, suggesting that the vibrotactors did not create a negative dependence.

In conclusion, vibrotactors are a valuable countermeasure to spatial disorientation, however the vibrotactors may lead to a feeling of conflict with one's feeling of orientation. Training through exposure to vibrotactors in Earth conditions may not be enough to overcome the conflict that will arise in spaceflight conditions. An effective training program will need to have a component where participants must exclusively rely on vibrotactile feedback to complete the task.

Table 1

	Control (n=10)	Vibrotactile (n=10)	Training (n=10)
Metric	Day2 Block 1	Day2 Block 1	Day2 Block 1
STD_{MARS} (deg)	22.3	16.8**	11.6***
Crashes (Hz)	2e-3	0.8e-3**	0.3e-3***
$\|Mag\|_{Pos}$ (deg)	17.1	12.8**	9.1***
$\|Mag\|_{Vel}$ (deg/s)	17.9	15.2	11.0**
$\|Mag\|_{Accel}$ (deg/s^2)	66.7	52.5	37.5**
$\|Mag\|_{Joy}$	0.22	0.18	0.13**
$\%Zero_{Joy}$	34	35	36
%Destab	1.7	0.37**	0.18**
D_S (deg^2/s)	116	103	63.4
D_L (deg^2/s)	38.7	19.2	7.5**
$Mean_{MSD}$(deg^2)	594	503	355

Table 1 shows several performance metrics for the 3 experimental groups on Day 2 (the spaceflight analog condition in the Horizontal Roll Plane) of Block 1 (initial exposure to the disorienting condition). Independent t-tests between the Vibrotactile and Control groups and the Training and Control groups are shown where * means p<0.05, ** means p<0.01, *** means p<0.001

REFERENCES

Vimal, V. P., Lackner, J. R., & DiZio, P. (2016). Experimental Brain Research, 234(2), 483-492.

Vimal, V. P., DiZio, P., & Lackner, J. R. (2017). Experimental Brain Research, 235(11), 3495-3503.

Vimal, V. P., Lackner, J. R., & DiZio, P. (2018). Experimental Brain Research, 236.5(5), 1321-30.

Vimal, V. P., DiZio, P., & Lackner, J. R. (2019). Experimental brain research, 1-13.

Vimal, V.P., Lackner, J.R., & DiZio, P. (2020). Aerospace medicine and human performance 91.6: 479-488.

Vimal, V. P., DiZio, P., & Lackner, J. R. (2021). Experimental brain research, 1-13.

Wang Y., Tang J., Vimal V., Lackner J. R., DiZio P., Hong P. (2022) Frontiers in physiology, 51.

14:45 Carole-Anne Vollette – "A wearable-based system to reduce space motion sickness by multi-sensory prehabituation"

A wearable-based system to reduce space motion sickness by multi-sensory pre-habituation

C.A. Vollette[1], C. Bockisch[1], D. Straumann[1], G. Bertolini[1]
[1]Vestibulo Oculomotor Lab, University Hospital Zürich, Zürich, Switzerland, dominik.straumann@usz.ch

INTRODUCTION

Motion sickness (MS) is a common disturbance occurring in healthy people exposed to specific real or illusory motion conditions. The most widely accepted hypothesis suggests a sustained conflict between expected and actual sensory inputs as the triggering factor (G. Bertolini et al. 2016). In space, these mismatches cannot be resolved into a stable self-motion perception as the brain cannot sense gravity. Accordingly, each transition between gravity levels implies space motion sickness SMS (M. Heer et al. 2006; HJ. Ortega et al. 2020) for roughly half of trained astronauts, significantly impairing missions and safety for days. Symptoms of motion sickness include vomiting and nausea, but also higher risk of disorientation, visual illusions and sopite syndrome. Although drugs diminish symptoms (e.g. meclizine, promethazine or scopolamine), they come with unwanted side-effects (sedation, drowsiness) (M. Heer et al. 2006; AP. Weerts et al. 2014) and risks related to intolerances, adaptation and addiction. An alternative to ameliorate MS symptoms are training programs employing centrifuges, rotating chairs and even rotating rooms that were proven effective in aircraft pilots, but not in astronauts (HJ. Ortega et al. 2020). The key problem is that how self-motion perception adapts to weightlessness is not yet established. Interestingly, astronauts with natural tendency to rely more on an ego-referenced frame (ideotropic vector) than on visual cues have been shown to have less SMS symptoms and a shorter adaptation time (DL. Harm et al. 1998). Pathological visual over-reliance may occur in patients after a transient vestibular insult and become chronic (Persistent postural-perceptual dizziness (PPPD) (S. Cousins et al. 2014)). A sudden exposure to weightlessness represents the equivalent of a strong vestibular insult: the vestibular sensors for gravity direction abruptly stop working. As for the PPPD patients, developing visual dependence is not uncommon in astronauts and has been related to higher and persistent SMS (DL. Harm et al. 1998). Multi-sensory cues to force reweighting of sensorial integration appear overall quite successful for vestibular patients (A. Viziano et al. 2019). Although visual dependence and maladaptation are also issues for astronaut, transfer of the know-how from these novel patient rehab in pre-flight habituation has yet to be evaluated.

The main aim is to develop a pre-rehabilitation lessening space motion sickness (SMS) by simultaneous manipulation of different sensory cues to create sensory conflict conditions that can be resolved when the subject adopts our desired reference frame. In practice, as astronauts with natural tendency to adopt an ego-referenced frame have been shown to suffer less SMS and adapt faster, the pre-rehabilitation should reinforce this reference frame against a visual-based one. A training paradigm successfully achieving this adapted state will have a double advantage: it will prevent overreliance on visual cues and promote a rapid switch to this learned strategy when gravity cues are absent (i.e. in orbit).

METHODS

The project is divided into three steps. The first one is to identify the optimal combination of non-vestibular stimuli (visual and haptic) that successfully manipulate the perceived "down" in a gravity-related illusion. Then we aim to create the Pre-Rehabilitation paradigm that reinforces the reliance on the ego-centric reference by combining the stimuli from the previous step (visual and/or haptic) with a real angular tilt motion stimulus to induce a lasting reduction of the reliance on gravito-inertia as a cue for tilt. Finally, we will test efficacy and retention of adaptation in parabolic flights (0-g analog) organized by the UZH SpaceHub and using Galvanic Vestibular Stimulation to make the whole equipment wearable.

To wrap up the first step, using a motion simulator (Stewart platform), we exposed 4 participants to a "Hilltop" illusion (i.e., lateral translations at 0.16 Hz in darkness are interpreted as tilt-while-translating). The illusion is due to uncertainty in the sensed angular motion at low frequency, which leads to errors in the estimated gravity direction. Random angular tilts (-6° to 6°) were also added to the motions, expecting participants to overestimate such tilts due to the Hilltop illusion. During the basic experimental stimulus, perception of gravity was measured using a continuous Haptic Vertical (HV) alignment by the participant, as well as a Subjective Visual Vertical

(SVV) test. Then, visual information was manipulated (polarity cues via VR Headset to provide verticality cues in contrast with gravitational reference (i.e. coherent with the illusion of an angular tilt). Finally, perception of verticality was measured once more during a second basic experimental stimulus to compare data before and after VR exposure.

PRELIMINARY DATA

In Fig1, preliminary Hilltop illusion parameters assessment was done in 15 participants collecting both HV and SVV.

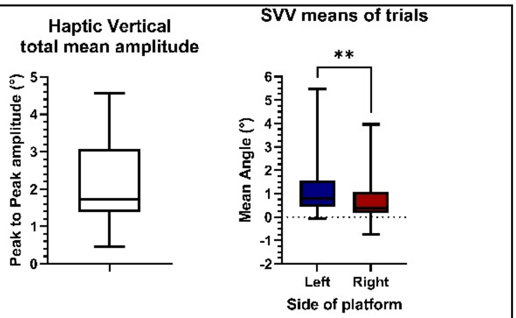


Figure 4: Average estimation error of HV was 2,2°±1,2. Difference of SVV values reported in left-most and right-most positions of the platform (0,51±0,57) was significant (p<0,05).

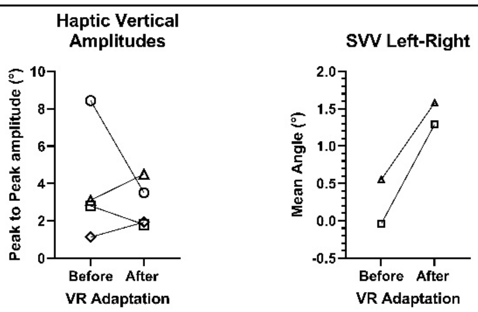


Figure 2: Both HV and SVV measurements showed an increased estimated angle error after VR exposure (in valid subjects).

After demonstrating that perception of gravity could be altered with our setup, Fig2 shows how optokinetic stimuli can increase the error in verticality angle perception with 4 participants. VR environment visually displayed a 1.5, 2 and 2.5 gain in the physical tilt in 3 trials, perception of gravity was measured before and after VR exposure. Out of 4 participants, 2 displayed signs of motion sickness (tiredness and drowsiness) and were not able to complete SVV task.

CONCLUSION

Using the Hilltop illusion allowed us to alter perception of verticality using optokinetic cues. VR exposure to increased visual tilt is expected to increase the error in perceived tilt angle, and to be retained in time. Only 2 subjects showed such results and more are being tested in order to observe a significant error. The experiment is being limited by its length in time (1h30) that might induce mild motion sickness and loss of focus in some participants, thus dividing it into two shorter sessions is being considered.

REFERENCES

1. G. Bertolini, D. Straumann, *Moving in a moving world: A review on vestibular motion sickness*, Front Neurol, 2016
2. M. Heer, WH. Paloski, *Space motion sickness: Incidence, etiology, and countermeasures*, Auton Neurosci Basic Clin, 2006
3. HJ. Ortega et al., *Space and entry motion sickness*, Principles of Clinical Medicine for Space Flight, 2020
4. AP. Weerts et al., *Space motion sickness countermeasures: A pharmacological double-blind, placebo-controlled study*, Aviat Sp Environ Med, 2014
5. DL. Harm, et al., *Relationship between selected orientation rest frame, circular vection and space motion sickness,* Brain Res Bull, 1998
6. S. Cousins et al., *Visual dependency and dizziness after vestibular neuritis*, PLoS One, 2014
7. A. Viziano et al., *Long-term effects of vestibular rehabilitation and headmounted gaming task procedure in unilateral vestibular hypofunction: a 12-month follow-up of a randomized controlled trial*, Clin Rehabil, 2019

15:00 Jonathan Scott – "Effects of body size and countermeasure exercise on estimates of life support resources during all-female crewed exploration missions"

Effects of body size and countermeasure exercise on estimates of life support resources during all-female crewed exploration missions

Jonathan P. R. Scott[1, 2] *, David A. Green[2, 3, 4], Guillaume Weerts[2], and Samuel N. Cheuvront[5]

[1] Institut Médecine Physiologie Spatiale (MEDES), Toulouse, France; [2] Space Medicine Team, European Astronaut Centre, European Space Agency, Cologne, Germany; [3] KBR GmbH, Cologne, Germany; [4] Centre of Human and Applied Physiological Sciences, King's College London, London, UK; [5] Sports Science Synergy, LLC, Franklin, MA, USA.

INTRODUCTION

Human basal metabolism is, in absolute terms, proportional with body size, with larger individuals possessing higher resting oxygen consumption (VO2), carbon dioxide production (VCO2) and metabolic heat production (Hprod) [White and Seymour, 2003]. These differences persist during physical activity (e.g., 75% maximal oxygen uptake [VO2max]), assuming equal aerobic fitness (VO2max relative to body mass) [Cramer and Jay, 2014]. Previously, we [Scott et al. 2020] estimated the implications of body size, drawn from a stature range of 1.50- to 1.90-m, representative of current space agency requirements (which exist for stature, but not for body mass) and the use of ISS-like countermeasure (CM) exercise in an all-male crew upon mission resources. Increasing stature alone increased 24-h total energy expenditure (TEE, +44%), VO2, VCO2 and Hprod (+60%), and water required for hydration (+19%). CM exercise further increased TEE (+29–32%), VO2 (+31%), VCO2 (+35%) and Hprod (+42%), and water requirements (+23–33%) across the stature range. Employing the methodology from the study of Scott et al., [2020], the present study estimated the effects of body size and CM exercise in a four-person, all-female crew composed of individuals drawn from an identical stature range upon the same parameters.

METHODS

Assuming geometric similarity across the stature range (1.50- to 1.90-m), estimates were derived using available female astronaut data (Moore et al., 2015: mean age: 40-y; BMI: 22.7-kg·m-2; resting VO2 and VO2max: 3.3- and 40.5-mL·kg-1·min-1), without and with, ISS-like countermeasure exercise (modelled as 2x30-min aerobic exercise at 75% VO_2max, 6-d·wk-1). Where spaceflight-specific data/equations were not available, terrestrial equivalents were used. To examine the effect of sex, these estimates were qualitatively compared to the estimates from our previous paper on theoretical males [Scott et al. 2020], as well using the 5th, 25th, 50th, 75th and 95th stature percentiles (female: 1.495-, 1.566-, 1.615-, 1.667- and 1.725-m; male: 1.625-, 1.702-, 1.757-, 1.804-, and 1.873-m) from adults in the United States Centre for Disease Control (CDC)'s 2015-16 NHANES survey [US CDC, 2017].

RESULTS

In theoretical females, body size alone increased 24-h TEE (+30%), VO_2 (+60%), VCO_2 (+60%) and Hprod (+60%), and water requirements (+17%). With CM exercise, the increases were 25–31%, 29%, 32%, 38% and 17–25% across the stature range. Extrapolated to 'per month' (30-d) for a four-person all-female crew, together these differences translated to an additional 678-MJ of energy expended, 43.6x103-L of O2 consumed, 140.9-L of water required, and 35.9x103-L of CO2 and 727-MJ of heat produced. Compared to the previous study of theoretical males [Scott et al., 2020], the effect of body size on TEE was markedly less in females, and, at equivalent statures, all parameter estimates were lower for females, with relative differences ranging from -5% to -29% (Table 1). When compared at the 50th percentile for stature for US females and males, these differences increased to -11% to -41% (Table 1) and translated to larger differences in TEE, O2 and water requirements, and CO2 and heat produced, during extended exploration missions using CM exercise.

Table 1. Relative (%) difference of theoretical female from theoretical male astronaut populations [Scott et al., 2020] at the lower (1.50-m) and upper (1.90-m) ends of the stature comparison range, and at the 50th percentile for stature for United States females (1.615-m) and males (1.757-m).*

	Relative (%) difference of females from males		
Stature (m)	1.50-m	1.90-m	50th Percentile*
Characteristics			
Body mass (kg)	-14	-14	-28
VO_{2max} ($L \cdot min^{-1}$)	-20	-20	-32
RMR ($MJ \cdot d^{-1}$)	-12	-21	-27
Basal H_{prod} ($J \cdot s^{-1}$)	-13	-13	-26
Basal fluid needs ($L \cdot d^{-1}$)	-5	-6	-11
1x 30-min bout of aerobic CM Ex.			
EE (MJ)	-21	-21	-33
O_2 (L)	-20	-20	-33
CO_2 (L)	-23	-23	-35
H_{prod} (MJ)	-21	-21	-33
Water requirements (L)	-29	-28	-41
24-h values without CM Ex.			
EE (MJ)	-10	-19	-25
O_2 (L)	-14	-14	-28
CO_2 (L)	-14	-14	-28
H_{prod} (MJ)	-12	-12	-26
Water requirements (L)	-5	-6	-11
24-h values with CM Ex.			
EE (MJ)	-13	-20	-26
O_2 (L)	-16	-16	-29
CO_2 (L)	-17	-17	-29
H_{prod} (MJ)	-15	-15	-28
Water requirements (L)	-9	-11	-18

VO_{2max}, maximal rate of oxygen uptake; RMR, resting metabolic rate; Mprod, metabolic heat production; EE, energy expenditure; O2, total oxygen consumed; CO2, total carbon dioxide produced. * For United States females and males based on United States Centre for Disease Control (CDC) 2015-16 National Health and Nutrition Examination Survey (NHANES) [US CDC, 2017].

DISCUSSION

This paper provides the first estimates of the effects of increasing body size (indexed to stature) and the use of CM exercise on life support resources required for theoretical all-female crews. Estimated differences between theoretical females and males result from lower resting and exercising O2 requirements (based on available female astronaut data) of theoretical female astronauts, who are lighter than theoretical male astronauts at equivalent statures and have lower relative VO_2max values. These data, combined with the move towards smaller diameter space habitat modules, suggest that there may be a number of operational advantages to all-female crews during future human space exploration missions.

REFERENCES

1. White, C.R. & Seymour, R.S., 2003, Proc. Natl. Acad. Sci. U S A. 100, 4046-4049.
2. Cramer, M.N. & Jay, O., 2014, J. Appl. Physiol. (1985). 116, 1123-32.
3. Scott, J., Green, D. A., Weerts, G., & Cheuvront, S. N., 2020. Sci. Rep., 10, 13836
4. Moore, A. et al., 2015, Aerosp. Med. Hum. Perform. 86, A78-A86
5. United States (US) Centre for Disease Control (CDC) 2015-16 NHANES Survey https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015

15:15 Jack van Loon – “Reduce Cost by Permanent Artificial Gravity”

Reduce Cost by Permanent Artificial Gravity

Jack J.W.A. van Loon[1], Cesare Lobascio[2], Giorgio Boscheri[2], Clement Goujon[2], Stefano Voglino[2], Eleonora Zeminiani[2], Ricard González-Cinca[3], Reinhold Ewald[4]

1: Department Oral & Maxillofacial Surgery/Pathology, Amsterdam Movement Sciences & Amsterdam Bone Center (ABC), Amsterdam University Medical Center Location VUmc & Academic Center for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands and European Space Agency (ESA), European Space Research and Technology Centre (ESTEC), TEC-MMG, Keplerlaan 1, 2201 AZ Noordwijk, The Netherlands.
2: Thales Alenia Space Italia SpA, Strada Antica di Collegno, 253 – 10146, Turin, Italy.
3: Department of Physics, Universitat Politècnica de Catalunya – BarcelonaTech, c/Esteve Terradas 5, 08860 Castelldefels, Barcelona, Spain.
4: Institute of Space Systems, University of Stuttgart, Pfaffenwaldring 29, 70569 Stuttgart, Germany.

Spaceflight is often a synonym for microgravity. Such a near weightlessness environment is used to explore the impact of microgravity in various life and physical sciences and technical applications. However, a microgravity environment can also be a challenging and even a disadvantaged situation.

For humans living in a near weightlessness setting for a long period of time can even be morbid. In order to counteract the various pathological symptoms of sustained microgravity specific in-flight countermeasure devices and protocols have been developed. However, over the years it has been shown that these protocols are not, or not fully, effective e.g. (Scott, Feiveson et al. 2023). In recent years there is a growing interest to explore artificial gravity (AG) in the form of short arm human centrifugation (SAHC) as microgravity countermeasure (Young, Yajima et al. 2009, Clement, Bukley et al. 2015). These ground-based studies make use of the microgravity analogue of head down tilt bed rest. But also these AG protocols are not sufficiently effective and needed to be complemented with exercise moduli such as ergometers or jumping devices e.g. (Kramer, Kümmel et al. 2020). Although the latter improve the countermeasure efficacy it is still not clear whether such systems would counteract microgravity phenomena other than the often explored cardiovascular and musculoskeletal deprivations. The SAHC application are similar to current in-flight protocols where crew are exposed to the treatment for a relatively short period of time, ~ 30-60 minutes, per day. But what are the benefits of a chronic AG system for long duration missions?

The answer to this question requires a multi- disciplinary approach. It will be beneficial for crew health (van Loon, Cras et al. 2020) but also affects other aspects of a mission such as the engineering of the spacecraft especially in various two-phase systems, the spacecraft’s operations, its life support systems (Butz and Herring 2019, Fili, Gòdia et al. 2021), its safety but also its cost (see Figure 1). Based on crew time we expect to save at least some 5.4 billion US$ when the crew did not have to counteract the negative effects of near weightlessness like they do in ISS. Also other aspects will significantly benefit from an AG environment.

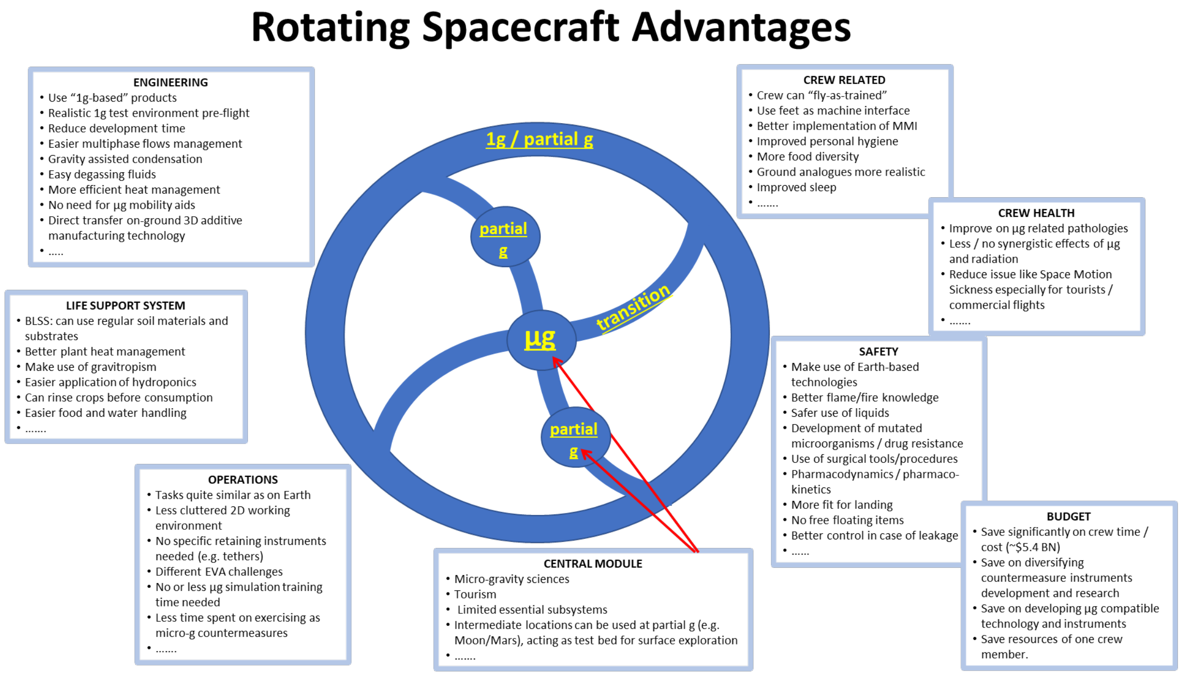


Figure 1: Overview of some main parts of the development and use of a large rotating spacecraft to be applied for human exploration and the various benefits that Artificial Gravity would imply for such a system.

So going towards long duration space missions one could save crew time and even require less crew members and related resources when one would make use of artificial gravity.

References

Butz, I. and A. Herring (2019). "Growing Plants in Space: Manipulating Medium Wettability to Create Different Saturation Conditions." Transport in Porous Media **130**(2): 463-485.

Clement, G. R., A. P. Bukley and W. H. Paloski (2015). "Artificial gravity as a countermeasure for mitigating physiological deconditioning during long-duration space missions." Front Syst Neurosci **9**: 92.

Fili, T., F. Gòdia and R. González-Cinca (2021). "Trade-off analysis of phase separation techniques for advanced life support systems in space." Acta Astronautica **178**: 571-583.

Kramer, A., J. Kümmel, M. Dreiner, S. Willwacher, T. Frett, A. Niehoff and M. Gruber (2020). "Adaptability of a jump movement pattern to a non-constant force field elicited via centrifugation." PloS one **15**(4): e0230854.

Scott, J. M., A. H. Feiveson, K. L. English, E. R. Spector, J. D. Sibonga, E. Lichar Dillon, L. Ploutz-Snyder and M. E. Everett (2023). "Effects of exercise countermeasures on multisystem function in long duration spaceflight astronauts." npj Microgravity **9**(1): 11.

van Loon, J. J., P. Cras, W. Roozendaal, W. Bouwens and J. Vernikos (2020). "Gravity deprivation: is it ethical for optimal physiology?" Frontiers in Physiology **11**: 470.

Young, L., K. Yajima and W. Paloski (2009). Artificial gravity research to enable human space exploration. Paris, France, International Academy of Astronautics**:** 1-37.

14:00 Elena Gorbacheva – "Hormonal Status Of Woman And Structural Characteristics Of The Ovaries And Uterus After A 5-Day "Dry" Immersion"

Hormonal Status Of Women And Structural Characteristics Of The Ovaries And Uterus After A 5-Day "Dry" Immersion

E. Yu. Gorbacheva [1,2], K. A. Toniyan [1,2], Yu. A. Biriukova [2], N. A. Lukicheva [1], O. I. Orlov [1], V. V. Boyarintsev [3]

and I. V. Ogneva [1]

[1] Cell Biophysics Lab, State Scientific Center of the Russian Federation Institute of Biomedical Problems of the Russian Academy of Sciences, 123007 Moscow, Russia; elenagorbacheva22@gmail.com, ktoniyan@mail.ru, luckichyowa.n@yandex.ru, olegtm@bk.ru, iogneva@yandex.ru, [2] Gynecology Department, FGBU KB1 (Volynskaya) UDP RF, 121352, Moscow, Russia; elenagorbacheva22@gmail.com, ktoniyan@mail.ru, julia@inbox.ru, [3] Emergency and Extreme Medicine Department, FGBU DPO CGMA UDP RF, 121359, Moscow, Russia; wpx@mail.ru

AIM OF THE STUDY

The impact of long-term space flight on the female reproductive system haven`t been studied enough, although it can be assumed that deep space exploration will require additional measures to protect women's health. The data presented in the literature indicate that women often postpone the implementation of the reproductive function until they have completed several space flights (Ronca A. et al., 2014; Mishra B., Luderer U., 2019). In addition, subsequent healthy aging depends on hormonal levels, which can be changed after space flight effects. The complexity of organizing research under real space flight conditions does not allow receiving a sufficient number for reliable conclusions. Therefore, the use of ground models, in particular, "dry" immersion, allows us to conduct experiments with a sufficient sample of human subjects. The purpose of this work is to study the effect of a 5-day "dry" immersion on the state of the hormonal background and the structural characteristics of the ovaries and uterus.

MATERIALS AND METHODS

The study involved 12 women, whose average age was 27.8 years (from 22.7 to 40.8 years). Stay in the conditions of "dry" immersion, which was carried out on the basis of the SSC RF IBMP RAS, was carried out in the same way as before (Tomilovskaya E.S. et al., 2021). Before and after immersion on the 4th day of the menstrual cycle, the hormonal status was estimated, specifically the content of anti-Müllerian hormone (AMH), inhibin B, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone in the blood. On the 4th and 9th days of the menstrual cycle, before and after immersion, the structure of the organs of the reproductive system was estimated using transvaginal ultrasound. The beginning of the exposure under the conditions of "dry" immersion, basically, coincided with the 10th day of the menstrual cycle. The determination of all parameters presented in this study was carried out on the basis of the Gynecological department of the Clinical Hospital No. 1 (Volynskaya, Moscow, Russia). All subjects were in stable clinical condition with no clinical, microbiological, or laboratory evidence of infection, encephalopathy, renal failure, or comorbidities including heart failure, pulmonary disease, malignancy, or diabetes mellitus. Written informed consent was obtained from each subject prior to participation in the study. The study design and procedures were approved by the Biomedicine Ethics Committee of the Institute of Biomedical Problems, Russian Academy of Sciences (Physiology Section of the Russian Bioethics Committee, Russian Federation National Commission for UNESCO, Permit #615/MSK/06/06/22) and conformed to the Declaration of Helsinki.

RESULTS AND DISCUSSION

As a result of the study, it turned out that the median value of AMH concentration before and after immersion did not differ significantly. In two human subjects, the AMH concentration before immersion was above the upper limit of normal, after immersion it decreased, but did not reach the normal level. After immersion the concentration of inhibin B in the blood significantly increased: the median value after immersion was higher by 35% ($p < 0.05$) than before immersion. However, the FSH concentration before and after immersion did not differ and was within the age norm. At the same time, the concentration of LH significantly decreased after immersion by 12% ($p < 0.05$), as well as progesterone – by 52% ($p < 0.05$).

Conducted ultrasound studies showed that the volume of the uterus before and after immersion did not change both on the 4th and on the 9th day of the menstrual cycle. The thickness of the endometrium also did not change after being in the conditions of "dry" immersion. The average volume of the ovary of the human subjects on the 4th day of the menstrual cycle did not differ before and after the 5-day "dry" immersion. However, on the 9th day of the menstrual cycle, the ovarian volume after immersion decreased on average for the group: the median value after immersion was lower by 22% ($p < 0.05$) than before immersion. The average diameter of antral follicles on the 4th day of the menstrual cycle before and after immersion did not differ significantly, but on the 9th day of the cycle after immersion it increased by 14% ($p < 0.05$), as well as the diameter of the dominant follicle – by 22% ($p < 0.05$).

Summarizing the above, the obtained results may indicate that a 5-day stay in the conditions of "dry" immersion changes the hormonal status and structural and functional characteristics of the female reproductive system.

This work was supported by the program of fundamental research of the SSC RF – IBMP RAS 65.4. and UNU “Transgenbank”.

REFERENCES

Ronca A.E., Baker E.S., Bavendam T.G., Beck K.D., Miller V.M., Tash J.S., Jenkins M., 2014, J Womens Health (Larchmt). 2014, 23(11), 967-974.

Mishra B., Luderer U., 2019, Nat Rev Endocrinol, 15(12), 713-730.

Tomilovskaya E, Amirova L, Nosikova I, Rukavishnikov I, Chernogorov R, Lebedeva S, Saveko A, Ermakov I, Ponomarev I, Zelenskaya I, Shigueva T, Shishkin N, Kitov V, Riabova A, Brykov V, Abu Sheli N, Vassilieva G, Orlov O., Front Physiol, 2021,12, 661959.

14:15 Irina V. Ogneva – "Cell Stiffness And Protein Content In Drosophila Melanogaster' Oocytes After Space Flight"

Cell Stiffness And Protein Content In *Drosophila Melanogaster'* Oocytes After Space Flight

I. V. Ogneva[1] and N. S. Biryukov[1]
[1] Cell Biophysics Lab, State Scientific Center of the Russian Federation Institute of Biomedical Problems of the Russian Academy of Sciences, 123007 Moscow, Russia; iogneva@yandex.ru, biryukovns@gmail.com

AIM OF THE STUDY

The deep space exploration requires the development of methods for the protection of human health, and for almost all body systems. The reproductive system has rarely been the focus of attention, however, maintaining its normal functional state, especially for the female body, is of great importance in the long-term health consequences after a space flight (Ronca AE et al., 2014; Mishra B., Luderer U., 2019). Maintaining an adequate level of hormones of the hypothalamic-pituitary-gonadal axis depends, among other things, on the functional state of the maturing oocyte. Experiments in space flight with the study of mammals are associated with a large number of technical difficulties. Therefore, the aim of the study was to evaluate the structural changes in the oocytes of the fruit fly Drosophila melanogaster, which were collected immediately after the space flight. Due to the limited number of cells obtained, the cytoskeleton structure was assessed by determining the integrative parameter - cell stiffness by atomic force microscopy.

MATERIALS AND METHODS

The "Cytomechanarium" space experiment, as part of a real space flight aboard the ISS RS, was carried out from September 21 to 29, 2022 (ISS-67 expedition) (Ogneva I.V. et al., 2022a). Five days before the start of the space flight, fifty female larvae of the 3rd age of the fruit fly Drosophila melanogaster of the Canton S line (flight group and synchronous control) were placed in test tubes with a breathable lid and a nutrient medium. At the landing site (L), the flies from the flight group and the synchronous control group were transplanted into a cage with agar plates for oocytes' collection. Within 12 hours after landing (period L - L + 12 hours), cages with flies were transported to the laboratory at a temperature of +250C. After arrival at the laboratory, the flies were transplanted into cages with fresh agar plates and continued to collect oocytes for the next 12 hours (period L+12 hours – L+24 hours). Cell stiffness was determined by atomic force microscopy by taking force curves (NTEGRA NEXT II, NT-MDT, Moscow, Russia) and the content of cytoskeletal proteins by Western blotting.All the experimental procedures were approved by the Commission on Biomedical Ethics of the Institute of Biomedical Problems (IBMP) (Minutes No. 521 dated September 25, 2019).

RESULTS AND DISCUSSION

The measurements performed showed that the stiffness of the cortical cytoskeleton of oocytes collected during the first 12 hours is reduced compared to the corresponding control (26.5 ± 1.1 pN/nm vs 31.0 ± 1.8 pN/nm), which indicates a change in the structure of the cytoskeleton (Ogneva I.V. et al., 2022a). Moreover, in oocytes collected in the next 12 hours, the stiffness of the submembrane cytoskeleton remains below the control level, which indicates that the structure is not restored. Considering that the cortical cytoskeleton plays a key role in the formation of the contractile ring, it can be assumed that such changes in the structure of oocytes can disrupt cytokinesis in early embryos.

A decrease in cell stiffness is more often associated with the destruction of microfilaments (Collinsworth A.M. et al., 2002; Costa K.D. et al., 2006), which is associated with a decrease in the content of actin and actin-binding proteins, including during the action of weightlessness and in the early period of readaptation (Ogneva I.V. et al., 2014). During the second period of oocyte collection (between L+12 hours and L+24 hours), a sufficient number of cells were obtained to perform both stiffness measurements and to evaluate the content of various cytoskeletal proteins.

Indeed, the relative content of actin and some actin-binding proteins in oocytes collected during the period L+12 hours – L+24 hours after landing was lower than in control oocytes by about 30-50%. The relative content of microtubule components and intermediate filaments was reduced much more dramatically, by 70-80%. This comparison is more likely to support the view that cell stiffness is due to the structure of the microfilament network, since it does not decrease so dramatically. If the stiffness of oocytes were determined by microtubules, then a sharper decrease could be expected. However, such a proposal is speculative and requires a separate analysis.

Interestingly, there was the decrease in the content of actin-binding proteins involved in the formation of a developed network of microfilaments (alpha-actinin and spectrin alpha), but the content of the singed protein (fascin homologue), which binds microfilaments into dense bundles, remained at the control level. Taking into account the formation of oocytes under weightless conditions, the effect of overloads during the landing of the spacecraft and the period of readaptation to 1 g can be considered as an increase in external mechanical stress on the cells. The results obtained support the previously proposed model of interaction between the cell and the gravitational field, in which an increase and decrease in external mechanical stress cause deformation of the cortical cytoskeleton and dissociation of various proteins from it (Ogneva I.V., 2013, 2022b).

REFERENCES

Ronca A.E., Baker E.S., Bavendam T.G., Beck K.D., Miller V.M., Tash J.S., Jenkins M., 2014, J Womens Health (Larchmt). 2014, 23(11), 967-974.

Mishra B., Luderer U., 2019, Nat Rev Endocrinol, 15(12), 713-730.

Ogneva I.V., Golubkova M.A., Biryukov N.S., Kotov O.V., Cells, 2022a, 11 (23), 3871.

Collinsworth A.M., Zhang S., Kraus W.E., Truskey G.A., 2002, Am J Physiol Cell Physiol, 283(4), C1219-1227.

Costa K.D., Methods Mol Biol, 2006, 319, 331-361.

Ogneva I.V., Biryukov N.S., Leinsoo T.A., Larina I.M., PloS ONE, 2014, 9(4), e96395.

Ogneva I.V., Biomed Res Int, 2013, 2013, 598461.

Ogneva I.V., Life (Basel), 2022b, 12(a10), 1601.

14:30 Dagmara Stasiowska – "Impact of honey bee (Apis mellifera) queen exposure to hypergravity on colony development - case study"

Impact of honey bee (*Apis mellifera*) queen exposure to hypergravity on colony development – case study

D. Stasiowska[1]
[1] AGH University of Science and Technology, Faculty of Electrical Engineering, Automatics, Computer Science, and Biomedical Engineering, Department of Biocybernetics and Biomedical Engineering, stasiowska@agh.edu.pl, al. Mickiewicza 30, 30-059 Kraków, Poland

INTRODUCTION

Since the beginning of the Space Age, the issue of the impact of hypergravity on living organisms has been raised. A relatively low number of them focus on insects which may be an important part of the food production system, e.g. as pollinators. Such a species are honey bees (A. mellifera) which were studied in terms of comb construction abilities (Vandenberg et al. 1985), flight patterns (STS-107 2003) and microgravity's impact on the drones' semen quality (Jun et al. 2009). A. mellifera usefulness as a pollinator will depend, among others, from the correctness of the colony development after space travel. This paper presents a case study on honey bee colony development with the queen bee exposed to a rocket-launch acceleration pattern generated by the large-scale centrifuge.

MATERIALS AND METHODS

Four honey bee queens (A. mellifera carnica) were given to the acceleration pattern of launching Soyuz-type rocket on the Human Training Centrifuge at the Military Institute of Aviation Medicine in Warsaw, Poland. Then, along with non-centrifuged queens from the control sample, they were given to the experimental hives for observation. During regularly performed controls, from August 2021 to April 2022, data on the number of eggs, larvae and pupae were gathered by the variation of the Liebefeld method (Imdorf et al. 1987). Stores amount was observed and the hives weighting procedure before the overwintering season was performed.

RESULTS

Only two out of four queens from each sample were finally observed in the experimental apiary. The rest did not start to lay eggs or were not accepted to the colony. All colonies' development generally followed a similar pattern. Some differences were visible for one of the colonies with centrifuged queen – eggs number increased more rapidly and more drone pupae were noticed. What is more, a sudden drop in the number of stores was noted during the last control before the overwintering, while keeping the colony mass similar to the other ones. All colonies successfully survived overwintering and queens started egg-laying in the spring.

SUMMARY

The results suggest that giving the queen bee to hypergravity may influence later colony development. However, more data is needed to confirm it and exclude the impact of individual traits on the obtained results. The impact of a specific acceleration pattern, with a maximum peak value at 4 G, was investigated. To verify if there exists a boundary G-force value not causing negative effects on the development of the bee colony, analogous studies covering specific values of hypergravity should be done.

REFERENCES

Imdorf A, Bueahlmann G, Gerig L, Kilchelmann V, Wille H. 1987. Überprüfung Der Schätzmethode Zur Ermittlung Der Brutfläche Und Der Anzahl Arbeiterinnen In Freifliegenden Bienenvölkern. Apidologie. 18(2). https://doi.org/10.1051/apido:19870204

Jun G, Wei S, Guiling D, Liping L, Zhiguang L. 2009. A primary report on honeybee space-flight breeding. Acta Agric Nucleatae Sin. 23.

STS-107. 2003. [accessed 2020 May 13]. https://www.nasa.gov/missionpages/shuttle/shuttlemissions/archives/sts-107.html

Vandenberg JD, Massie DR, Shimanuki H, Peterson JR, Poskevich DM. 1985. Survival, behavior and comb construction by honey bees, Apis mellifera, in zero gravity aboard NASA shuttle mission STS-13. Apidologie. 16(4):369–384.

14:45 Simon Wuest – “Analyzing Calcium Signaling by CaMPARI2 during Parabolic Flights”

Analyzing Calcium Signaling by CaMPARI2 during Parabolic Flights

S. Wuest[1], A. Hammer[2], G. Cerretti[1], D. Ricciardi[2], D. Schiffmann[1], S. Maranda[1], R. Kummer[1], C. Zumbühl[1], K. Rattenbacher-Kiser[1], S. von Arx[1], S. Ammann[1], F. Strobl[2], R Berkane[2], A. Stolz[2], E. Stelzer[2], E. Schleiff[2], M. Egli[1] and M. Böhmer[2]

[1]Lucerne School of Engineering and Architecture, Horw, Switzerland (simon.wueest@hslu.ch);
[2]Johann Wolfgang Goethe University, Frankfurt am Main, Germany (boehmer@bio.uni-frankfurt.de)

Introduction

Calcium is an essential secondary messenger in many cellular processes, including diseases and adaptation to environmental stimuli, such as gravitational load (Wuest et al. 2018). Mapping and quantifying calcium signaling with a high spatiotemporal resolution is a key challenge. Moreover, on microgravity platforms, experiment time, volume and weight are limited, allowing only a small number of replicates. Furthermore, experiment hardware is exposed to changes in gravity levels, causing experimental artifacts unless appropriately controlled (Wuest et al. 2022). In this project we introduced a new experimental setup based on the fluorescent calcium reporter CaMPARI2, onboard LED arrays and subsequent microscopic analysis on the ground (Hammer et al. 2022).

CaMPARI and Flight Hardware

CaMPARI (calcium-modulated photoactivatable ratiometric integrator) is an engineered protein, which irreversibly converts from green to red emission when illuminated with near UV light at 405 nm in the presence of calcium (Moeyaert et al. 2018). We seeded transfected cells in 96-well plates prior a parabolic flight and irradiated the samples with near UV light at predefined time points during the flight, using a newly developed hardware (Figure 1). The hardware featured a LED array that allowed irradiating each well individually. A special challenge was the thermal management, as mammalian cells are temperature sensitive. For safety reasons, the experiment could not be powered during take-off and landing which prohibits active heating during this period. In addition, the LEDs produced excessive heat, which had to be conducted away from the samples reliably. This problem could be solved by a combination of electrical heating elements, thermal insulation and a thermal buffer containing a paraffin wax with a melting point at ca. 36 °C. This stabilized the temperature two-fold: First, during the unpowered condition, it slowed down cooling in combination with an insulation. Second, it also absorbed the excessive heat generated during illumination. After the flight, the 96-well plates were removed from the flight hardware and subsequently imaged in the lab on site.

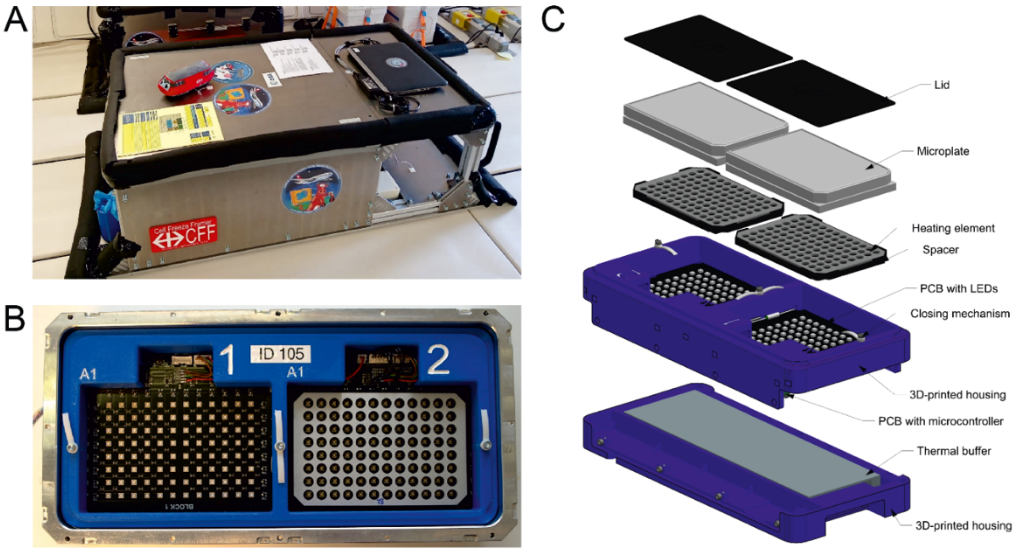


Figure 1: Parabolic flight hardware. (A) Flight rack mounted inside Novespace’s aircraft. (B) Top view on the hardware units, showing the empty payload bays for the two 96-well plates. The spacer with the heating element was removed in the left bay, exposing the LED array. (C) Explosion view of the hardware, highlighting the most relevant elements. Published in (Hammer et al. 2022).

Conclusions

Due to the separation of photoconversion in-flight and microscopy on ground, our novel approach allows accurate, higher throughput calcium recordings on microgravity platforms, such as parabolic flights. The excellent performance of CaMPARI2 was demonstrated with human chondrocytes during the 75th ESA parabolic flight campaign. CaMPARI2 revealed a strong calcium response triggered by histamine in articular chondrocytes. However, calcium was not affected by the alternating gravitational load of a parabolic flight (Figure 2). (Hammer et al. 2022)

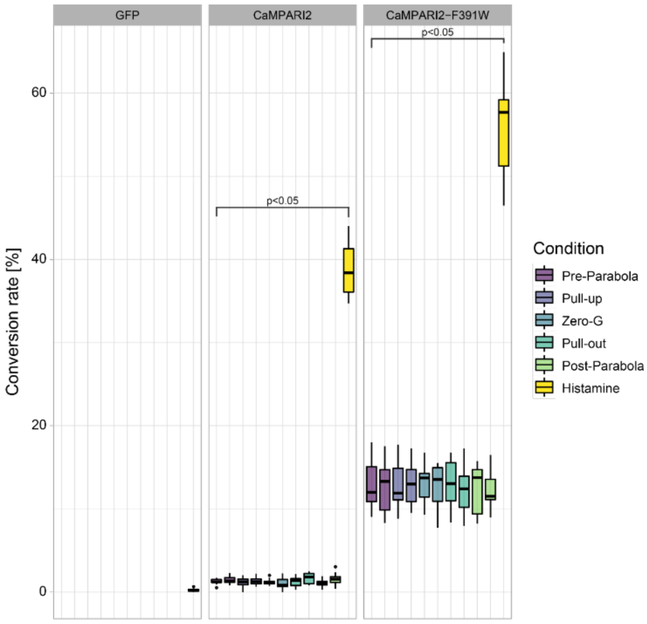


Figure 2: Conversion rates of human chondrocyte cells expressing CaMPARI2 or CaM-PARI2-F391W. The two constructs have different calcium sensitivity. The cells were illuminated during the indicated flight phase for conversion. The cells expressing GFP (negative control) were illuminated directly before a parabola. For each parabola and CaMPARI2-construct, eight wells per 96-well plate were treated with 100 µM histamine and subsequently illuminated (positive control). Published in (Hammer et al. 2022).

Acknowledgements

We thank Sartorius for providing the IncuCyte S3 and personnel and their support in analyzing the data. We thank the European Space Agency (ESA) and Novespace (Bordeaux, France) for the organization and support during the parabolic flight campaign. We gratefully acknowledge the financial support through PRODEX (ESA), and the Swiss Space Office (SSO), as well as through the German Space Agency (DLR).

References

Hammer, A., Cerretti, G., Ricciardi, D.A., Schiffmann, D., Maranda, S., Kummer, R., Zumbuhl, C., Rattenbacher-Kiser, K.F., von Arx, S., Ammann, S., Strobl, F., Berkane, R., Stolz, A., Stelzer, E.H.K., Egli, M., Schleiff, E., Wuest, S.L., Bohmer, M.: Retrograde Analysis of Calcium Signaling by CaMPARI2 Shows Cytosolic Calcium in Chondrocytes Is Unaffected by Parabolic Flights. Biomedicines 10(1) (2022). doi:10.3390/biomedicines10010138

Moeyaert, B., Holt, G., Madangopal, R., Perez-Alvarez, A., Fearey, B.C., Trojanowski, N.F., Ledderose, J., Zolnik, T.A., Das, A., Patel, D., Brown, T.A., Sachdev, R.N.S., Eickholt, B.J., Larkum, M.E., Turrigiano, G.G., Dana, H., Gee, C.E., Oertner, T.G., Hope, B.T., Schreiter, E.R.: Improved methods for marking active neuron populations. Nat Commun 9(1), 4440 (2018). doi:10.1038/s41467-018-06935-2

Wuest, S.L., Cerretti, G., Wadsworth, J.L., Follonier, C., Rattenbacher-Kiser, K.F., Bradley, T., Egli, M., Ille, F.: Cytosolic calcium and membrane potential in articular chondrocytes during parabolic flight. Acta astronautica 193, 287-302 (2022). doi:https://doi.org/10.1016/j.actaastro.2022.01.016

Wuest, S.L., Gantenbein, B., Ille, F., Egli, M.: Electrophysiological experiments in microgravity: lessons learned and future challenges. NPJ microgravity 4, 7 (2018). doi:10.1038/s41526-018-0042-3

15:00 Christian Liemersdorf – "Human Neural Network Activity Reacts to Gravity Changes"

Human Neural Network Activity Reacts to Gravity Changes

L. Kalinski[1], J. Striebel[2], M. Sturm[1], Y. Lichterfeld[1], N. Drouvé[3], S.L. Peters[3], S. El Sheikh[3], V. Busskamp[2], R. Habibey[2], J. Hauslage[1], S. Schunk[1], P. Lau[1], C. Liemersdorf[1]

[1]German Aerospace Center, Institute of Aerospace Medicine, Linder Hoehe, D-51147 Cologne, Germany
Christian.Liemersdorf@dlr.de; Laura.Kalinski@dlr.de; Maximilian.Sturm@dlr.de; Jens.Hauslage@dlr.de; Yannick.Lichterfeld@dlr.de; Sarah.Schunk@dlr.de; Patrick.Lau@dlr.de

[2]University of Bonn, Department of Ophthalmology, Medical Faculty, Bonn, Germany
Volker.Busskamp@uni-bonn.de; Johannes.Striebel@ukbonn.de; Rouhollah.Habibey@ukbonn.de

[3]Cologne University of Applied Sciences, Faculty of Applied Natural Sciences, InnovAGe Institute, Leverkusen, Germany
Sherif.elSheikh@th-koeln.de; Nils.Drouve@th-koeln.de; Stefan.Peters@th-koeln.de

During spaceflight, humans are subjected to a variety of environmental factors which deviate from Earth conditions. Especially the lack of gravity poses a major challenge to the human body. Space flight conditions have been identified as a major trigger of various detrimental effects observed either in returning astronauts but also in subjects of spaceflight-analog studies. Structural alterations within the brain as well as declines in cognitive performance have been reported, which has brought the topic of brain health under microgravity into the focus of space research. However, the physiological mechanisms underlying these observations remain elusive.
Every aspect of human cognition, behavior and psychomotor function is processed by the brain based on electro-chemical signals of billions of neurons, which relay information via neuronal networks throughout the body. Alterations in neuronal activity are characteristic of a variety of mental disorders and changed neuronal transmission may further lead to diminished human performance in space. Thus, understanding the functioning of these fundamental processes under the influence of altered gravity conditions on a cellular level is of high importance for further manned space mission. Notably, previous electrophysiological experiments using patch clamp have shown that propagation velocity of action potentials (APs) is dependent on gravity.

Here, we hypothesized that neuronal network activity is influenced by alterations in mechanical loading as experienced by altered gravity conditions. Furthermore, investigations aimed to understand the role of mechanical (de-)coupling on the functional level of human neurons that could be translated to individual patient-specific applications and therapeutic approaches in space and on Earth.

With this project, we advanced the electrophysiological approach from a single-cell to a network level by employing microelectrode array (MEA) technology. MEAs feature the advantage of real-time electrophysiological recording of human neuronal network in vitro, without the need for invasive patch clamp insertion into single cells. Using a custom-built experiment module, we were able to integrate and conduct our experiment on the ZARM Drop Tower, the DLR human centrifuge and the MAPHEUS-12 sounding rocket platforms.
Constantly neuronal activity of human stem-cell derived neurons (hiPSC) were measured throughout all gravitational loading phases. Even throughout the 4.7 sec of microgravity during free fall in the drop tower, the mean AP frequency and burst rate across the neuronal networks was significantly elevated. Interestingly, hypergravity of up to 6g had opposing effects on neuronal activity in both firing and burst rate. Additionally, neuronal activity re-adapted back to baseline level after intermediate time periods.

The MEA module was therefore validated on various gravity research platforms for autonomous recording of electrophysiological data from human neuronal networks. Notably, gravity-induced differences in neuronal activity could be detected already within the second range of exposure to different gravity levels.

REFERENCES

Human neural network activity reacts to gravity changes in vitro. Striebel J, Kalinski L, Sturm M, Drouvé N, Peters S, Lichterfeld Y, Habibey R, Hauslage J, El Sheikh S, Busskamp V and Liemersdorf C Front. Neurosci. 17:1085282. (2023) doi: 10.3389/fnins.2023.1085282

15:15 Jess Bunchek – "VEG-04: How Lighting Affects Crop Production, Quality, And Acceptability of Mizuna Mustard Grown on the International Space Station"

VEG-04: How Lighting Affects Crop Production, Quality, and Acceptability of Mizuna Mustard Grown on the International Space Station

J. M. Bunchek[1], M. E. Hummerick[2], L. E. Spencer[3], M. W. Romeyn[4], M. Young[5], G. L. Douglas[6], R. C. Morrow[7], C. A. Mitchell[8], R. M. Wheeler[9], and G. D. Massa[10]

[1]LASSO, Kennedy Space Center, FL, USA; German Aerospace Center (DLR), Bremen, Germany (jess.bunchek@dlr.de), [2]LASSO, Kennedy Space Center, FL, USA (mary.e.hummerick@nasa.gov), [3]LASSO, Kennedy Space Center, FL, USA (lashelle.e.spencer@nasa.gov), [4]NASA, Kennedy Space Center, FL, USA (matthew.w.romeyn@nasa.gov), [5]NASA, Johnson Space Center, Houston, TX, USA (millennia.young@nasa.gov), [6]NASA, Johnson Space Center, Houston, TX, USA (grace.l.douglas@nasa.gov), [7]Sierra Space, Madison, WI, USA (robert.morrow@sierraspace.com), [8]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA (cmitchel@purdue.edu), [9]NASA, Kennedy Space Center, FL, USA (raymond.m.wheeler@nasa.gov), [10]NASA, Kennedy Space Center, FL, USA (gioia.massa@nasa.gov)

As we prepare for longer-duration missions, growing produce that is fresh, nutritious, safe, and palatable for crew consumption during spaceflight may provide health-promoting, bioavailable nutrients and enhance the astronaut dietary experience. However, requirements to support consistent growth of a variety of high-quality crops under spaceflight environmental and physical conditions remain unclear. The VEG-04 study explored the potential to grow crops for astronaut consumption on the International Space Station (ISS) using the Veggie vegetable-production system. VEG-04A and VEG-04B were two flight tests conducted with the leafy green crop Mizuna mustard, which was selected based on its nutritional quality, palatability, and compatibility with the Veggie system and ISS environmental conditions. Mizuna was grown in two Veggie chambers simultaneously both in spaceflight and in a ground control at the Kennedy Space Center (KSC), with the Veggie systems set to different red-to-blue-to-green light formulations. For both the flight and ground components, one Veggie was programmed as "red-rich" with an average of 270 µmol m-2 s-1 of 630 nm red light, 30 µmol m-2 s-1 of 455 nm blue light, and 30 µmol m-2 s-1 of 530 nm green light. The other Veggie was "blue-rich" with an average of 150 µmol m-2 s-1 of 630 nm red light, 150 µmol m-2 s-1 of 455 nm blue light, and 30 µmol m-2 s-1 of 530 nm green light. Light quality is known to impact plant growth, nutrition, microbiology, and organoleptic characteristics on Earth, and the Veggie flight tests examined how these impacts might differ in microgravity. VEG-04A, a 35-day growth test with a single harvest, was initiated in June and harvested in July 2019. VEG-04B, a 56-day test with three harvests from the same plants, assessed sustained productivity. VEG-04B was initiated in October 2019 with harvests at four, six, and eight weeks after initiation. At all of the harvests, the astronauts froze half of the edible plant tissue to return to Earth and weighed the remaining half using the Mass Measurement Device (MMD). Weighed samples were then cleaned with produce-sanitizing wipes, and consenting crew members participated in organoleptic evaluation of the fresh produce. The remaining sanitized produce was available for crew consumption as desired. Frozen flight samples were returned to Earth for microbial and chemical analyses to assess food safety and nutritional quality. No pathogens were detected in flight or ground control samples from either experiment. On average, bacterial and fungal counts were significantly lower on ground control samples than flight samples, and yield and chemistry differed between ground and flight samples and between red-rich and blue-rich light treatments. Additional data collection included environmental conditions (temperature, relative humidity, and CO_2), water use, and crew time. It is our hope that these tests on the ISS will help mitigate the risk of an inadequate food supply for long-duration missions by adding fresh produce to the crew diet, and that the VEG-04 study can improve our understanding of lighting selection and the water and crew time required for growing future crops in spaceflight.
This research was co-funded by the NASA Human Research Program and Space Biology (MTL#1075) in the ILSRA 2015 NRA call.

15:30 Jennifer-Vernice Pauly – "Veggie on ICE: The Effects of Plant Production on Human Behavioral Health in Long-Duration Antarctic Overwintering Missions"

Brains on ICE – Hippocampal Changes in Response to Prolonged Isolation and Confinement

J.V. Pauly[1], A. Friedl-Werner[1], K. Brauns[1], P.C. Barsby[1], S. Kühn[2], and A. C. Stahn[3]
[1]Charité University Medicine Berlin, Germany (jennifer-vernice.pauly@charite.de, anika.werner@charite.de, katharina.brauns@charite.de, poppy.barsby@charite.de), [2]Max-Planck-Institute for Human Development Berlin, Germany (kuehn@mpib-berlin.mpg.de), and [3]University of Pennsylvania, Philadelphia, PA (astahn@pennmedicine.upenn.edu).

Long-duration space missions (LDSM) can pose a considerable risk for developing adverse behavioral conditions and psychiatric disorders. The neural signatures of these risks are currently not well understood. Previous data have shown that prolonged isolation and confinement can lead to significant structural brain changes that are associated with key neurotrophins and translate to impaired cognitive performance (Stahn et al., 2019). Importantly, the response to isolation and confinement is characterized by significant inter-individual differences, and it remains to be determined how different crews and crew dynamics respond to prolonged isolation and confinement and how these relate to phenotypic vulnerabilities.

One brain area impacted by stress is the hippocampus as shown by scientific studies. The hippocampus is involved in memory formation, spatial navigation as well as mood regulation, which makes it of particular interest if negatively affected by LDSM. As a subregion of the hippocampus, the dentate gyrus is one of the two known regions in the adult human brain where active neurogenesis can occur. Whereas physical activity and environmental enrichment can stimulate the dentate gyrus, it has been shown that various conditions and stressors can have adverse effects on hippocampal function and structure.

With this study we will investigate the impact of long-duration Antarctic missions on hippocampal structure and shape. We hypothesize that the isolation and confinement experienced during such missions results in changes specific to different hippocampal subfields such as the dentate gyrus. A total of 4 crews (each comprising N=9 crew members) overwintering at Neumayer-Station III in Antarctica for 12 to 14 months will be investigated. To asses neurostructural and neurofunctional changes crew members underwent neuroimaging once before and once after the mission. The magnetic resonance imaging (MRI) data (Siemens Tim Trio 3T scanner) obtained includes high-resolution T1- and T2-weighted sequences. Changes in the volumes of hippocampal subfields will be analyzed in the post-processing of those sequences using the automated segmentation of hippocampal subfield (ASHS) tool (Yushkevich et al., 2010). The algorithm allows for analysis of various hippocampal segments, including the head, tail, CA1-3, dentate gyrus, subiculum, entorhinal cortex, and parahippocampal gyrus. Data analyses are currently ongoing and preliminary findings will be presented at the conference.

With this study we will relevantly contribute to ESA's and NASA's goal to provide knowledge, technologies, and tools to enable safe, reliable, and productive human space exploration by providing data on critical neurobehavioral functions. Our findings relate to the relevance for facilitating effective countermeasure tools critical in support of exploration class missions to monitor and mitigate crew health and performance risks.

SUPPORT

Supported by DLR through grant 50WB1525 and 50WB1330.

REFERENCES

Stahn A.C., Gunga H.C., Kohlberg E., Gallinat J., Dinges D.F. and Kühn S., 2019, New England Journal of Medicine, 381(23), 2273–2275.

Yushkevich P.A., Wang H., Pluta J., Das S.R., Craige C., Avants B.B., Weiner M.W. and Mueller S., 2010, NeuroImage, 53(4), 1208–1224.

16:15 Dag Linnarsson – "Lung diffusing capacity for nitric oxide in space: microgravity gas density interactions"

Lung diffusing capacity for nitric oxide in space: microgravity gas density interactions

D. Linnarsson[1], L. L. Karlsson[1], and A. Van Muylem[2]
[1]Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden, dag.linnarsson@ki.se, lars.karlsson@ki.se, [2]Chest Department, Erasme University Hospital, Brussels, Belgium, alain.vanmuylem@hubruxelles.be

During manned space exploration, lung health is threatened by toxic planetary dust (Linnarsson D. et al, 2012) and radiation (Delp M.D. et al, 2016). Thus, tests such as lung diffusing capacity (DL) are likely be used in planetary habitats to monitor lung health. Earlier studies of DL in microgravity have been performed during relatively short Shuttle flights and in normal atmospheric pressure (Prisk G.K. et al, 1993, Verbanck S. et al, 1997).Therefore, the aim of this study was to investigate DL during long-term microgravity and at the same time study the influence of a reduced atmospheric pressure on the results, since the atmos-pheric pressure in a habitat on the moon or Mars is planned to be lower than on earth.

Lung diffusing capacity for nitric oxide (DLNO) was determined in 11 subjects on the ground and in microgravity on the International Space Station. Experiments were performed at both normal (1.0 atmospheres absolute, ata) and reduced (0.7 ata) atmospheric pressures. On the ground, DLNO did not differ between pressures, but in microgravity DLNO was increased by 9.8% (9.5) (mean [SD]) and 18.3% (15.8) at 1.0 and 0.7 ata respectively, compared to normal gravity, 1.0 ata. There was a significant interaction between pressure and gravity (P=0.0135).

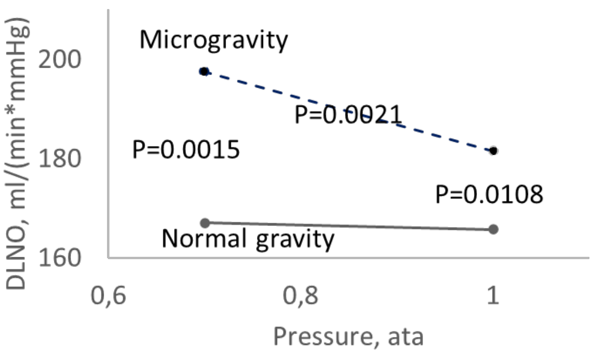


Figure 1. Lung diffusion capacity for nitric oxide as a function of cabin pressure. P values between lines refer to differences between gravity conditions, and P value on a line refers to difference between pressure conditions

Estimates of the membrane (DmNO) and gas phase (DgNO) components of DLNO (Linnarsson D. et al, 2013) suggested that at normal gravity a reduced pressure led to opposing effects in convective and diffusive transport in the gas phase, with no net effect of pressure. In contrast, a DLNO increase with reduced pressure at microgravity is compatible with a substantial increase of DmNO partially offset by reduced DgNO, the latter being compatible with interstitial edema (Permutt S. 1967) with narrowing of peripheral diffusion pathways. In microgravity therefore, DmNO would be proportionally underestimated from DLNO. We also conclude that normal values for DL in anticipation of planetary exploration should be determined not only on the ground but also at the gravity and pressure conditions of a future planetary habitat.

REFERENCES

Linnarsson D., Carpenter J., Fubini B., et al., 2012, *Planet Space Sci*, 74: 57–71. DOI: 10.1016/j.pss.2012.05.023.
Delp M.D., Charvat J.M., Limoli C.L., Globus R.K., Ghosh P., 2016, *Sci Rep,* Jul 28;6:29901. DOI: 10.1038/srep29901.
Prisk G.K., Guy H.J.B., Elliott A.R., et al., 1993, *J Appl Physiol 1993*, 75: 15–26. DOI: 10.1152/jappl.1993.75.1.15.
Verbanck S., Larsson H., Linnarsson D., et al., 1997, *J Appl Physiol 1997*, 83: 810–816. DOI: 10.1152/jappl.1997.83.3.810.
Linnarsson D., Hemmingsson T.E., Frostell C., Van Muylem A., Kerckx Y., Gustafsson L.E.., 2012, *Respir Physiol Neurobiol*, 189(3):552-7. DOI: 10.1016/j.resp.2013.08.008.
Permutt S.,1967, Washington: *NAS NRC* 1485B; 1967. pp. 38–56.

16:30 Paniz Balali – “Adaptive Changes in Heart Rate Variability and Cardiac Function during LongTerm Spaceflight: Insights from Wearable Devices”

Adaptive Changes in Heart Rate Variability and Cardiac Function during Long-term Spaceflight: Insights from Wearable Devices

Paniz Balali[1,2*], Cyril Tordeur[1], Jeremy Rabineau[1], Vitalie Faoro[3], Irina Funtova[4], Olivier Debeir[2], Elena Luchitskaya[5], Pierre-Francois Migeotte[6], Jens Tank[7], Philippe van de Borne[8]

[1] Laboratory of Physics and Physiology, Université Libre de Bruxelles (paniz.balali@ulb.be, Cyril.Tordeur@ulb.be, Jeremy.Rabineau@ulb.be), [2] Laboratory of Image Synthesis and Analysis, Université Libre de Bruxelles (Olivier.Debeir@ulb.be), [3] Faculty of Motricity Sciences (Vitalie.Faoro@ulb.be), [4] Russian academy of sciences (funtova.imbp@mail.ru), [5] Institute of Biomedical Problems RAS, Russian Federation (e.luchitskaya@gmail.com), [6] HeartKinetics (pierre-francois@heartkinetics.com), [7] German Aerospace Center (jens.tank@dlr.de), [8] Department of Cardiology, Erasme Hospital, Université Libre de Bruxelles (Philippe.Van.de.Borne@erasme.ulb.ac.be).

* Correspondence: paniz.balali@ulb.be

ABSTRACT

Exposure to microgravity during spaceflight significantly affects the human body, including alterations of the autonomic nervous system, changes in heart rate variability (HRV), and redistribution of blood flow in the chest area. The objective of this study was to use a wearable device to investigate the changes in the cardiovascular system of cosmonauts during their mission on the International Space Station (ISS).

Data was collected from eight cosmonauts (one female) using a device measuring electrocardiography (ECG) and impedance cardiography (ICG). The subjects were asked to perform controlled 5-second breathing cycles, and recordings were made before (-66 ±19 days), during (7 ±5, 37 ±4, and 158 ±5 days), and after (+3 ±1 days) spaceflight. The measurements on Earth were done in sitting position, while in space they were done in free-floating. HRV features included root mean square of successive differences between normal heartbeats (RMSSD) and the ratio of power in the low and high frequency ranges (LF/HF). In addition, stroke volume (SV) normalized by baseline value, pre-ejection period (PEP), left ventricular ejection time corrected for heart rate and gender (LVETi), and their ratio (PEP/LVETi) were computed for each beat using the ECG and ICG signals. They were then averaged over the whole record. We consider $p < 0.05$ as statistically significant. Data is expressed as median and interquartile range.

From baseline to the first week in space, we observed an increase in RMSSD from 0.018 [0.013; 0.022] s to 0.030 [0.024; 0.037] s, and SV by 35% [6%; 48%], and a decrease in LF/HF from 1.28 [1.04; 2.78] to 0.63 [0.30; 1.10], PEP from 68.11 [56.16; 72.79] ms to 55.94 [50.46; 60.65] ms, and PEP/LVETi from 0.15 [0.13; 0.16] to 0.12 [0.11; 0.13]. Besides, within 6 months in space all parameters tended to return to baseline levels.

In space, astronauts experience a microgravity-induced head-ward fluid shift. This shift causes hemodynamic and autonomic changes. An increase in cardiac preload and a reflex sympathetic withdrawal causing a reduction in systemic vascular resistance ipso facto produce an increase in SV, a reduction in PEP and a tendency to increase LVETi, as compared to sitting position on Earth. These changes happen early during the flight, but afterwards these features tend to come back to baseline levels. The findings of this study confirm that the cardiovascular system can adjust to the microgravity environment within a few months, at least with the countermeasures used onboard the ISS.

16:45 Jason Fisher – "Cardiovascular And Cutaneous Blood Flow Responses To Artificial Gravity And Temperature: A Pilot Study"

Cardiovascular And Cutaneous Blood Flow Responses To Artificial Gravity And Temperature: A Pilot Study

J. T. Fisher[1], V. Faivre-Rampant[2], J. P. Fortune[3], and I. B. Mekjavic[4]
[1] Department of Automatics, Biocybernetics and Robotics, Jozef Stefan Institute, Jamova Cesta 39, Ljubljana, SI-1000, Slovenia. E: Jason.fisher@ijs.si
[2] Department of Automatics, Biocybernetics and Robotics, Jozef Stefan Institute. E: victorien14@live.fr
[3] Department of Automatics, Biocybernetics and Robotics, Jozef Stefan Institute. E: Jack.Patrick.Fortune@ijs.si
[3] Department of Automatics, Biocybernetics and Robotics, Jozef Stefan Institute. E: Igor.mekjavic@ijs.si

INTRODUCTION

The use of artificial gravity (AG) has been discussed as a suitable method of reducing microgravity-induced cardiovascular deconditioning. However, the extent to which hypergravity and ambient temperature experienced during AG must be evaluated. Previous research has considered the effect of differing levels of AG on cardiovascular and cerebrovascular responses, indicating a relationship between standing and 1G exposure (Goswami, N. et al., 2015). However, this study did not measure regional vascular responses, or the impact of differing ambient conditions. Therefore, the present pilot study assessed the impact of AG in the head-to-foot direction, and ambient temperature, on the cardiovascular system and cutaneous blood flow. It is anticipated that both cardiovascular and cutaneous blood flow responses to normal gravity (NG) and 1G will be comparable, and higher ambient temperature will have a negative impact on the regulation of blood flow in response to environmental stressors.

METHODS

Three male participants (age: 24.3 ± 1.2 years, height: 180.7 ± 7.4 cm, weight: 80.0 ± 13.9 kg) completed two sessions where cardiovascular responses (heart rate, blood pressure), cutaneous blood flow measured at the forearm (armBF) and calf (legBF), and skin temperature (T_{skin}) were assessed in four positions; standing (ST), supine (SU), supine 1G (1G), supine 2G (2G). Cutaneous blood flow was measured by laser doppler flowmetry, and skin temperature by four thermistors (chest, bicep, thigh, calf) averaged via the Ramanathan (1964) four-site equation. The two sessions were alternated between either cold (20.5 ± 0.7 °C) or warm (29.2 ± 0.9 °C) ambient conditions with 30% relative humidity.

RESULTS

AG appeared to produce similar responses in heart rate to those observed during NG. Heart rate in the ST condition was 84.2 ± 12.1 b·min-1, and in the 1G condition was 87.9 ± 12.6 b·min^{-1}; interestingly, 2G AG caused a lower heart rate of 74.5 ± 17.15 b·min-1. Mean blood pressure, however, observed a large increase between the ST and 1G conditions (SU: 86.7 ± 7.9 mmHg, 1G: 106.2 ± 10.2 mmHg). Similarly, to heart rate, armBF between NG and AG were similar (SU: 15.3 $_{\%max}$, 1G: 13.0 $_{\%max}$), however legBF was considerably higher during AG (SU: 12.2 $_{\%max}$, 1G: 23.1 $_{\%max}$). The ambient temperature had a clear impact on all variables. The largest increase in cardiovascular responses occurred in the ST position; heart rate was 17.9 b·min^{-1} higher, whilst mean blood pressure increased by 43.9 mmHg. Cutaneous blood flow observed the greatest increase during the SU position; armBF was 11.1 $_{\%max}$ higher and legBF was 15.8 $_{\%max}$ higher in the warm ambient conditions. As expected, Tskin was considerably higher in the warm condition (cold: 29.3 °C, warm: 34.0 °C), and AG caused a decrease in the Tskin in both warm (-0.9 °C) and cold (-1.9 °C) conditions.

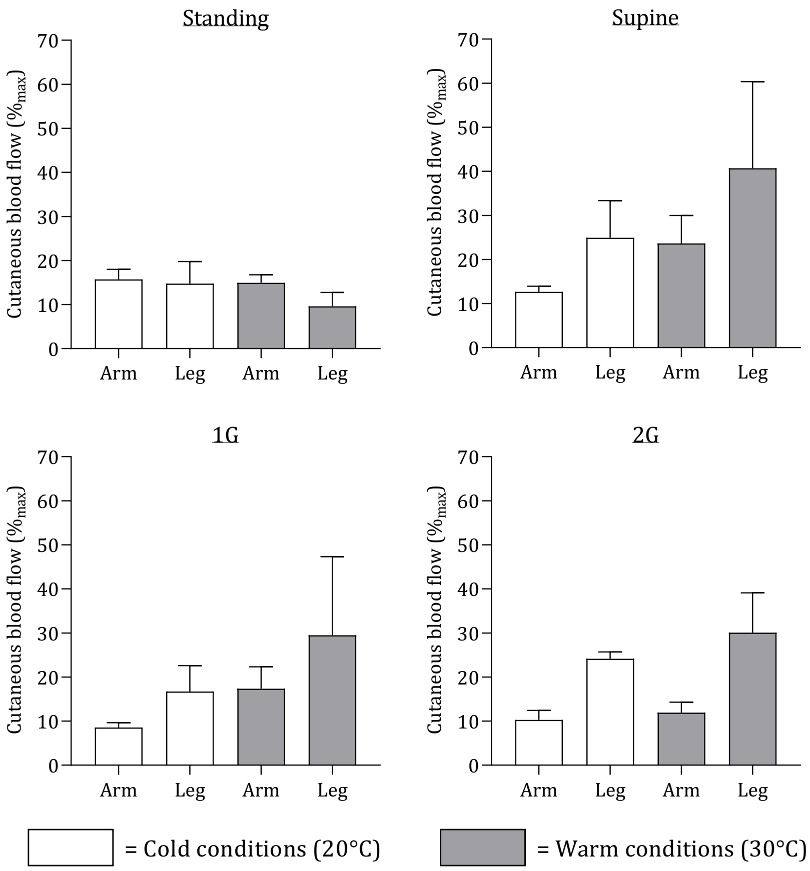


Figure 1. Mean ± SD regional cutaneous blood flow responses ($\%_{max}$) to different positions and AG load, in both cold (white bars) and warm (grey bars) ambient conditions.

DISCUSSION AND CONCLUSION

It is clear that while AG appears to cause little change with regards to the control of heart rate, the distribution of blood within the body is affected more greatly. Large increases in blood pressure and legBF in particular indicate the stress of AG causes a considerable shift of blood in the foot direction, which must be countered in order to avoid syncope. In addition, higher ambient temperatures appear to exacerbate these issues by redirecting partial control of the blood flow towards regulation of the thermal status of the body. It is the therefore essential that additional research is conducted to fully understand the regional regulation of blood flow during exposure to AG, particularly with reference to increasing hypergravity load and ambient temperature.

REFERENCE LIST

Goswami, N., Bruner, M., Xu, D., Bareille, M. P., Beck, A., Hinghofer-Szalkay, H., & Blaber, A. P. (2015). *European Journal of Applied Physiology*, 115, 1569-1575.

Ramanathan, N. L. (1964). A new weighting system for mean surface temperature of the human body. *Journal of Applied Physiology*, 19(3), 531-533.

17:00 Damien Lanéellé – "Cerebral Blood flow distribution according to cerebral arterial pattern variations during hemodynamic stress induced by simulated hypovolemia or hypercapia: a triplex ultrasound and magnetic resonance imaging study"

Cerebral Blood flow distribution according to cerebral arterial pattern variations during hemodynamic stress induced by simulated hypovolemia or hypercapia: a triplex ultrasound and magnetic resonance imaging study

D. Lanéelle[1] and H. Normand[2]
[1] Normandie Université, UNICAEN, INSERM, COMETE, CYCERON, CHU Caen Normandie, Service de médecine vasculaire, 14000 Caen, France (laneelle-d@chu-caen.fr), [2] Normandie Université, UNICAEN, INSERM, COMETE, CYCERON, CHU Caen Normandie, Service des explorations fonctionnelles, 14000 Caen, France (herve.normand@unicaen.fr)

BACKGROUND

On return to Earth, space travellers heterogeneously develop Orthostatic Intolerance (OI), the aetiology of which is not yet resolved. As the symptoms of OI seem to be related to a decrease in cerebral blood flow (CBF), an anatomical reason could explain this heterogeneity. The Circle of Willis, a network of arterial anastomoses located at the base of the brain, shows heterogeneity in its conformation, in particular hypoplasias of the posterior communicating arteries (AComP). It is possible that this heterogeneity is related to this variability in response to orthostatism (figure 1).

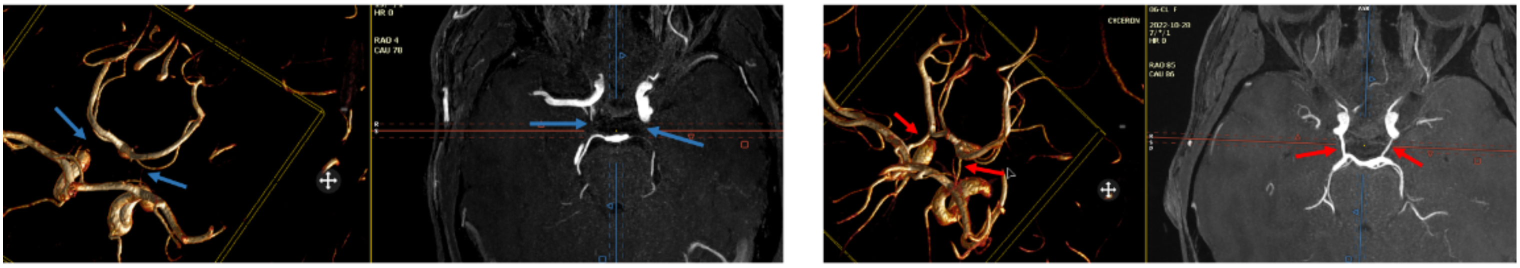


Figure 5. MRI of the Circle of Willis with incomplete arterial pattern to the left and full arterial pattern to the right

The aim of this study will be to create two groups of subjects with different arterial pattern variations, using a Doppler ultrasound technique, and to assess the CBF according to this arterial pattern during hemodynamic stress induced by simulated hypovolemia or hypercapia.

METHODS

A GE Vivid-i Doppler ultrasound machine was used in combination with a short (5s) carotid compression to distinguish two groups: full arterial pattern and incomplete arterial pattern. CBF distribution was assessed with 3 Tesla (SIGNA, GE MEDICAL SYSTEMS) time-resolved phase contrast MRI with three-directional flow velocity encoding, referred to as 4D flow MRI, for 24 subjects during baseline, inhalation of 5% carbon dyoxid (CO_2) and lower body negative pressure (LBNP). Segmentation, artery contouring, velocity, area and flow measurements were performed with CVI42 software and 4D FLOW module (v5.16-2022, Circle Vascular Imaging) for 11 flow measurements sites per subject (figure 2).

Figure 6. The measurement sites are indicated in red

RESULTS

The global CBG (gCBF), calculated by adding the flow of both Internal Carotid arteries ($\dot{Q}$ICA) and the flow of Basilar artery ($\dot{Q}$BA), was 595 (±125) mL/min at baseline, increased to 774 (±169) mL/min for 5% of CO2 and decreased to 529 (±92) mL/min for LBNP ($P \leq 0.001$ and $P = 0.007$ respectively). There was no change in $\dot{Q}$BA / gCBF (24.7%, 25.2% and 24% at baseline, 5% of CO2 and LBNP respectively). There was no difference in gCBF or CBF distribution by arterial pattern, at baseline, CO2 or LBNP (table 1).

Stimulation	Characteristic	Incomplete Pattern N = 11	Full Pattern N = 12	95% CI	p-value
inhalation of 5% CO_2	Δ gCBF (%)	35% (±21%)	24% (±14%)	[-4.7, 27]	0.2
	- Δ $\dot{Q}_{BA}$ (%)	47% (±33%)	28% (±28%)	[-8.1, 46]	0.2
	- Δ $\dot{Q}_{ICA}$ (%)	34% (±26%)	23% (±15%)	[-8.5, 29]	0.3
	Δ $\dot{Q}_{BA}$ / gCBF (%)	4% (±24%)	-1% (±21%)	[-15, 25]	0.6
LBNP -40 mmHg	Δ gCBF (%)	-5% (±12%)	-12% (±13%)	[-4.6, 18]	0.2
	- Δ $\dot{Q}_{BA}$ (%)	-10% (±19%)	-12% (±23%)	[-18, 21]	0.9
	- Δ $\dot{Q}_{ICA}$ (%)	-3% (±13%)	-10% (±16%)	[-5.5, 21]	0.2
	Δ $\dot{Q}_{BA}$ / gCBF (%)	-5% (±15%)	1% (±31%)	[-29, 16]	0.5

Table 1. Effect of hypercapnia (5% CO2) and simulated hypovolemia (LBNP – 40 mmHg) on CBF distribution according to the polygone

CONCLUSIONS

The heterogeneity of the cerebral arterial pattern does not appear to result in changes in cerebral flow distribution when CBF is increased by hypercapnia or decreased by simulated hypovolemia.

17:15 Danielle Greaves – "Robotic Ultrasound on ISS Operated from Canadian University's Mission Control Center - Results from Vascular Echo Experiment"

Robotic Ultrasound on ISS Operated from Canadian University's Mission Control Center - Results from Vascular Echo Experiment

Danielle K. Greaves[1], Richard L. Hughson[2] and Philippe Arbeille[3]
[1]dgreaves@uwaterloo.ca, Faculty of Health, University of Waterloo, Canada, [2]hughson@uwaterloo.ca, Schlegel-University of Waterloo Research Institute for Aging, Waterloo, Canada, and [3]arbeille@med.univ-tours.fr, CERCOM-UMPS, Faculté de Médecine Université de Tours, France

For the first time, the ESA ECHO robotic ultrasound device was successfully tele-operated from a Mission Control site outside of the French Space Agency (CNES). On-orbit data were collected for the Canadian experiment, Vascular Echo, using a purpose-built Mission Control Center at the University of Waterloo in Canada. Internal jugular vein (IJV) congestion was measured using two different ultrasound methods: first, using a conventional 2D method and then the measurements were repeated using a newly-launched 3D motorized probe. When novice sonographers such as astronauts use conventional 2D imaging to scan the jugular vein, the scans are susceptible to poor reproducibility due to errors in hold-down pressure, positioning, and angle. The 3D motorized probe was launched to mitigate these types of user errors. In addition to comparing the two methods, the congestion level in the IJV was experimentally manipulated using venoconstrictive thigh cuffs. Thigh cuffs, or Braslets, are a countermeasure used to reduce IJV congestion during spaceflight and space analogs. The area (cm2) of consecutive cross-sectional slices taken along the length of the neck was reported before and after having worn the cuffs for 4 hours, and compared using both methods. These data are the first to compare the outputs of both 2D and 3D ultrasound methods during spaceflight in the same participants. The 2D and 3D ultrasound results were not congruent in all astronauts. 3D ultrasound confirmed that four hours of thigh-cuff use reduced IJV volume in three astronauts by approximately 35%, whereas 2D data were more equivocal as to the impact of the thigh-cuffs. We believe that motorized 3D ultrasound provides less error-prone quantitative data on astronaut fluid shifts on ISS. The motorized 3D ultrasound technology mitigates human scanning errors created by non-expert sonographers under remote guidance, by equalizing down pressure, controlling probe sweep speed, and correcting the angle of insonation of the irregularly shaped IJV. The current results demonstrate that motorized 3D ultrasound should be the preferred imaging method when trying to measure venous congestion in the IJV, and that 2D ultrasound results should be interpreted with caution.

17:30 Philippe Arbeille – "Spaceflight and Dry Immersion Increases the Reflectivity of the Arterial Wall to Ultrasound Radio Frequency Waves"

Spaceflight and Dry Immersion Increases the Reflectivity of the Arterial Wall to Ultrasound Radio Frequency Waves

Philippe Arbeille[1] (MD-PhD), Danielle Greaves[2] (PhD), Laurent Guillon[3] (MD), and Richard L Hughson[4] (PhD).

[1]arbeille@med.univ-tours.fr, CERCOM-UMPS, Faculté de Médecine Université de Tours, France, [2]dgreaves@uwaterloo.ca, Faculty of Health, University of Waterloo, Canada, [3]guillon@med.univ-tours.fr, CERCOM-UMPS, Faculté de Médecine Université de Tours, France, and [4]hughson@uwaterloo.ca, Schlegel-University of Waterloo Research Institute for Aging, Waterloo, Canada

Recent studies have reported a significant increase in common carotid artery (CCA) intima media thickness, wall stiffness and reflectivity to ultrasound, in astronauts, after six months of spaceflight. The hypothesis was that 4 days in dry immersion (subjects under bags of water) will be sufficient to change the CCA wall reflectivity to ultrasound similar to what observed after spaceflight. Such response would be quantified using the amplitude of the ultrasound signal returned to the probe by the target concerned. (coefficient of signal return (Rs)).
The Rs for anterior and posterior CCA wall, sternocleidomastoid muscle, intima layer and CCA lumen were calculated from the ultrasound radio frequency (RF) data displayed along each echographic line.
After four days of DI, Rs increased in the CCA posterior wall (+15% +/- 10 from pre DI, $p < 0.05$), while no significant change was observed in the other targets. The observed increase in Rs with DI was approximately half compared to what was observed after six months of space flight (+34% +/- 14). This difference may be explained by unequal dose response; dry immersion lasting only four days in duration and spaceflight missions lasting 6 months. While the equivalency between dry immersion and spaceflight is unlikely 1:1, it seems reasonable to assume that 4 days is less stimulating than 6 months. As a marker of tissue-level physical changes, Rs provide complimentary information alongside previously observed CCA wall thickness and stiffness.

17:45 Damian Bailey – "Gravitational transitions increase blood-brain barrier permeability in humans; focus on the redox-regulation of cerebral hyper-fusion"

Gravitational transitions increase blood–brain barrier permeability in humans; focus on the redox-regulation of cerebral hyper-perfusion

D. M. Bailey[1*], D Lanéelle[2,3], J.E. Trihan[4], N. Marchi[5], B.S. Stacey[1], K. Tamiya[6], T. Washio[6], E. Tuaillon[7], C Hirtz[8], S. Lehmann[8], S. Ogoh[1,6] and H. Normand[3]

[1]Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Glamorgan, UK (damian.bailey@southwales.ac.uk; benjamin.stacey@southwales.ac.uk); [2]Service de Médecine Vasculaire, Centre Hospitalo-Universitaire, Caen (damien.laneelle@unicaen.fr; herve.normand@unicaen.fr); [3]Normandie University, UNICAEN, INSERM, COMETE, GIP CYCERON, Caen, France; [4]Service de Médecine Vasculaire, Centre Hospitalo-Universitaire, Poitiers (j.eudes.trihan@gmail.com); [5]UMR, Laboratory of Cerebrovascular and Glia Research, Department of Neuroscience, Institute of Functional Genomics (CNRS Unit Mixte de Recherche 5203; INSERM U1191), University of Montpellier, France (Nicola.Marchi@igf.cnrs.fr); [6]Department of Biomedical Engineering, Toyo University, Kawagoe-Shi, Saitama, Japan (tamichan.0615@gmail.com; t.wasshie@gmail.com; ogoh@toyo.jp); [7]Unit Mixte de Recherche, INSERM l'Etablissement Français du Sang, Université Montpellier 1, Montpellier, France (e-tuaillon@chu-montpellier.fr); [8]Laboratoire de Biochimie et Protéomique Clinique, University of Montpellier, Institute of Regenerative Medicine-Biotherapy IRMB, CHU Montpellier, INSERM, Montpellier, France (christophe.hirtz@umontpellier.fr; sylvain.lehmann@umontpellier.fr); [8]Normandie University, France; [9]UNICAEN, COMETE, Caen, France; [10]INSERM, U 1075 COMETE, Caen, France and [11]CHU de Caen, Department of Clinical Physiology, Caen, France.

BACKGROUND

Alterations in gravitational fluid-pressure gradients caused by the microgravity of orbital spaceflight and hypergravity associated with take-off/landing thus pose unique physiological challenges for the astronaut brain with recent interest focused on the complex pathophysiology underlying a constellation of debilitating neurological, ophthalmological and neurovestibular symptoms known collectively as the spaceflight-associated neuro-ocular syndrome (SANS, formerly the visual impairment/intracranial pressure syndrome). As a ground-based spaceflight analogue, parabolic flight (PF) provides a unique opportunity to induce rapidly alternating shifts in central blood volume (CBV) subsequent to repeated exposures to microgravity (0 Gz) interspersed with hypergravity (1.8 Gz) to provide insight into cerebral haemodynamic function. However, to what extent intermittent alterations in CBV during PF impacts cerebral blood flow (CBF) remains inconclusive, complicated in part by the logistical challenges associated with data collection, absence of normogravity control comparators and technical limitations associated with indirect measurement techniques. Furthermore, there are no published human studies that have examined how altered CBF impacts the blood-brain barrier (BBB) or structural integrity of the neurovascular unit (NVU) including its functional regulation by free radicals and associated reactive oxygen-nitrogen species.

AIM

The present study examined if repeated bouts of micro- and hypergravity during parabolic flight (PF) alter structural integrity of the NVU subsequent to free radical-mediated changes in regional CBF.

METHODOLOGY

Six participants (5♂, 1♀) aged 29 ± 11 years were examined before, during and after a 3h PF and compared to six sex and age-matched (27 ± 6 years) normogravity controls. Blood flow was measured in the anterior (middle cerebral artery, MCA; internal carotid artery, ICA) and posterior (vertebral artery, VA) circulation (duplex ultrasound) in-flight over the course of 15 parabolas. Venous blood was directly assayed for free radicals (electron paramagnetic resonance spectroscopy), nitric oxide (NO, ozone-based chemiluminescence) and NVU integrity biomarkers (chemiluminescence/ELISA) in normogravity before and after exposure to 31 parabolas.

RESULTS

While MCA velocity did not change ($P > 0.05$), a selective increase in VA flow was observed during the most marked gravitational transition from micro- to hypergravity ($P < 0.05$). Increased oxidative-nitrosative stress defined by a free radical-mediated reduction in NO and elevations in glial fibrillary acidic protein (GFAP) and S100B were observed after PF ($P < 0.05$), the latter proportional to the increase in VA flow ($r = 0.908$, $P < 0.05$) and CBV. In contrast, biomarkers of neuronal-axonal damage (neuron-specific enolase, neurofilament light-chain, ubiquitin carboxy-terminal hydrolase L1 and tau) did not change ($P > 0.05$).

DISCUSSION

The present study has identified three important findings. First, while microgravity per se had no demonstrable impact on CBF, a selective albeit modest increase in perfusion to the posterior circulation was observed during the most marked gravitational differential imposed by the micro- to hypergravity transition. Second, this was associated with an elevation in systemic oxidative-nitrosative stress confirmed by a free radical-mediated reduction in vascular NO bioavailability that was detectable 'post-flight' upon return to normogravity. Third, despite no molecular evidence for structural neuronal-axonal damage, the selective elevations in GFAP and S100B post-flight were taken to reflect ongoing, albeit mild disruption of the BBB, changes that were proportional to the hypergravity-induced increase in posterior CBF.

CONCLUSIONS

Collectively, these findings are the first to suggest that PF-induced gravitational transitions may promote minor BBB disruption, potentially related to the combined effects of haemodynamic (posterior cerebral hyperperfusion) and molecular (systemic oxidative-nitrosative) stress. Future studies employing pharmacological manipulation of free radicals combined with contrast-enhanced MRI are encouraged to confirm our preliminary findings. While we appreciate that PF is an entirely different stimulus dominated by hypergravity, our findings may nonetheless provide some background for understanding the long-term health risks associated with the prolonged microgravity of deep spaceflight given that increased BBB permeability directly impacts neuronal function, eliciting or accelerating neurological sequelae and brain disease.

16:15 Ivan Ponomarev – "Effect of 5-day dry immersion on viscoelastic and contractile properties of the lower extremities muscles in women"

EFFECT OF 5-DAY DRY IMMERSION ON VISCOELASTIC AND CONTRACTILE PROPERTIES OF THE LOWER EXTREMITIES MUSCLES IN WOMEN

I. I. Ponomarev[1], A. A. Saveko[1], M. P. Bekreneva[1], and E. S. Tomilovskaya[1]
[1] Institute of Biomedical Problems of the Russian Academy of Sciences; ponom.96@mail.ru

INTRODUCTION

Microgravity has a significant impact on the human body, including the musculoskeletal system. For example, the strength and mass of skeletal muscles decrease by 8% in the soleus muscle during 7 days of space flight (SF) (LeBlanc A. et al., 1995; Grigoryeva L.S. and Kozlovskaya I.B., 1987) and continue to decrease with long-term stay in microgravity (Adams et al., 2003). Dry immersion (DI) conditions leads to the development of similar to SF structural and functional changes in postural muscles due to a decrease in their contractile activity (Shenkman B.S. et. al., 2004) - type I fibers (slow) are more sensitive to changes compared to fast type II fibers. It has also been shown that a decrease in support and motion loads causes a cascade of reflex reactions, which accompanied by a drop in the tone of postural (the most gravity-dependent, slow) muscles (Kozlovskaya I.B. et al., 2007). Currently, DI model is a widely used ground-based model of the physiological effects of microgravity (Tomilovskaya E.S. et al., 2019), however, most of the data using DI was obtained with the participation of men, since women did not participate in such experiments until 2020 (Tomilovskaya E.S. et al., 2021). The study of the DI effects on viscoelastic and contractile properties of the lower extremities muscles in the female group is of particular interest in the question of the gender specificities in the reactions of the musculoskeletal system to support unloading.

METHODS

The study involved 16 healthy women of reproductive age (from 26 to 30 years) with a natural menstrual cycle. The support unloading was created using 5-days DI model (Shul'zhenko E.B. and Vill-Villiams I.F., 1976). DI exposure started in the first half of the cycle for all participants - on 10-15 day of the cycle. Investigation of the contractile and viscoelastic properties of the lower extremities' muscles (mm. biceps femoris, rectus femoris, tibialis anterior, soleus, gastrocnemius) were carried out in the first half of the day; twice before the start of DI exposure and twice after its completion – on the 1st and 2nd days of recovery. The MyotonPro surface palpation method (Myoton AS, Estonia) was used to assess Stiffness in N/m by processing the frequency of the muscle response on the three short mechanical pulses (15 ms) of stable force (0.4 N). The high frequency of the tissue response corresponds to the high tension and stiffness of this tissue – i.e. high muscle tone (Nguyen A.P. et.al, 2022). The tensomyography method (TMG-BMC Ltd.; Ljubljana, Slovenia) was used to assess the morpho-functional potential of muscles and their contractile properties, which we evaluated by the characteristics of radial muscle belly displacement under electrically induced isometric contraction – Maximal displacement amplitude (Dm) and Contraction time (Tc; Šimunič B., 2012). The data were analyzed using repeated measures ANOVA (GraphPad Prism version 8).

RESULTS

The most remarkable and stable changes were observed in the most gravity-dependent muscle – the soleus muscle. If, before DI exposure, the Tc of this muscle was 60.68±1.06 ms, then on the first day after DI it significantly decreased by 6.02±2.91 ms ($p < 0.05$; Fig. 1A). At the same time, Dm did not change (Fig. 1B) and Stiffness significantly dropped by 31.98±10.74 N/m ($p < 0.05$; Fig. 1C). Interestingly, a full recovery of these characteristics was not observed even on the 2nd day after DI. Thus, the results demonstrate that after DI the soleus muscle began to respond to an electrical stimulus faster, while the tone of this muscle decreased. This phenomenon is consistent with current ideas about the gravitational mechanisms of the tonic muscle activity (Kozlovskaya I.B. et al., 2007), and also confirms the similarity of physiological reactions to support unloading in women and men.

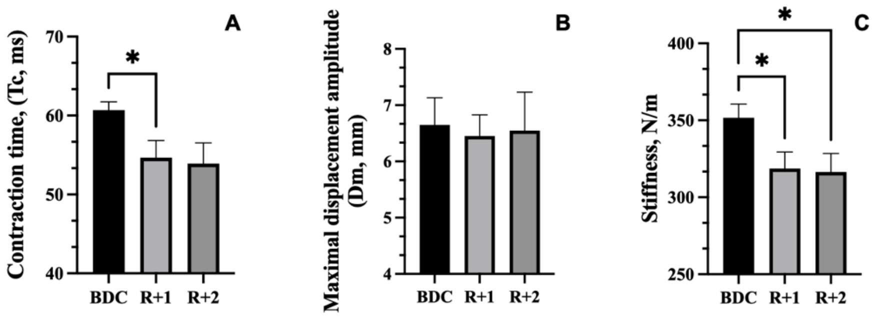


***Figure 1**. Change in the evoked responses of the soleus muscle. A – Contraction time (Tc), B – Maximal displacement amplitude (Dm), C – Stiffness. BDC – data collected before DI exposure. R+1-2 – post-DI results during 1st and 2nd days of recovery. M±SEM, * – significant changes compared to the initial values, p<0.05.*

FUNDING

The study was supported by a grant from the Russian Science Foundation № 19-15-00435P, https://rscf.ru/en/project/19-15-00435/.

REFERENCES

LeBlanc A., Rowe R., Schneider V., Evans H. et al., 1995, Aviat Space Environ Med, 66, 1151-1154.

Grigoryeva L.S. and Kozlovskaya I.B., 1987, Kosm Biol Aviakosm Med, 21, 27-30.

Adams G.R., Caiozzo V.J. and Baldwin K.M., 2003, J. Appl Physiol, 95, 2185-2201.

Shenkman B.S. and Kozlovskaya I.B., 2019, Front Physiol, 10, 187.

Kozlovskaya I.B., Sayenko I.V., Sayenko D.G., Miller T.F. et al., 2007, Acta Astronaut, 60, 285-294.

Tomilovskaya E., Shigueva T., Sayenko D., Rukavishnikov I. et al., 2019, Front Physiol, 10, 284.

Tomilovskaya E., Amirova L., Nosikova I., Rukavishnikov I. et al., 2021, Front. Physiol, 12, 661959.

Shul'zhenko E.B. and Vill-Villiams I.F., 1976, Kosm Biol Aviakosm Med, 10, 82-84.

Nguyen A.P., Detrembleur C., Fisette P., Selves C., et al., 2022, Front. Sports. Act Living, 21(4), 797975.

Šimunič B., 2012, J. Electromyogr Kinesiol, 22, 527-530.

16:30 Alexandra Ryabova – “Effects of 5-day dry immersion on characteristics of shin muscles motor evoked potentials in women”

Effects of 5-day Dry Immersion on characteristics of shin muscles motor evoked potentials in women

A. M. Riabova[1], I. N. Nosikova[1], V. V. Kitov[1] and E. S. Tomilovskaya[1]
[1]Institute of Biomedical Problems of Russian Academy of Sciences, Moscow, Russia; aleksriabova@yandex.ru

Currently, as women astronauts participate in space flights more frequently, the research on how the female body adapts to microgravity conditions is required. One of the important topics in the field of gravitational physiology is the study of sensorimotor system reactions to the factors of space flight, such as weightlessness, hypodynamia, and support withdrawal. It is know that exposure to microgravity leads to the development of hypogravitational hyperreflexia, which manifests as a decrease in thresholds and an increase in amplitudes of motor responses (Kozlovskaya et al., 1988). With that in mind, we conducted the experiment using Dry Immersion (DI) as a ground-based model of the main physiological effects of space flight and studied changes in motor evoked potentials (MEPs) of the lower leg gravity-dependent muscles (mm. gastrocnemius and soleus) in women after 5-day exposure to DI. Sixteen healthy female volunteers participated in this study. Motor responses were evoked with transcranial magnetic stimulation (MS), which was delivered to the area of cortical motor projections of the right lower leg muscles, and with trans-spinal MS, which was applied to the lumbar spine at the level of L5–S1 segments. MEPs to transcranial and trans-spinal MS were referred to as “cortical MEPs” and “spinal MEPs,” respectively. MEP characteristics that were analyzed included thresholds, amplitudes, and latencies. We observed the decrease in both spinal and cortical MEP thresholds and the increase in spinal MEP amplitudes in the majority of the group after DI. This finding reflects the phenomenon of the hypogravitational hyperreflexia (Kozlovskaya et al., 1988) and suggests that hyperreflexia develops on both spinal and cortical levels. In addition, these results generally are consisted with the data from a similar experiment conducted with the participation of male volunteers (Nosikova et al., 2021a). In the present study, we also showed the increase in spinal MEP latencies and, by contrast, the decrease in cortical MEP latencies after DI; therefore, central motor conduction time decreased, which means that corticospinal conductibility increased, by the end of immersion exposure. MEP amplitudes in general varied between subjects and experimental sessions, more so in case of transcranial MS, which could be explained by a number of factors, such as shift in the functional state of the subjects and possibly even hormonal fluctuations during menstrual cycle. A similar amplitudes variability was previously observed in women after 3-day DI (Nosikova et al., 2021b). Further research is warranted to identify the origin of the discovered phenomena and reveal the mechanisms behind them.

The study was supported by a grant from the Russian Science Foundation № 19-15-00435, https://rscf.ru/en/project/19-15-00435/.

REFERENCES

Kozlovskaya I., Dmitrieva I., Grigorieva L. et al., 1988, Stance and Motion (New York: Plenum), 37-48.

Nosikova I.N., Ryabova A.M., Dmitrieva L.E. et al., 2021a, Human Physiology, 47, 282-288.

Nosikova I., Riabova A., Amirova L. et al, 2021b, Front Hum Neurosci, 15, 753259.

16:45 Maren Dreiner – "Sex-specific cartilage biomarker response to 5 days of dry immersion"

Sex-specific cartilage biomarker response to 5 days of dry immersion

M. Dreiner[1], E.-T. Godonou[2,3], A-M. Liphardt[2,3] and A. Niehoff[1,4]

[1]Institute of Biomechanics and Orthopaedics, German Sport University Cologne, Cologne, Germany (m.dreiner@dshs-koeln.de); [2]Department of Internal Medicine 3, Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nuremberg & University Hospital Erlangen, Erlangen, Germany (elie-tino.godonou@uk-erlangen.de); [3]German Center for Immunotherapy (DZI), Friedrich-Alexander-University Erlangen-Nuremberg & University Hospital Erlangen, Erlangen, Germany (anna-maria.liphardt@uk-erlangen.de); [4]Cologne Center for Musculoskeletal Biomechanics (CCMB), Faculty of Medicine, University of Cologne, Cologne, Germany (niehoff@dshs-koeln.de)

INTRODUCTION

Human spaceflight is associated with the deconditioning of multiple physiological systems due to the absence of Earth gravity (Shelhamer M. et al., 2020). To unravel the underlying mechanisms of the adaptation to microgravity, ground-based models have been developed, with the objective of simulating physiological effects of spaceflight on the human body on Earth, primarily by immobilization and unloading. Commonly applied ground-based models are dry immersion (DI) or -6° head-down-tilt bed rest (HDT). Cartilage remodeling can be evaluated by quantifying metabolites, so called biomarkers, generated during cartilage synthesis and degradation (Lotz et al., 2013) and microgravity can result in alterations in biomarker assessed cartilage metabolism, that have the potential to initiate catabolic processes (Niehoff et al., 2016). Type II collagen is a major component of articular cartilage and the serum biomarkers C2C and CPII indicate degradation and synthesis of this protein. Previous studies indicate changes in cartilage biomarker concentrations in male study participants exposed to HDT (Liphardt et al., 2018; Liphardt et al., 2020). However, it is unknown if the impact of immobilization by DI on serum biomarker concentration is different in women compared to men. Thereto, the purpose of the study was to investigate sex-dependent effects of five days of immobilization by DI on serum concentrations of C2C and CPII.

METHODS

The study was part of a research program of the European Space Agency (ESA) and was carried out at the Institute de Médecine et de Physiologie Spatiales (MEDES) in Toulouse, France. Eighteen healthy female participants (29±5 years, 165±6 cm, 59±6 kg) and nineteen male participants (28±4 years, 177±4 cm, 72±7 kg) were subjected to four days of baseline data collection (BDC), five days of DI, and three days of recovery (R). During DI, participants were fully immersed in a tub filled with water for 24 hours per day, while being protected against direct water contact by a specialized elastic fabric. This immobilization model limits movement, in particular of the lower extremities and reduces ground support. Fasting, venous blood samples were taken on four days at 7 am: BDC-26h, DI46h, DI118h and R+46h (Figure 1). We analyzed serum concentrations of C2C and CPII with commercially available ELISAs (IBEX Pharmaceuticals Inc., Montreal, Québec, Canada). A two-factor repeated measures ANOVA, followed by a LSD post-hoc test, were carried out to detected significant differences over time and between sexes.

RESULTS

The serum C2C concentration was significantly different between sexes at all time points ($p=0.006$), with higher concentrations for male participants compared to females (e.g., BDC-26h: 184.92±43.85 ng/mL (female), 223.33±47.22 ng/mL (male), $p=0.015$). The serum C2C concentration of female participants increased significantly ($p=0.013$) from BDC-26h to DI46h by +10% and by +7% from DI46h to DI118h ($p=0.02$), resulting in an overall increase of +17% compared to baseline (DI118h vs. BDC-26h, $p=0.002$). During recovery the serum C2C concentration remained elevated compared to baseline (R+46h vs. BDC-26h, $p=0.006$). In male participant, progression of serum C2C concentration was similar, starting with a significant increase ($p=0.012$) from BDC-26h to DI46h by +10% and an additional significant increase ($p=0.029$) of +9% from DI46h to DI118h, causing an overall increase of +19% compared to baseline (DI118h vs. BDC-26h, $p<0.001$). Surprisingly, serum C2C concentration decreased by -9% during recovery in male participants (R+46h vs. DI118h, $p=0.025$), but values were still significantly higher compared to baseline (R+46h vs. BDC-26h, $p<0.001$).

The serum CPII concentration was not significantly different between sexes at all time points (p=0.853), thereto female and male concentration were considered together (e.g., BDC-26h: 1434.50±473.74 ng/mL (female), 1421.51±347.44 ng/mL (male), p=0.924). The serum CPII concentration, mean of both sexes, increased significantly (p=0.004) from BDC-26h to DI46h by +8%. Concentrations further increase (p=0.005) by +12% from DI46h to DI118h, resulting in an overall increase of +20% compared to baseline (DI118h vs. BDC-26h, p<0.001). During recovery the serum CPII concentration remained elevated and was still significantly increased compared to baseline (R+46h vs. BDC-26h, p<0.001).

DISCUSSION

The C2C biomarker concentrations were significantly higher in male than in female participants, which might be caused by higher overall cartilage volume in men compared to women (Otterness I.G. and Eckstein F., 2007). Both, serum C2C and CPII concentrations increased significantly after only 46 hours of DI and increased further until the end of DI (DI118h). These findings indicate that five days of DI elicits substantial changes in the assessed cartilage biomarkers. However, the observation that both degradation and synthesis biomarkers of type II collagen were increased may imply a rescue mechanism to balance catabolic and anabolic processes.
Nevertheless, both biomarkers stayed elevated even after 46 hours of recovery. These results suggest that even a brief period of immobilization and unloading can have sustained consequences on articular cartilage metabolism in both healthy females and males. This emphasizes the importance of implementing effective countermeasures to preserve cartilage function for long-duration space missions.

REFERENCES

Shelhamer M., Bloomberg J., LeBlanc A., et al., 2020, *NPJ Microgravity 6*, 5.
Niehoff A., Brüggemann G.-P., Zaucke F., et al., 2016, *Osteoarthritis Cartilage*, 24(1), S144-S145.
Lotz, M., Martel-Pelletier J., Christiansen C., et al., 2013, *Ann Rheum Dis*, 72(11), 1756-1763.
Liphardt A.-M., Mündermann A., Andriacchi T.P., et al., 2018, *J. Orthop. Res.*, 36(5), 1465-1471.
Liphardt A.-M., Mündermann A., Heer M., et al., 2020, *J. Orthop. Res.*, 38(11), 2373-2382.
Otterness I.G. and Eckstein F., 2007, *Osteoarthritis Cartilage*, 15(6), 666-672.

Funding: Federal Ministry of Economic Affairs and Climate Action, Germany (DLR 50WB2021, DLR 50WB2022).

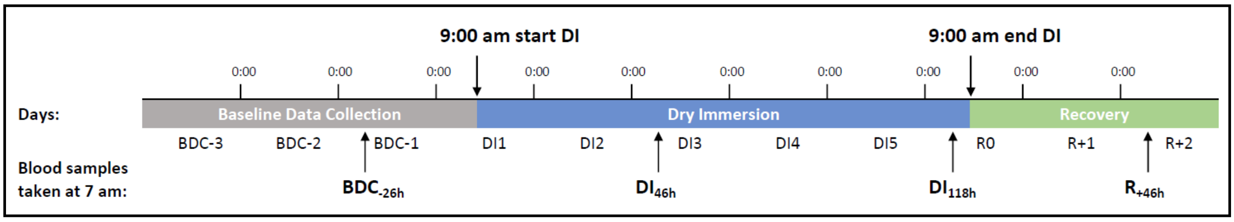


Figure 1: Study timeline to illustrate blood sampling time points. BDC: Baseline Data Collection (grey area), DI: Dry Immersion (blue area), R: Recovery (green area)

17:00 Alessandra Bosutti – "Impact of 60-bed rest and human artificial gravity on serum oxidative stress biomarkers and skeletal muscle protein oxidative modifications"

Impact of 60-day bed rest and human artificial gravity on serum oxidative stress biomarkers and skeletal muscle protein oxidative modifications

Alessandra Bosutti[1], Bergita Ganse[2], Ifigeneia Giakoumaki[3], Edwin Mulder[4], Jörn Rittweger[4], Moritz Eggelbusch[5], Rob C.I. Wüst[5], Paul Hendrickse[6] and Hans Degens[3]

[1]University of Trieste, Department of Life Sciences, Via A. Fleming 22, 34127, Trieste, Italy (e-mail:bosutti@units.it)
[2]Saarland University, Werner Siemens Foundation Endowed Chair for Innovative Implant Development, Homburg, Germany (e-mail: Bergita.Ganse@uks.eu)
[3]Manchester Metropolitan University, Department of Life Sciences, Manchester, UK (e-mail: I. Giakoumaki: ifigeneiagd@hotmail.com; H. Degens: h.degens@mmu.ac.uk)
[4]German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne, Germany (e-mails:E. Mulder: Edwin.Mulder@dlr.de; J. Rittweger: Joern.Rittweger@dlr.de)
[5]Vrije Universiteit Amsterdam, Faculty of Behavioural and Movement Sciences, The Netherlands (e-mail:M. Eggelbusch: m.eggelbusch@amsterdamumc.nl; R. Wüst: r.wust@vu.nl)
[6]University of Leeds, School of Biomedical Sciences, Faculty of Biological Sciences, UK (e:mail: p.w.hendrickse@leeds.ac.uk)

Introduction

Microgravity during space flight significantly affects the muscular system, resulting in the gradual loss of muscle mass and strength in astronauts. Similar changes occur during prolonged period of immobilisation in hospitalized patients or in elderly people. Oxidative modifications of myofibrillar proteins generated by oxidative stress contribute to the observed reduced force and power generating capacity of the muscle. Our previous data from the 60-day Artificial Gravity Bed Rest – European Space Agency (AGBRESA) study did show that 60 days of bed rest induced muscle fibre atrophy (P. Hendrickse et al, 2022) and negatively affected specific tension and the maximum velocity of unloaded shortening of muscle fibres (B. Ganse et al, NASA HRP IWS Conference 2020) irrespective of artificial gravity (AG). Here, we studied how bed rest (and artificial gravity as countermeasure) affected markers of systemic oxidative stress, skeletal muscle protein carbonylation and protein breakdown, as part of the 60-day AGBRESA bed rest (BR) study. Additionally, we quantified the relative amounts of circulating stress-related microRNAs and their relation to oxidative stress and fibre atrophy of vastus lateralis and soleus muscles (P. Hendrickse et al, 2022).

Methods

Twenty-four healthy subjects (16 men, 8 women, 33 ± 9 years) participated in the 60-day AGBRESA study and were randomly assigned to a control group (BR only), a continuous centrifugation group (cAG), or an intermittent centrifugation group (iAG) (n=8 in each group) to assess the efficacy of daily 30-min continuous or intermittent human centrifugation (AG) on attenuating adverse effects of bed rest-induced disuse. Blood samples and vastus lateralis (VL) muscle biopsies were collected before (BDC-1), and on day 6 (HDT6) and day 57 (HDT57) of bed rest. Concentrations of circulating 8-iso-PGF2α (marker of oxidative stress; n=72) and skeletal muscle protein carbonyls (BDC-1, n=21; HDT6, n=20; HDT57, n=17) were assessed by ELISAs. Urine 3-methylhistidine (3-MH; protein breakdown) data were obtained as part of the international standard measures (n=72). Serum miRNAs (myo-miRs 133a-3p and -206, miR-126-3p and miR-21-5p), which are involved in the modulation of oxidative stress and myogenic processes, were quantified by Real-Time PCR and ΔCT (2^- ΔCT) method (n=72). Significance was assessed via two-way ANOVA (mixed effects model with Dunnett's or Šidák's correction for multiple comparisons). Spearman linear regression analyses were also performed. Statistical significance was set at $P<0.05$.

Results

Bed rest plus daily centrifugation led to an increase in urine 3-MH levels (iAG+cAG, pooled: HDT57 vs BDC-1, $P<0.05$), while no significant alterations were observed in the control group (only bed rest). Bed rest or daily centrifugation (iAG+cAG, pooled) did not significantly affect the 8-iso-PGF2α levels, nor the muscle carbonyl contents in VL muscle biopsies ($P>0.05$). However, following bed rest (HDT57) the carbonylation levels were significantly higher in the cAG group (P=0.017) compared to BDC-1, and the fold change (% on BDC-1) of 8-iso-PGF2α levels was lower (P=0.018) in iAG group compared to the cAG group, indicating cAG had a larger impact on systemic oxidative stress than iAG. Bed rest and bed rest plus daily centrifugation (iAG+cAG, pooled groups) significantly decreased the circulating levels of miR21-5p (P=0.045) and myo-miR206 (P=0.0094), respectively. Myo-miR206 and miR133 were (P<0.05) less abundant in the iAG group at both the HDT6 and HDT57 time points, suggesting a possible impact on skeletal muscle regeneration cascade. Overall, we did not find any significant correlation between 8-iso-PGF2α and carbonyl levels. A significant positive correlation between 8-iso-PGF2α and miR126 (r=0.785) and 21-5p (r=0.952) was seen at HDT57 in the iAG group, suggesting a possible role of these microRNAs in modulating oxidative stress under intermittent bouts of AG exposure. An overall positive relationship between miR21-5p, miR126 and miR133a-3p with average of FCSA of soleus muscle (P<0.01) in all pooled conditions (BR+iAG+cAG groups) was found at the end of bed rest, which may indicate an involvement of these miRNAs in the regulation of muscle mass in bed rest.

Conclusions

Our results indicate that the three study groups showed a different response to 60 days of bed rest with regard to stress biomarkers and serum miRNAs analysed. While bed rest alone did not significantly affect oxidative stress, the centrifugation-induced stress effect was less pronounced with the iAG protocol compared to the continuous centrifugation. The continuous centrifugation was not suitable to mitigate alterations in muscle protein carbonylation levels. Serum miRNAs could be useful biomarkers to study systemic and muscle adaptation following prolonged period of disuse, in simulated or real microgravity.

Acknowledgements

Italian Space Agency (MIAG project ASI n.2021-13-U.0), German Research Foundation (DFG, grant n. GA 2420/1-1), German Aerospace Center (DLR, grant n. 50WB1928), UK Space Agency (ST/S0001735/1 & ST/T00066X/1), European Space Agency (ESA, 16 16ESA AGBR-0013, contract n. 4000113871/15/NL/PG), and National Aeronautics and Space Administration (NASA, contract n. 80JSC018P0078).

REFERENCES

P. Hendrickse et al., RCI Wüst, B. Ganse, I. Giakoumaki, J. Rittweger, A. Bosutti, H. Degens., 2022, J Cachexia Sarcopenia Muscle. 2022, 13(6), 2712-2723.
B. Ganse, RCI Wüst, I. Giakoumaki, A. Bosutti, J. Rittweger, M. Drey, H. Degens A.,2020, NASA HRP IWS Conference

17:15 Peter Fernandez – “Insights and Conundrums Involving Spaceflight and Bone - An 18-Month Perspective”

Insights and Conundrums Involving Spaceflight and Bone- An 18-Month Perspective

P.Fernandez[1], K.Gordienko[2], H.Locrelle[1], M.Linossier[1], P.Lau[3], J.Rittweger[3], G.Vassilieva[2], L.Vico[1]

[1]Université Jean Monnet Saint-Étienne, Mines Saint Etienne, INSERM, SAINBIOSE U1059, F-42023, Saint-Etienne, France, peter.fernandez@kcl.ac.uk, herve.locrelle@chu-st-etienne.fr, linossier@univ-st-etienne.fr, vico@univ-st-etienne.fr
[2]Russian Federation State Scientific Centre - Institute of Biomedical Problems of the Russian Academy of Sciences, Russia, k.vl.gordienko@gmail.com, galvassilieva@mail.ru
[3]Institute of Aerospace Medicine, German Aerospace Centre, Germany, patrick.lau@dlr.de , joern.rittweger@dlr.de

Introduction

Prolonged exposure to the microgravity environment is known to elevate the rate of bone resorption in astronauts. (Vico L. et al., 2017; Gabel L. et al.,2021). Despite various forms of exercise countermeasures to regulate this rate of loss, the effect on bone continues to pose a significant challenge for both the present and future of long-duration spaceflight. While international space agencies continue to work towards developing a lunar habitat and eventually the colonisation of Mars, the unresolved consequences of spaceflight physiology on bone need to be understood so that effective countermeasures can be developed. For this reason, a longitudinal study (EDOS-2) examining the change in bone geometry and bone architecture is underway with a focus on determining reversibility, the quantity of deterioration, and identifying factors associated with intra-individual responses to spaceflight.

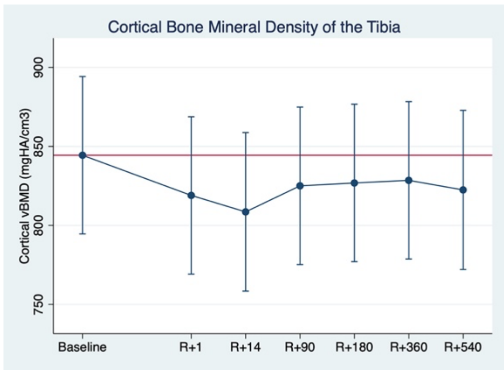


Figure 7: Cortical vBMD changes over time at the distal tibia. Red line represents the reference line to baseline values.

Methods

12 male participants (47.33±5.96 years), eight of whom were repeat flyers, had their volumetric BMD and microarchitecture parameters measured at the non-dominant distal radius and tibia using HRpQCT imaging (Xtreme CT, SCANCO Medical). Pre-flight measurements were taken at (1 or 2 months pre-flight, or at both time points) for a baseline reference and followed up for 1.5 years after landing. Post-flight measurements were obtained at the following time points: 1 day (R+1); 14 days (R+14), 3 months (R+90); 6 months (R+180); 12 months (R+360); and 18 months (R+540).

Results & Conclusion

Preliminary results demonstrated a decrease in cortical volumetric BMD from baseline values in both the distal radius and tibia upon return, with more pronounced differences in the tibia (Fig. 1). Multiple flights negatively impacted cortical bone volume at the tibia and demonstrated lack of recovery over time (Fig.2). In addition, these values were also below pre-flight levels even 18-months later (Fig. 1). Cortical porosity levels followed a similar trend with elevated levels upon return and no signs of recovery to pre-flight values. These preliminary results are consistent with a one-year longitudinal follow-up (Vico L. et al., 2017; Gabel L. et al.,2021) and further highlight that a return to baseline levels is currently not observed even after 18-months.

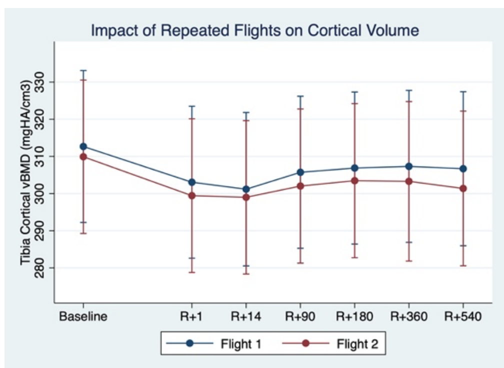


Figure 2: Cortical vBMD changes at the distal tibia for multiple flights.

REFERENCES

L.Vico., (2017), JBMR, Vol. 32, pp 2010–2021

L.Gabel., (2021) BJSM, Vol 56, pp 196–203

17:30 Anna-Maria Liphardt – "Effect of microgravity during long-duration spaceflight on transverse relaxation times of the femorotibial cartilage (MRI T2) - the ESA Cartilage Health study"

Effect of microgravity during long-duration spaceflight on transverse relaxation times of the femorotibial cartilage (MRI T2) – the ESA Cartilage Health study

A-M. Liphardt[1,2], E-T. Godonou[1,2], A. Mündermann[3], M. Dreiner[4], F. Zaucke[5], W. Wirth[6,7], A. Wisser[6,7], S. Maschek[6,7], F. Eckstein[6,7], G. Schett[1,2], A. Niehoff[4,8]

[1]Dep. of Internal Medicine 3 – Rheumatology & Immunology (Anna-Maria.Liphardt@uk-erlangen.de, Elie-Tino.Godonou@uk-erlangen.de, Georg.Schett@uk-erlangen.de), Universitätsklinikum Erlangen & Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Erlangen, Germany [2]Deutsches Zentrum für Immuntherapie (DZI), Universitätsklinikum Erlangen & FAU Erlangen-Nürnberg, Erlangen, Germany [3]Dep. of Orthopaedics and Traumatology, University Hospital Basel, Dep. Of Biomedical Engineering, University of Basel (annegret.muendermann@unibas.ch), Basel Switzerland, [4]Inst. of Biomechanics & Orthopaedics(m.dreiner@dshs-koeln.de), German Sport University Cologne, Köln, Germany [5]Research Unit for Osteoarthritis, Dep. ofOrthopaedics (Friedrichsheim) (Frank.Zaucke@kgu.de), University Hospital Frankfurt, Goethe University, Frankfurt/Main, Germany, [6]Chondrometrics GmbH (eckstein@chondrometrics.de, maschek@chondrometrics.de, wirth@chondrometrics.de, wisser@chondrometrics.de), Freilassing, Germany, [7]Paracelsus Medizinische Privatuniversität, Salzburg, Austria, [8]Cologne Center for Musculoskeletal Biomechanics (CCMB), Medical Faculty (Niehoff@dshs-koeln.de), University of Cologne, Köln, Germany.

BACKGROUND

Immobilization alters the morphological and biomechanical characteristics of synovial joint components, including cartilage. Findings from spaceflight analogue model research, such as bed rest studies, indicate that unloading exposure is associated with changes in cartilage metabolism (Liphardt et al., 2009, 2018). This study aims to investigate the effect of microgravity during long-duration spaceflight on knee cartilage quality by magnetic resonance imaging (MRI) transverse relaxation time (T2) in medial and lateral femorotibial cartilage of crew members of the International Space Station (ISS).

METHODS

For the ESA (European Space Agency)-funded ESA CARTILAGE HEALTH study, 12 USOS (United States Operational Segment) crew members, with a mission length of 4 – 6 months on the ISS, were recruited for this study after giving written informed consent. A 2DMESE (multiple echo spin echo) T2 mapping sequence (TR = 1500 ms, TE = 10, 20, 30, 40, 50, 60, 70 ms, flip angle = 180°, field of view = 159 mm, matrix = 269 × 384 pixels, slice thickness = 3 mm, in-plane resolution = 0.31 mm^2) of the knee joint was obtained pre-flight (launch (L)-60) and at three time points after landing (return (R)+7 days, +30 days and +365 days) after a 45 minutes supine rest using a 3 tesla MRI scanner (Siemens Healthineers, Erlangen, Germany or Philipps Archieva, Hamburg, Germany). Mean T2 time (miliseconds, ms) was computed for the deep and superficial cartilage layers for all medial and lateral femorotibial cartilage compartments. Linear Mixed-Effects Models were used to detect significant changes in the MRI T2 of knee articular cartilage in response to 4 -6 months on the ISS, considering time points as predictors.

RESULTS

Baseline (pre-flight), T2 relaxation times are summarized in Table 1. T2 relaxation times of the medial tibia superficial layer ($p=0.024$) was affected by time points. Mean T2 was increased after landing for the medial tibia superficial layer (3.1% at R+7 ($\beta=0.76$, 95% CI: 0.11 – 1.42) $p=0.024$; 3% at R+30 ($\beta=0.73$, 95% CI: 0.05 – 1.41) $p=0.035$) compared to preflight values. No effect for T2 mean was detected for superficial layer of the lateral tibia, medial and lateral femur and for the deep layer of all compartments.

Table 1: Baseline T2 relaxation times at pre-flight in milliseconds (ms) reported as means and standard deviation (SD) for 12 crew members.

Compartment			T2 relaxation time (ms) mean(SD)
Femur	medial	deep layer T2	21.3(3.5)
		superficial T2	33.7(5.7)
	lateral	deep layer T2	21.9(2.8)
		superficial layer T2	32.8(3.6)
Tibia	medial	deep layer T2	16.2(1.9)
		superficial layer T2	22.8(3.0)
	lateral	deep layer T2	18.1(2.4)
		superficial layer T2	29.4(3.4)

CONCLUSION

Immobilization by microgravity during 4 – 6 months spaceflight results in regional increases in T2 relaxation times of knee articular cartilage. Greater T2 relaxation times for the medial tibia superficial layer may reflect an increase of relative water content that can be indicative of tissue matrix perturbation. These results support our previous findings of a shift of cartilage metabolism towards cartilage degradation in response to immobilization measured by urine and serum biomarkers. Further research is needed to better understand the role of mission duration for the magnitude of tissue matrix perturbations and to identify countermeasures against these changes.

REFERENCES

Liphardt et al. 2009, Dec;17(12):1598-603; Liphardt et al. 2018, J Orthop Res. May;36(5):1465-1471.

Acknowledgements

This work was funded by the Federal Ministry for Economic Affairs and Climate Action, Germany & German Aerospace Centre (DLR e.V.) – German Space Agency (Project #: DLR 50WB0913, DLR 50WB1217, DLR 50WB1719, DLR 50WB1520, DLR 50WB2021, DLR 50WB2022) and the European Space Agency and partially supported by the German Research Foundation (DFG) under Grant CRC 1483 EmpkinS - Project-ID 442419336.

11:30 Roxy Fournier – "Increased cerebrovascular tone following long duration spaceflight is associated with increased pulsatility and reduced conductance"

Increased cerebrovascular tone following long duration spaceflight is associated with increased pulsatility and reduced conductance

R. Fournier[1], R.L. Hughson[1], J.K. Shoemaker[2], P. Arbeille[3], A.D. Robertson[1*]
[1]Schlegel-UW Research Institute for Aging, Waterloo, ON, Canada
[2] School of Kinesiology, University of Western Ontario, London, ON, Canada
[3]CERCOM-UMPS, Faculté de Médecine Université de Tours, Tours, France
*andrew.d.robertson@uwaterloo.ca

INTRODUCTION

Long-duration habitation on the International Space Station (ISS) is associated with a shift of fluid towards the neck and head, and vascular impairment suggestive of central artery stiffening, among other physiological alterations. These changes are consistent with intracranial congestion and hemodynamic pulsatility, which can be deleterious to brain health. While considerable effort is being directed toward spaceflight-associated neuro-ocular syndrome, little evidence exists with respect to altered cerebrovascular regulation that could have bearing on long-term brain health. This study aimed to determine the impact of spaceflight on cerebrovascular pulsatility and compliance.

METHODS

Subjects and study design

Data were collected as part of the Cardiovascular and Cerebrovascular Control on Return from the International Space Station (CCISS) study (2007-2010). This was a repeated measures observational study of 7 astronauts (1 woman) with an average age of 48 ± 4 years. Pre-flight data were collected 36 ± 22 days before launch. Post-flight data were collected within ~4 h (n = 4) or ~36 h of landing (n = 3), depending on the return vehicle (i.e., shuttle vs. Russian Soyuz). Measures were taken during supine rest, as well as two levels of lower body negative pressure (LBNP; -10 and -20 mmHg in sequential order).

Outcome measures

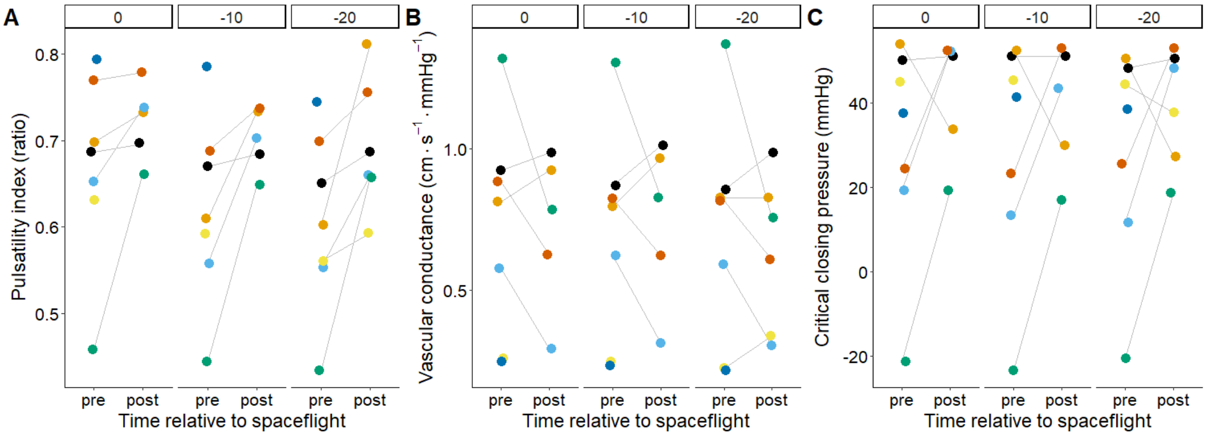
We monitored arterial blood pressure using the finger-cuff volume-clamp technique and reconstructed brachial and aortic pressure waveforms using Beatscope® algorithms (Finapres Medical Systems). We monitored middle cerebral artery (MCAv) and brachial artery blood velocity by Doppler ultrasound. The partial pressure of end-tidal carbon dioxide was also collected. Data segments (30 s) were extracted at rest and after ~90 s of LBNP exposure at each level, and were averaged, beat-by-beat, within each participant. Mean and pulsatile components of pressure and velocity were calculated. Cerebrovascular pulsatility was calculated as the pulsatility index (PI; velocity pulse amplitude divided by mean velocity). Vascular conductance was calculated as mean velocity divided by mean pressure. Cerebrovascular critical closing pressure (CrCP) and resistance area product were calculated using the 2-point method.

Statistics

We used linear mixed-effect models with repeated measures, including hemodynamic characteristics as response variables, session (pre-flight, post-flight) and LBNP level as a fixed-effect factors, and participant number as a random-effect factor to account for between-subject variability.

RESULTS

Both brachial and central mean arterial blood pressures were elevated post-flight ($p < .001$); however, pulse pressures were unchanged ($p > .13$). Neither brachial nor central pressures were affected by LBNP ($p > .83$). Mean MCAv was reduced post-flight ($p = .034$). PI was elevated post-flight ($p < .001$; Figure 1A – each dot represents one individual, with pre- and post-flight values connected by grey lines). The increased post-flight PI was primarily driven by the decrease in mean MCAv, as pulse amplitude did not change ($p = .43$). Corresponding to the contrasting changes in mean velocity and pressure, cerebrovascular conductance was reduced post-flight ($p = .011$; Figure 1B). Furthermore, while resistance area product was unchanged ($p = .28$), post-flight CrCP was elevated ($p = .006$; Figure 1C). In contrast to brachial blood velocity, which was reduced during LBNP ($p = .006$), all cerebrovascular metrics were stable during LBNP both pre- and post-flight ($p > 0.08$).



CONCLUSION

We observed increased CrCP following long duration spaceflight which is indicative of increased vascular tone. This change in tone may contribute to the observation of increased PI within the cerebral circulation despite no change in central pulsatile pressures. Changes in the ability of the cerebral circulation to dampen hemodynamic pulsatility appears to occur in the acute period following spaceflight. Despite this, cerebral hemodynamics remained stable in response to mild orthostatic stress.

Funded by the Canadian Space Agency.

11:45 Emil Rehnberg – "Compartimentalized chip for improved formation of human cardiac spheroids using simulated microgravity"

Compartmentalized chip for improved formation of human cardiac spheroids using simulated microgravity

Emil Rehnberg[1,2,*] , Bjorn Baselet[2], Sarah Baatout[1,2], Lorenzo Moroni[3], Kevin Tabury[2]
[1]Department of Molecular Biotechnology, Ghent University, Belgium, [2]Belgian Nuclear Research Centre, Belgium,
[3]Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands; *Emil.Rehnberg@sckcen.be

Introduction

Future human space exploration is threatened by negative health effects of prolonged exposure to the space environment. Cardiovascular effects have been identified as one of the main health risks, potentially jeopardizing future space missions. Human cardiac three-dimensional (3D) models play a crucial role in understanding the biological effects during long-term missions. Recently, evidence suggesting microgravity (µg) aids pluripotency maintenance and cardiac differentiation of human induced pluripotent stem cells (hIPSCs) has emerged. Furthermore, µg and simulated microgravity (s-µg) can aid to the formation of 3D structures. However, the generation of these structures under s-µg is so far uncontrolled and inconsistent. Therefore we set out to design a device to control the formation of cardiac 3D models using s-µg.

Methods

We prepared spheroids using different compositions of human cardiomyocytes, cardiac fibroblasts and endothelial cells at normal gravity (1g). Through immunofluorescent (IF) imaging and scanning electron microscopy (SEM), we determined the composition which most accurately represented the human heart (which was used for further experiments). Continuing, we designed and fabricated a compartmentalized chip through 3D-printing to control the formation of cardiac spheroids at s-µg using the random positioning machine (RPM). We thereafter compared spheroids formation at 1g and s-µg during 24h and 72h with the help of IF imaging and SEM imaging.

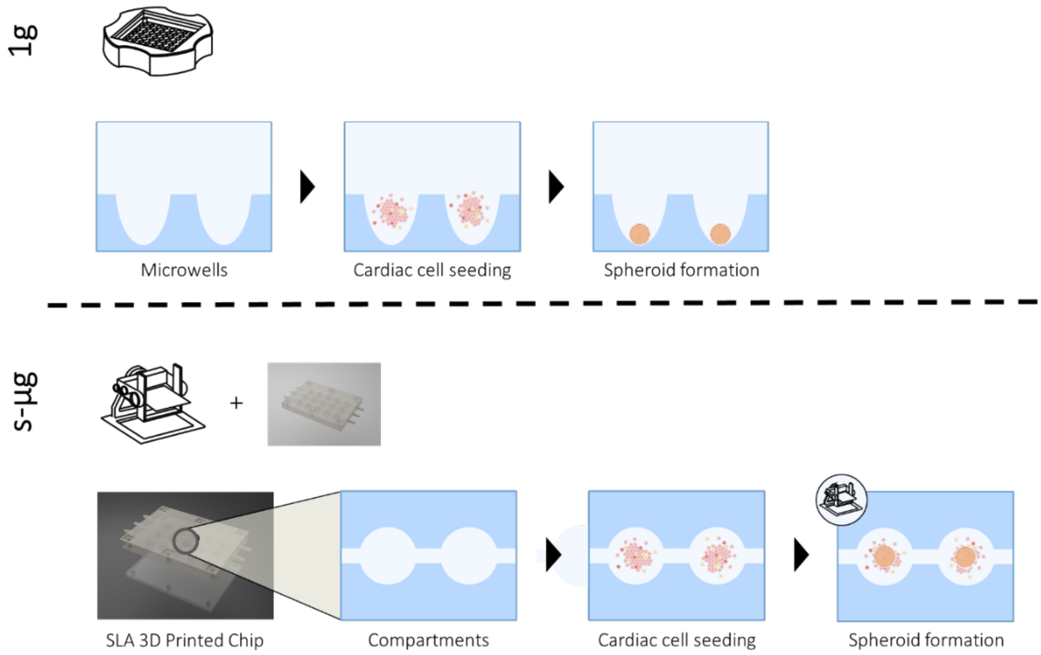


Figure 1. Schematic illustration of spheroid formation using 1g and s-µg.

Results

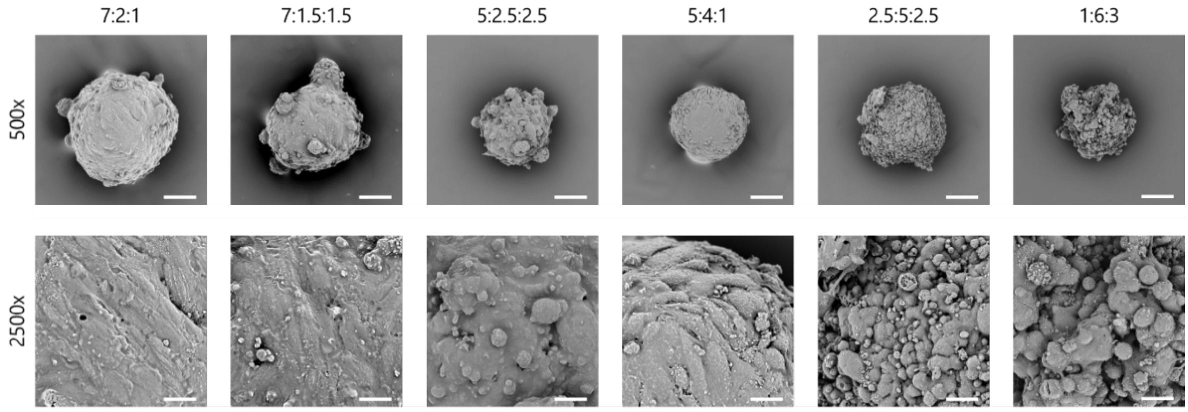


Figure 2. SEM images of spheroids with different compositions of cardiomyocytes (CM), cardiac fibroblasts (CF) and endothelial cells (EC) formed using 1g (CM:CF:EC). Scale bar 100µm (500x), 10µm (2500x).

From IF and SEM images (figure 2), we observed an optimal composition of 70% cardiomyocytes, 20% cardiac fibroblasts and 10% endothelial cells, based on spheroid morphology, size and expression of cell type specific markers. We further designed and fabricated a 3D-printable chip which successfully aided the formation of cardiac spheroids with consistent size and shape, using s-µg. We observed morphological differences between the spheroids formed at 1g and s-µg, using both bright field and SEM images. Furthermore, molecular differences in terms of different cell specific marker expression was observed using IF imaging.

Discussion & Conclusions

Considering the already promising applications of µg in terms of 3D structure formation, generation of these in a controlled and scalable manner will be crucial for tissue engineering applications. In this study, we showed that our 3D-printable chip could be used for the formation of cardiac spheroids with consistent size and shape by means of s-µg using the RPM. Furthermore, we show different expression of cell type specific markers between 1g and s-µg spheroids. In the future, we further aim to investigate the gene expression using RNA sequencing to identify molecular differences between 1g and s-µg spheroids, central to tissue formation, viability and function. All in all, this supports the use of our chip as a proof-of-concept to enable spheroid formation at µg and its advantages here on Earth. The next step is to further increase the complexity of the model through the use of hIPSC derived cells to help develop the astronaut 2.0 of the future.

12:00 Timo Frett – “Comparison between maximal rowing ergometry in artificial gravity and terrestrial conditions"

COMPARISON BETWEEN MAXIMAL ROWING ERGOMETRY IN ARTIFICIAL GRAVITY AND TERRESTRIAL CONDITIONS

T. Frett[1], L. Lecheler[1], M. Arz[1], F. Mommsen[2], J. Breuer[1], & D. A. Green[2, 3, 4]

1 Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany; 2 Space Medicine Team, European Astronaut Centre, European Space Agency, Cologne, Germany. 3 Centre of Human and Applied Physiological Sciences, King’s College London, London, UK. 4 KBRwyle GmbH, Albin-Köbis-Straße 4, Cologne, 51147, Germany.

(timo.frett@dlr.de, michael@arz.de, David.Green@ext.esa.int)

BACKGROUND

Despite daily countermeasures in microgravity (µg) including treadmill running, spaceflight is associated with loss of skeletal integrity, muscle atrophy and reduced exercise capacity. Provision of passive 1g via short-arm human centrifugation (SAHC) appears insufficient to ameliorate physiological deconditioning induced by head down bed rest – an analogue of µg – suggesting concurrent exercise is required. On Earth, rowing ergometry is a popular and effective exercise requiring the coordinated recruitment of upper and lower body musculature, promoting aerobic fitness. As a result, rowing during SAHC is a novel candidate multi-system spaceflight countermeasure. Therefore, we evaluated the feasibility, tolerability, performance and cardiovascular response to seated rowing performance during SAHC centrifugation with 0.5 g at the Center of Mass (CoM), with that performed with an equivalent resultant g vector in 1g.

SPECIFIC AIMS

Aim 1: Develop an exercise system to facilitate rowing during SAHC, and in equivalent resultant g levels on Earth.

Aim 2: Evaluate the feasibility, tolerability and rowing performance during matched resultant 0.5g.

Aim 3: Determine the cardiovascular response to rowing during matched resultant 0.5g.

METHODS

8 men and 4 women (27.2 ± 7.4 years, 179 ± 0.07 cm, 73.7 ± 9.4 kg) who were experienced rowers and recreationally active (at least twice weekly) performed upright seated self-paced rowing ergometry until exhaustion (3 minute 30 Watts steps) during centrifugation with 0.5 g at the CoM, via clockwise or counter-clockwise SAHC, and when in 1g with a 26.6° inclination. Motion sickness, rating of perceived exertion (BORG), rowing performance (maximal wattage and total distance), blood lactate and cardiovascular response (ECG, blood pressure) were compared between conditions.

RESULTS

Participants completed all the sessions with no difference in motion sickness ($p = 0.382$) between conditions. Whilst BORG score increased in both conditions (Earth $p < 0.001$; SAHC; $p < 0.001$) – there was no difference post conditions ($p = 0.764$). Whereas, total distance ($p = 0.003$) and maximal wattage ($p = 0.008$) were significant lower during SAHC rowing, no difference in heart rate ($p = 0.136$) or blood pressure responses (systolic: $p = 0.088$, diastolic: $p = 0.184$) were observed between conditions. Blood lactate concentrations increased over time (($F (9, 103) = 6.002$, $p < 0.0001$) but did not differ between conditions ($F (1, 103) = 4.933$, $p = 0.03$).

DISCUSSION

We demonstrate for the first time that graded rowing to exhaustion during SAHC is feasible and tolerable – with BORG and motion sickness comparable with the matched Earth condition. Whilst, total distance and average wattage during SAHC rowing were lower, the cardiovascular responses – and thus aerobic stimulus were similar. Thus, biomechanical (kinematic and kinetic) and electromyographic evaluation of SAHC rowing is warranted to assess musculoskeletal suitability.

12:15 Carmen Possnig – "Influence of gravitational gradients on cerebral and ocular blood flow"

INFLUENCE OF GRAVITATIONAL GRADIENTS ON CEREBRAL AND OCULAR BLOOD FLOW

Carmen Possnig[1], Hendrik Mugele[1], Ronja Mittermeier[1], Justin S. Lawley[1]

[1] Department of Sport Science, Division of Performance Physiology and Prevention, University of Innsbruck, Austria

Some astronauts return from long-term spaceflights with changes in vision and the structure of the eye. These symptoms have been summarized as Spaceflight-Associated Neuro-Ocular Syndrome (SANS). While their etiology is unclear, an engorgement of the choroid is a prominent feature that develops during both simulated (bed rest) and real microgravity (Lawley et al 2020; Laurie et al. 2017; Shinojima et al. 2012; Mader et al. 2011; Anderson et al. 2016). As the choroid has limited capacity for autoregulation (Nickla & Wallman 2010), gravitational effects on ocular blood flow and/or blood volume may be a key driver of these volume changes. We therefore examined the influence of different gravitational gradients on blood flow in the posterior ciliary artery and contrasted these to changes in brain blood flow, where autoregulation is typically effective. In addition, as low-level lower body negative pressure (LBNP) attenuates choroid engorgement associated with short-term bed rest, we examined if -20 mmHg LBNP reduces posterior ciliary artery velocity, and to what extent relative to normal gravitational gradients on Earth. We hypothesized that blood flow to the eye, as opposed to blood flow to the brain, is more sensitive to changes in gravitational gradients due to changes in perfusion pressure and/or cephalad fluid shifts, and is reduced by LBNP.

Methods

To test our hypotheses, we performed 2 studies. Firstly, 11 men and 3 women spent 1 hour in 4 different body positions: 1) seated upright, 2) supine, 3) lateral, and 4) prone. We assessed blood flow in the internal carotid artery and vertebral artery, and posterior ciliary artery velocity via ultrasonography. In our second study, participants (3 men and 2 women so far) spent 20 minutes in a supine position, followed by an additional 10 minutes with -20mmHg LNBP applied. Posterior ciliary artery velocity was assessed via ultrasonography alongside beat-by-beat blood pressure (Finometer).

Results

Study 1. Compared to the supine position, total cerebral blood flow (CBF) was reduced while sitting upright (1071±178 to 966±187 ml·min-1, P=0.0009) and also in the prone position (968±160 ml·min-1, P=0.004), but not significantly changed while in a lateral position (1082±237 ml·min-1, P=0.75). These changes were mirrored in oxygen delivery and end-tidal CO2. In comparison, while blood velocity in the posterior ciliary artery showed a similar decrease when changing from a supine to an upright seated position (6.95±1.9 to 5.85±1.8 cm·s-1, P=0.034), there was no difference between supine to prone (7.3±1.9 cm·s-1, P=0.18), or the lateral position (7.68±2.1 cm·s-1, P=0.38).

Study 2. Our preliminary results from study 2 show a tendency for posterior ciliary artery conductance index to decrease within minutes of applying -20mmHg LBNP, returning towards the upright posture.

Conclusion

In study 1, we observed blood flow to the brain and eye to be sensitive to changes in the Gx-axis, potentially due to the slight haemoconcentration associated with the upright posture. As oxygen delivery remains slightly below the supine values, hypocapnia likely contributes to the lower cerebral blood flow in the upright position. Rotation around the Gz-axis had little effect on optic blood flow, but had an unexpected reduction in cerebral blood flow. Finally, the change in optic blood flow in study 2 could underlie the diminished increase in choroid thickness and area when low-level LBNP is applied during bed rest. Long duration studies in space will be necessary to determine if LBNP can be used as an effective countermeasure against SANS and its underlying mechanisms.

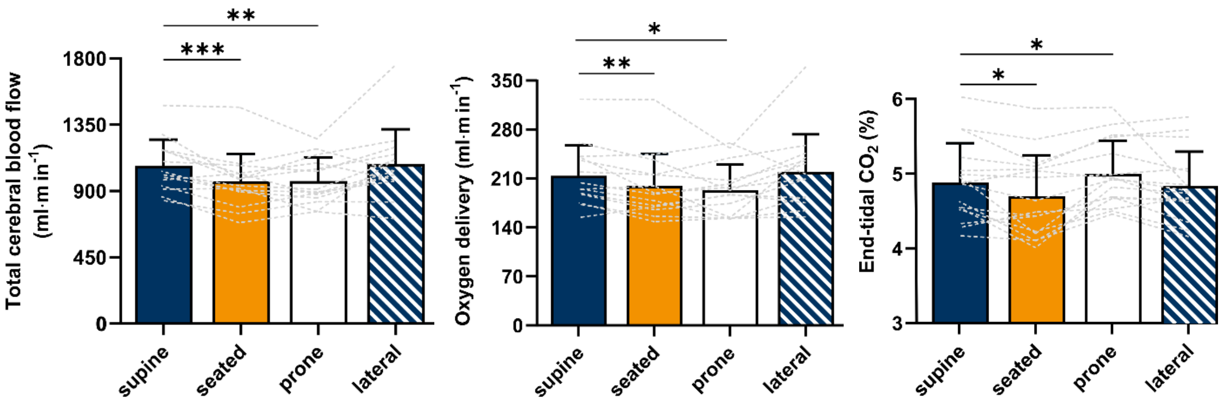


Fig. 1. Changes in cerebral blood flow, oxygen delivery and end-tidal CO_2 over 4 postures. Compared to supine: *p < 0.05, **p < 0.01, ***p < 0.001

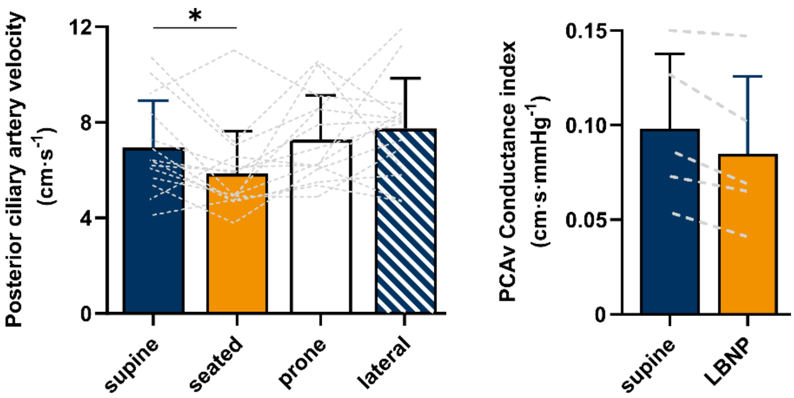


Fig. 2 & 3. Changes in posterior ciliary artery velocity over 4 positions (study 1) and posterior ciliary artery conductance index from a supine position to -20mmHg LBNP (study 2). *p < 0.05

References

Lawley JS, Babu G, et al 2020, et al. J Appl Physiol.129(5):1220–31.

Laurie SS, Vizzeri G et al 2017, Physiol Rep. 5(11).

Shinojima A, Iwasaki K ichi, et al 2012, Aviat Space Environ Med. 83(4):388–93.

Mader TH, Gibson CR, et al 2011, Ophthalmology. 118(10):2058–69.

Anderson AP, Swan JG, et al 2016, J Appl Physiol. 120(8):939–46.

Nickla DL, Wallman J. 2010, Prog Retin Eye Res. 29(2):144–68.

Patrick Lau – "FLUMIAS - high-resolution live-cell fluorescence microscopy and identification of gravity-dependent thresholds on-board the ISS"

FLUMIAS - high-resolution live-cell fluorescence microscopy and identification of gravity-dependent thresholds on-board the ISS

T. Schmakeit[1], Y. Lichterfeld[1], L. Kalinski[1], M. Braun[2], A. Carstens[2], S. Daali[2], S. Herbert[3], R. Sütterlin[3], R. Hemmersbach[1], P. Lau[1], C. Liemersdorf[1]

[1]German Aerospace Center, Institute of Aerospace Medicine, Linder Hoehe, D-51147 Cologne, Germany
Theresa.Schmakeit@dlr.de; Yannick.Lichterfeld@dlr.de; Laura.Kalinski@dlr.de; Ruth.Hemmersbach@dlr.de; Patrick.Lau@dlr.de; Christian.Liemersdorf@dlr.de
[2]DLR Raumfahrtagentur, Forschung unter Weltraumbedingungen, Deutsches Zentrum für Luft- und Raumfahrt (DLR)
Königswinterer Str. 522-524, D-53227 Bonn, Germany
Markus.Braun@dlr.de; Anna.Carstens@dlr.de; Samira.Daali@dlr.de
[3]Airbus Deutschland
Stefan.Herbert@airbus.com

Numerous biological research questions can be answered by using platforms providing short-duration microgravity (µg) conditions, such as drop towers, parabolic flights or sounding rockets. For many more reasons, prolonged time frames of µg are necessary to investigate how (micro-)gravity triggers cell differentiation, adaption processes and tissue development. The ISS provides constant high-quality µg and therefore grants an excellent research platform for gravitational science. Until now, the possibility to target research goals on the cellular level for various biological systems employing high-resolution live-cell microscopy in real µg was missing. FLUMIAS (FLUorescence MIcroscopy Analysis in Space, OpNom: Live Cell Imaging (LCI)) represents a novel research platform, developed by the German Space Agency at DLR as a national contribution to the ESA SciSpace program. It combines a structured illumination (SIM) laser microscope with a life support system for optimal environmental conditions. In addition, a centrifuge applies variable gravitational loads during the imaging process. Hence, fully automated transition from µg to 1g enables studies on the immediate impact of gravity changes on biological systems ranging from mammalian cells to microorganisms, three-dimensional (3D) cell culture systems or even whole tissue samples as well as small animals and plants.

The Concept

Microscopy-based investigations of cellular systems require spatiotemporal high-resolution imaging within living cells by various fluorescence-based techniques. FLUMIAS grants new possibilities for cell cultivation, since a novel life support system (LSS) enables medium exchange, incubation, pharmacological stimulation or live staining. With a variation of the SIM technique in a laser scanning microscope, spatial and temporal high-resolution fluorescent live-cell imaging is now possible to investigate novel questions that previously could not have been targeted. The acquired images and videos can be used to study the impact of gravity on fundamental biological processes.

A Science Reference Model (SRM) located at DLR, Cologne, Germany with its function as facility responsible center, provides the possibility for optimal testing and reference experiments with the identical setup as used on the ISS. This approach optimizes the scientific outcome.

DLR Cologne, Germany, as facility responsible center, provides access to the FLUMIAS SRM microscope system (SRM) and additionally provides well-established ground-based facilities for gravitational research. The DLR Hyperscope assembled to the human centrifuge at the :envihab research facility can be utilized for live-cell imaging at hypergravity conditions for increased gravitational loads of up to 4g. For simulation of microgravity, various models of clinostats can be employed for adherent or suspended cells, organoids, 3D cell cultures, in combination with irradiation for cell cultivation, online-kinetics and microscopy. FLUMIAS enables studies under altered gravity conditions with respect to e.g., adaptation processes, dynamic cytoskeletal rearrangements, visualization of Ca^{2+}-fluctuations, cell development progression, as well as pharmacokinetics.

FLUMIAS/LCI Capabilities

FLUMIAS/LCI provides the ability to incubate various samples inside of specific Experiment Blocks (EBs) with automated medium exchange and a controlled temperature environment (25-40°C, 0.5°C increment). Up to six EBs can be stored in the facility magazine for sequential processing, whilst being powered for uninterrupted life support functions. Perfusion of up to 4 different fluids is possible, e.g. nutrient and staining solutions, or pharmacological stimulants (1x100mL, 3x5mL). For the experiment runs, one EB at a time is transferred onto the FLUMIAS centrifuge for imaging. The hexapod-mounted centrifuge plate is designed for minimal vibration and capable to accelerate from µg to 1g within 3 sec and decelerate from 1g to µg within 7 sec.

Imaging parameters include a standard FoV (Field of View) of 400µm x 350µm (using a 40x air NA 0.95 objective), with multi-dimensional acquisition modes such as tile/mosaic imaging to create overview images of regions of interest or the whole slide, time lapse acquisition with a frame rate of up to 3 fps, as well as z-stacks. The microscope supports brightfield and fluorescence illumination with 4 excitation lasers (405 nm/488 nm/561 nm/640 nm) and 10 mW power each. Each EB will have one objective installed, with an air objective in the range of 10-40x to be chosen from.

Two different types of specialized EBs are under in development, a plant (EB-P) and a cell EB (EB-C). Within the EB-C, a 4-channel or 1-channel slide can be utilized. The EB-P grants a Phyto-LED with a spectrum equal to the spectrum of the cLED 4000K Leaflet within +/- 10% of spectral intensity. The concept for the plant EB foresees agar-filled microscopy slides and a closed gas-supply-loop for provision of the adequate fractions of O_2/Co_2.

Conclusions

Bringing advanced fluorescent microscopy to the ISS grants unique opportunities. FLUMIAS enables insights on the gravity-sensitivity of cellular processes that were preciously impossible to gain. With this microscope facility, scientists will now be able to decipher questions in a comprehensive manner that were targeted for decades. Countermeasure development and identification of gravity-sensitive pathways and target molecules will be significantly accelerated; thresholds of gravisensitivity can be determined and used to predict cellular behaviour on other planets.

Fuminori Kawano – “Histone chaperone SPT16 triggers histone turnover in mouse skeletal muscle”

Histone chaperone SPT16 triggers histone turnover in mouse skeletal muscle

F. Kawano, and T. Kobayashi
[1]Graduate School of Health Sciences, Matsumoto University, Matsumoto, Nagano, Japan
e-mail (FK): kawano@t.matsu.ac.jp

PURPOSE

Onboard exercise successfully prevents the loss of skeletal muscle mass and function which is caused by exposure to weightlessness environment during long term stay in the International Space Station. Future plans for deep space exploration include a manned mission to Mars as well as Moon in the Artemis program. Interplanetary spaceflight will limit the payloads including exercise instruments, and make any supplies from Earth impossible. Therefore, more effective exercise procedure is necessary to be developed for deep space exploration. Epigenetics is a theory that regulates the gene transcription by histone modifications and DNA methylation. We previously reported that exercise induced epigenetic changes in human (Lim and Shimizu et al., 2020) and mouse (Ohsawa and Kawano, 2021; Shimizu and Kawano, 2022) skeletal muscles. They suggested that exercise-associated histone modification enhanced the gene responsiveness of skeletal muscle to exercise. Activation of histone turnover is one of exercise-induced epigenetic changes in skeletal muscle in which the components of nucleosomes are exchanged to new histones. Although histone turnover enhances the accessibility of locus to transcriptional mediators, it is unknown how exercise activates histone turnover in skeletal muscle. In the present study, we investigated the role of histone chaperone SPT16 for the activation of histone turnover in mouse skeletal muscle.

METHODS

To obtain direct data regarding histone turnover, the present study used tetracycline-inducible H2B-GFP expression model mice. Forced SPT16 expression was performed by intramuscular injection of viral vector. Adeno-associated virus (serotype 9) vector was designed to express mouse SPT16 gene under control of skeletal muscle-specific ACTA1 promoter. Viral vector with non-coding sequence (stuffer) was used for the control group. Male H2B-GFP mice (8-wk-old) were injected with SPT16 vector into the left tibialis anterior muscle and stuffer vector into the right side. After 2 weeks of ambulation, supplementation of doxycycline-containing water was started. After further 2 weeks (4 weeks from vector injection), the mice were separated into control and exercise groups. The mice in the exercise group were assigned to perform a single bout of running exercise using a treadmill at 15 m/min for 30 min. Tibialis anterior muscles were sampled from both control and exercise groups 2 hours after the end of exercise. Gene expression and GFP incorporation at the loci were analyzed in 20 selected genes that were transcriptionally upregulated in response to acute exercise in the previous study (Ohsawa and Kawano, 2021).

RESULTS AND DISCUSSION

Successful induction of SPT16 was confirmed by western blotting. Localization of SPT16 in myonuclei was also detectable by immunohistochemical labeling. The expression of target genes was upregulated in response to acute exercise in the stuffer group, whereas the SPT16 group showed more enhanced upregulation in the gene expression compared to the stuffer group. GFP incorporation into nucleosomes was noted at these loci in the SPT16 group. The distribution of GFP was further increased in response to acute exercise in the SPT16 group, although GFP incorporation was not induced after exercise in the stuffer group. Furthermore, in the stuffer group, the distributions of total histone H3 and H2B at the loci were drastically reduced in response to acute exercise. Forced SPT16 expression suppressed the exercise-induced dissociation of histones from the loci. These results indicate that SPT16 triggered the activation of histone turnover in skeletal muscle. It is also suggested that active histone turnover lead to higher responsiveness of genes to exercise via rapid recovery of nucleosome deposition.

References

Lim C.H. and Shimizu J. *et al.* 2020. *PLoS ONE* 15: e0231321.
Ohsawa I. and Kawano F., 2021. *FASEB Journal* 35: e21453.
Shimizu J. and Kawano F., 2022. *Journal of Physiology* 600: 3331-3353.

Junya Shimizu – "Role of exercise-induced H3K27me3 for the gene response to exercise in mouse skeletal muscle"

Role of exercise-induced H3K27me3 for the gene response to exercise in mouse skeletal muscle

J. Shimizu and F. Kawano.
Graduate School of Health Sciences, Matsumoto University, Matsumoto, Nagano, Japan.
e-mail (JS): shimijun.shimi@gmail.com, e-mail (FK): kawano@t.matsu.ac.jp

PURPOSE

Deep space exploration, such as a manned mission to Mars, limits the payloads for onboard exercise. Although there is no doubt that exercise is beneficial to prevent the loss of skeletal muscle mass during long term stay in space, it is fact that the results of exercise were largely defferent in individuals (Fitts et al., 2010). Similar individual differences were also reported after resistance exercise training on the Earth (Bamman et al., 2007). Therefore, the development of effective onboard exercise procedures as well as preconditioning on the Earth will be the issues for the future manned mission. We previously reported that histone modifications, one of epigenetic systems, significantly altered in response to acute and chronic exercise in human subjects (Lim and Shimizu et al., 2020), suggesting that prior exercise causes the individual differences in the responsiveness to exercise. Histone H3 trimethylation at lysine 27 (H3K27me3) is known to act as a transcriptionally repressive histone modification via heterochromatin formation. Although H3K27me3 is enriched at the loci transcriptionally upregulated by exercise, the role of H3K27me3 for altering the responsiveness of genes to exercise is not fully understood. The present study investigated the effects of exercise training and detraining on H3K27me3 dynamics and the gene response to exercise in mouse skeletal muscle.

METHODS

Male C57BL/6J mice (8-wk-old) were assigned to perform acute treadmill running at 15 m/min for 30 min, or training (5 days/week) for 4 weeks. The half number of trained mice were detrained for 4 weeks after the end of training period. The mice were further separated into control and exercise groups. The mice in the exercise group performed a single bout of running exercise 3 days after the last session of exercise in the training study, or at the end of setraining period in the training + detraining study. Sedentary mice were also tested with same schedule. Tibialis anterior muscles of both control and exercise groups were sampled 2 hours after the end of acute exercise. Gene expression and histone distribution at the loci were determined in 20 selected genes that were transcriptionally upregulated in response to acute exercise in the previous study (Ohsawa and Kawano, 2021).

RESULTS AND DISCUSSION

The results obtained in chromatin immunoprecipitation followed by next generation sequencing analysis show that the target loci were marked by both H3K27me3 and H3K4me3, which were further enhanced in response to acute exercise (Figure 1, also published in Shimizu and Kawano, 2022).

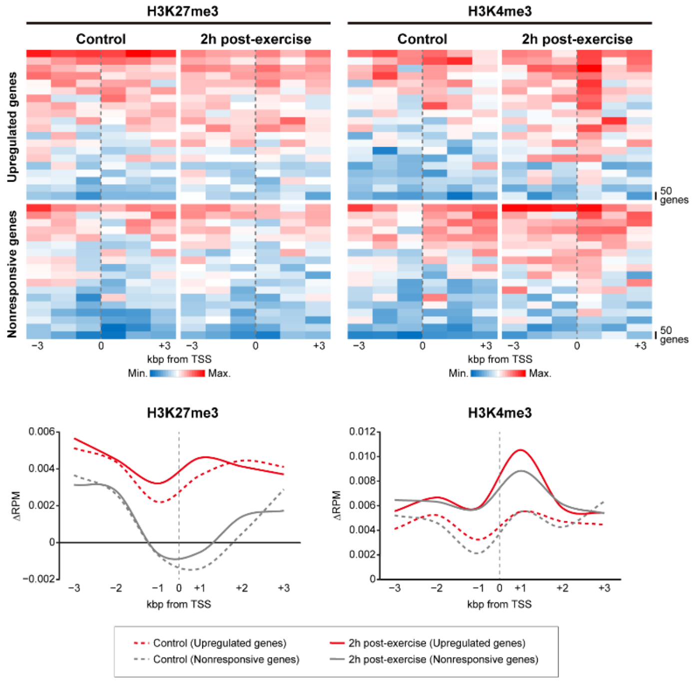


Figure 1: Responses of H3K27me3 and H3K4me3 distributions at the target loci to exercise. In heat maps, a single block showing the occupancy in every 50 genes was aligned by the order of RNA polymerase II distribution, e.g. top row is most prevalent. Mean occupancies in all upregulated (n=1,100) or non-responsive (n=935) genes are plotted in the bottom graphs.

Exercise training promoted to accumulate both H3K27me3 and H3K4me3 at the loci. Upregulation of gene expression in response to a single bout of exercise was more pronounced in the trained mice. Interestingly, both H3K27me3 and H3K4me3 distributions were decreased by exercise in the trained mice. The enhanced gene response to exercise was disappeared after detraining. H3K27me3 and H3K4me3 did not respond to exercise in the detrained mice. These results indicate that exercise-induced H3K27me3 plays a transcriptionally active role in association with H3K4me3 in skeletal muscle. The present study also showed that the histone modifications by the current exercise protocol did not persist during detraining period.

References

Bamman M. et al,, 2007. Journal of Applied Physiology 102: 2232–2239.

Fitts R.H. et al., 2010. Journal of Physiology 18: 3567-3592.

Lim C.H. and Shimizu J. et al. 2020. PLoS ONE 15: e0231321.

Ohsawa I. and Kawano F., 2021. FASEB Journal 35: e21453.

Shimizu J. and Kawano F., 2022. Journal of Physiology 600: 3331-3353.

Directional accuracy of point-to-point arm movements in different body orientations with respect to gravity

S. Vandergooten[1], L. Opsomer[2], J.L. Thonnard[3] , J. McIntyre[4] and P. Lefèvre[5]
[1]Institute of Neuroscience and ICTEAM, UCLouvain, Belgium (simon.vandergooten@uclouvain.be),
[2] Institute of Neuroscience and ICTEAM, UCLouvain, Belgium (laurent.opsomer@uclouvain.be),
[3] Institute of Neuroscience and ICTEAM, UCLouvain, Belgium (jean-louis.thonnard@uclouvain.be),
[4]Tecnalia, Basque Research and Technology Alliance, San Sebastian, Spain & Centre National de la Recherche Scientifique, CNRS, Paris, France (joe.mcintyre@parisdescartes.fr)
And [5]Institute of Neuroscience and ICTEAM, UCLouvain, Belgium (philippe.lefevre@uclouvain.be).

ABSTRACT

On Earth, gravity plays a primordial role during motor planning and control, both as a reference axis for proprioception and as a driving force for motor optimization. To further explore how the central nervous system exploits sensory information related to gravity, we studied the influence of body orientation relative to gravity and vision on the accuracy and trajectory of point-to-point arm movements.

Twelve participants performed point-to-point arm movements parallel to the longitudinal body axis, with eyes open or with eyes closed, in three body orientations relative to gravity (0° Upright, 45° Intermediate and 90° Supine). A motion-tracking system was used to measure hand kinematics and an instrumented manipulandum was held between the thumb and the index finger during the tasks.

Body orientation had a significant impact on movement direction and accuracy when eyes were closed. More precisely, in the Intermediate and Supine orientations movement direction drifted progressively away from the target axis in the absence of visual feedback. The extent of the drift was the largest in the Supine orientation (See Figure 1).

These results show that a misalignment between gravity and the body impacts the trajectory of upper limb movements, particularly when the eyes are closed.

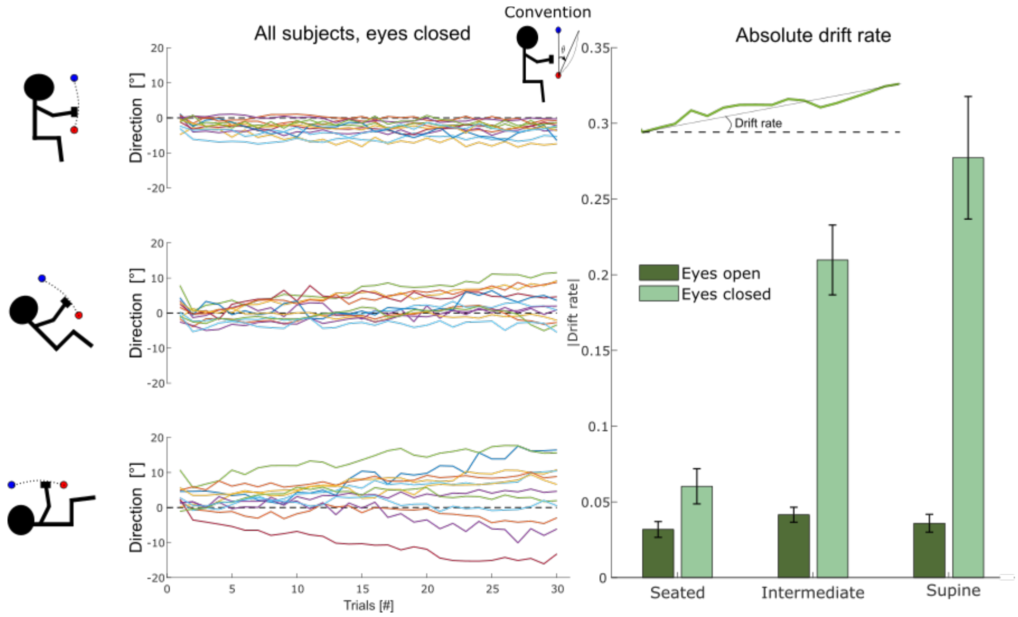


Figure 8 - Movement direction relative to the target axis during point-to-point arm movements performed with eyes closed in seated (top), intermediate (middle) and supine (bottom). Each curve shows the mean behavior for one participant. The angle was computed in the para-sagittal plane between a line connecting the start and end point of each movement, and a line connecting the targets (here parallel to the longitudinal body axis). (left)
The drift rate, taken as the absolute value of the slope of the linear regression of the direction angle, is shown for each body orientation and vision condition. (right)

Katharina Biere – "Isolation and lack of stimuli in Antarctica temporarily increase the alertness of the innate immunity"

Isolation and lack of stimuli in Antarctica temporarily increase the alertness of the innate immunity

K. Biere*[1], D. Moser[1], M. Feuerecker[1], C. Strewe[1], A. Choukér[1]
[1] Laboratory of Translational Research 'Stress and Immunity', Department of Anesthesiology, LMU Hospital, Ludwig-Maximilians-University, Munich, Germany (*Katharina.biere@med.uni-muenchen.de)

CONCORDIA STATION – LIVING AND WORKING UNDER EXTREME CONDITIONS

Concordia research station is located at 3233 m altitude in the interior of the Antarctic continent. Temperatures fall down to -80 °C and at least three months are in total darkness. Due to the altitude, the environment is dry and hypoxic. Added to these harsh outer influences, overwinterers are exposed to stress factors like isolation, lack of fresh food, plants and animals, monotony and the constant risk for their life as medical support is limited and during winter no evacuation of the station is possible. Social life is reduced to a small group of maximum 13 persons who get to know each other shortly before the start of the campaign. Due to this suit of unique factors, Concordia is an accepted ground analog platform for space-related research and especially exploration class missions.

Concordia timeline

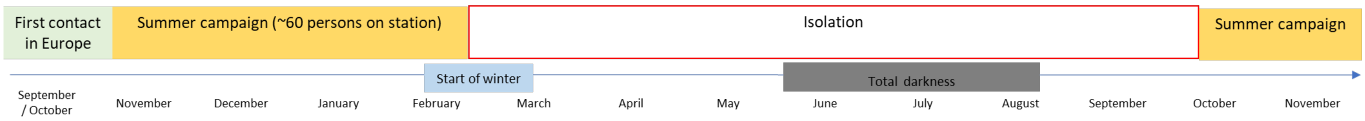


Figure 1: Visualization of the different phases the overwinterers experience during a full campaign.

CHOICE study

To evaluate the effects of all these stressful factors on the human immune system, the CHOICE study has been initiated.
Ex vivo investigations of innate immune functions showed an enhanced capacity for stimulated (TNF/fMLP or PMA) oxidative burst in granulocytes. Monocytes increased spontaneous cytokine release in incubation assays, which was augmented by stimulation with lipopolysaccharide. Both cell types displayed a rising intensity in immune response during winter with peak values after winter solstice, suggesting an immune cell sensitization and priming towards (hyper-)inflammation.

Based on these results, the expression of activation markers on the cell surface of monocytes was investigated in follow-up experiments. Incubation assays and subsequent flow cytometric analysis revealed an increase in basal expression of CD40, CD80 and CD69 during winter, which was further enhanced after incubation with bacterial antigens and heat-killed Listeria monocytogenes. At the same time, TLR2 expression decreased with the lowest level occurring towards the end of the winter. Antigen incubation partly restored TLR2 expression. These results support the previous observations. Moreover, they indicate that monocytes increase their alertness at the beginning and during Antarctic winter, which then – likely as a consequence of stimulus deprivation – declines over time which is accompanied by a partial immune anergy.

It remains to be confirmed whether TLR2 expression on monocytes is also reduced on the transcriptional level and in addition the contradictory surface expression of activation markers will be investigated more closely in future overwintering seasons at Concordia. This will increase the understanding of innate immune systems' adaptation to isolation in extreme environments.

Vivek Mann – “Blood vessels and microgravity”

Blood Vessels and Microgravity

Vivek Mann, and Alamelu Sundaresan

Author 1: Vivek Mann, Department of Biology, Texas Southern University, Houston, Texas, USA, Vivek.mann@tsu.edu
Author 2: Alamelu Sundaresan, Department of Biology, Texas Southern University, Houston, Texas, USA, Alamelu.sundaresan@tsu.edu

Space traveler’s subjection to microgravity environment results in alteration of various organ systems including headward fluid shift due to increased blood volume in head and trunk regions, decreased immune function, decreased red blood cell count, neurovestibular disturbances, muscular deconditioning, bone demineralization due to decreased weight bearing and cardiovascular adaptations characterized by orthostatic intolerance and decreased exercise capacity. Periodic changes in blood pressure and heart rate are well known in humans and recently confirmed in rats with little understanding of mechanisms causing periodic control of microgravity-induced damage of the cardiovascular system. Current research has shown release of nitric oxide from the endothelium alters with biological time, alluding its association in the modulation of circadian functions. Microgravity can lead to modifications in endothelial cell morphology, specifically leading to alterations in the formation and expression of vasoactive and inflammatory mediators and adhesion molecules, which are due to changes in the remodeling of the cytoskeleton.

It has been recently revealed that the angiogenic protein, Placental induced growth factor (PlGf) was significantly up- regulated in modeled microgravity conditions leading to possible atherogenesis and pathogenesis in microgravity and beyond low earth orbit. The relevance is enhanced because in life on earth, high levels indicate cardiovascular inflammation and acute coronary syndrome (ACS). PlGf levels were significantly higher in clinically high-risk populations, and this correlated inversely with HDL-cholesterol but directly with the triglyceride levels. Studies on the levels of PIGF in mammalian endothelial cells and lymphocytes will help to assess risk intensity of angina or heart failures in astronauts. Since PlGf uses the Flt2 receptor to mediate its effects, this model could be used to delineate the mechanism by which PlGf changes in microgravity, moon, and mars gravity. Since isoform 2 is released into the supernatant only when inflammation occurs, we will examine gene and protein regulation of the PlGf isoform 2 and the interaction with the Flt2 receptor. Upstream and downstream pathways affected will also be characterized.

Key words: Microgravity, Simulated microgravity analogue, endothelium, cardiovascular system, Inflammatory mediators.

Ryo Masuzawa – "Examination of histone variant induction as a novel countermeasure for long term stay in space using mouse model"

Examination of histone variant induction as a novel countermeasure for long term stay in space using mouse model

R. Masuzawa, F. Kawano
Graduate School of Health Sciences, Matsumoto University, Niimura, Matsumoto City, Nagano, Japan (e-mail: ryomasuzawa@outlook.jp)

BACKGROUND AND PURPOSE

Deep space exploration such as a manned mission to Mars will require a comprehensive medical care system to treat and maintain astronauts' health. The new generation of spacecraft that will be used for interplanetary missions limits a cargo capacity, which also restrict onboard exercise. In the future missions, gravitational unloading-induced loss of skeletal muscle mass and function will be more severe problem. Furthermore, in the case of Mars mission, crew is required to perform EVA tasks under partial gravity without the extensive rehabilitation support that was generally available after they return to Earth. Therefore, the development of new theory that prevents the loss of skeletal muscle mass and function is one of most urgent issues. Epigenetics is a current topic to understand the regulation of gene responsiveness to environmental stimuli. We previously reported using rats that tail suspension, a ground simulation model of spaceflight, drastically decreased the distribution of histone variant H3.3 at the loci that were transcriptionally upregulated during disuse atrophy in skeletal muscle (Nakamura et al., 2017). Histone H3.3 is also known to accumulate in tissues during aging. Although incorporation of histone H3.3 into nucleosomes may affect the gene responsiveness via the conformational change of chromatin, the role of histone H3.3 in differentiated cells and organs is unclear. The purpose of the present study is to clarify the role of H3.3 in mouse skeletal muscle using aging and forced expression models.

METHODS

In Experiment 1, tibialis anterior muscles were sampled from male C57BL mice at 8, 32, 53, and 75 weeks of age. Histochemical properties of muscle fibers and the level of histones by western blotting were analyzed. RNA sequencing analysis was also performed to identify the genes up- or down-regulated by aging, and the genes were targeted to the chromatin immunoprecipitation (ChIP) analysis. In Experiment 2, young male C57BL mice (8-wk-old) were injected with adeno-associated virus vector expressing histone H3.3 under control of skeletal muscle-specific ACTA1 promoter or carrying non-cording sequence (stuffer). Motor coordination function was evaluated using a rotarod every 2 weeks until 32 weeks of age.

RESULTS AND DISCUSSION

Experiment 1 showed that the tibialis anterior muscle weight relative to body weight significantly decreased after 53 weeks of age. Similar change was observed in the muscle fiber size. Myonuclear number was also decreased after 53 weeks of age. It was indicated that decline of tibialis anterior muscle was phenotypically detectable at middle age. Up- (n=15) and down- (n=14) regulated genes were selected by RNA sequencing. Interestingly, the expression of up-regulated genes gradually increased in association with age, although that of down-regulated genes significantly decreased at 32 weeks of age, suggesting that down-regulation of gene expression precedes age-related change in the transcriptome of skeletal muscle. The level of H3.3 relative to canonical variants H3.1/3.2 significantly increased in the tibialis anterior muscle during aging, although the increase of H3.3 level reached to peak at 53 weeks of age. Age-related H3.3 increase was correlated with H3K27me3 modification, not the other histone modifications. The histone distribution at the target loci analyzed by ChIP also showed the results similar to western blotting. It was suggested that transcriptionally repressive H3K27me3 accumulated in association with the increase of H3.3 incorporation. In Experiment 2, the mice expressing H3.3 showed longer latency to fall in the rotarod test compared to the stuffer group. These results suggest that H3.3 itself play a positive role in skeletal muscle function, although it is still unclear how H3.3 is modified by K27 trimethylation during aging.

Nakamura K, Ohsawa I, Masuzawa R, Konno R, Watanabe A and Kawano F., 2017, *Journal of Applied Physiology*, 123, 902-913.

J.J.W.A. van Loon – "Effect of 30 day-3Gexposure on C57BL6J mice: neurobehavioural and biomolecular effects on ocular and brain tissues"

Effect of 30 day-3G-exposure on C57BL6J mice: neurobehavioural and biomolecular effects on ocular and brain tissues

A. Racca[1], V.V. Chiricuta[2], and L. Bruno[2], J.J. won Loon[3], A. Micera[2], and D. Santucci[1]

[1]Reference Center for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy. arianna.racca@iss.it, daniela.santucci@iss.it [2]Research and Development Laboratory for Biochemical, Molecular and Cellular Applications in Ophthalmological Sciences; IRCCS - Fondazione Bietti, Rome, Italy, and [3] Dutch Experiment Support Center (DESC), Amsterdam Bone Center (ABC), Amsterdam University Medical Center, Location VU University Medical Center (VUmc) and Academic Centre for Dentistry Amsterdam (ACTA), Department of Oral and Maxillofacial Surgery/Oral Pathology, Amsterdam, Netherlands.

Understanding the phenomena underlying tolerance and adaptation to altered environmental conditions such as un-physiological gravity represents a great opportunity to study coping strategies, mechanisms underlying neuroplasticity phenomena, and the individual vulnerability to stress. In addition, animals are sent into orbit as a useful tool to preserve potential human and animal health, predicting and developing adequate countermeasures in long-term space travel. The present study was aimed to investigate behavioural and biomolecular profiles in animal models in order to identify behavioural biomarkers for individual differences in coping with the hypergravity paradigm, and compared with data from animals who underwent the suspension paradigm, also trying to make progress in all these procedures to ensure and improve animal welfare. C57BL6J mice were placed in a Long Diameter Centrifuge (LDC) and subjected to 3g-rotation induced hypergravity up to 30 days. The behavioural profile was studied before, during and after hypergravity. Age-matched mice were used as controls (normogravity). Left half-brain and right eye ball were dissected out and used for biomolecular analysis (Ella microfluidic, real time RT-PCR). Right half brain and left eye ball were fixed and used for microscopical examination (HE) and immunofluorescent analysis. Corneal imprints and optic nerve were collected before eye dissection.

Specific behavioural items, such as vertical movements (rearing and wall rearing behaviours) or novel object exploration, were affected after being exposed to altered gravity conditions. Preliminary molecular data revealed changes in the expression of few neuromediators (Serotonin, Dopamine, Acetilcoline), neurotrophins (NGF, BDNF), vascular/angiogenic/angiostatic (VEGF isoforms and Angiotensin II, MAO-A) factors and some mediators of oxidative stress (iNOS/eNOS, Cav-1) in selected ocular tissues (retina/RPE cells, lens and cornea), vitreous and optic nerve dissected-out from 3G-exposed mice. Overall eye ball and lens size as well as vitreous volume were reduced in 3G-exposed mice. Changes were also observed for few proinflammatory mediators (IL8, IL10, IL17a) and a restricted number of metalloproteinases (MMP2/4/6/9) in the vitreal fluids as well as at the corneal level (muc1/muc5AC, goblet cells).

These preliminary data confirm that the behavioural, biomolecular and cytological changes might be most likely related to hypergravity exposure, although with different degrees of severity.

Practical Application Of Electrical Stimulation Space Mode With "Russian Currents" In Neurological Elderly Patients

N.M.A. Abu Sheli[1], A.A. Saveko[1], L.E. Amirova[1], M.A. Avdeeva[2], A.A. Gudkova[2], A.B. Guekht[2], and E.S. Tomilovskaya[1]
[1] Institute of Biomedical Problems of the Russian Academy of Sciences; abusheli.md@gmail.com
[2] Research and Clinical Center for Neuropsychiatry of Moscow Healthcare Department

INTRODUCTION

Biolo G. et al. made an interesting observation - many space-related physiological changes resemble those observed during aging (Biolo G. et al., 2003). Postural stability is affected by both spaceflight and aging (Demertzi et al., 2016). It is known that postural perturbations in the elderly may be associated with impaired perceptual processing of sensory afferent signals (Ozdemir et al., 2018). At the same time, changes in the support and proprioceptive afferent signals are a key factor in the many sensorimotor disorders during space flight (Kozlovskaya I.B. et al., 2007).

The effects of aging, especially with hypokinesia, are an important cause of loss of the contractile capacity and mass of the skeletal muscle in the elderly (Scicchitano et al., 2009; Vinciguerra et al., 2010). Such patients are not able to maintain a sufficient level of physical activity. In this case, electromyostimulation (EMS) can be an alternative to active physical training. We hypothesized that the EMS regime with the so-called Russian currents, which is an effective passive countermeasure in weightlessness, can also be effective for neurological elderly patients with impaired balance and walking functions.

METHODS

The study involved 38 patients (73.8±7.0 years) with confirmed balance and walking disorders and a chronic cerebral ischemia, 27 of whom were in the group with EMS, and 11 - in the control group. Patients of the EMS group passed a two-week course of EMS of the hip and shin muscles - from 3 to 9 procedures. For EMS we used two single-channel stimulators Amplipuls-5DS (Russia) and the mode of sinusoidal modulated, so–called Russian, currents – modulation of high-frequency current (5 kHz) with lower frequencies one (50 Hz). The stimulation amplitude was set according to patient tolerance (submaximal). The average stimulation amplitude was 22-24 mA. The duration of one EMS procedure was 20 minutes.
To assess the effectiveness of the EMS course, we used clinical questionnaires and tests (Performance Oriented Mobility Assessment or Tinetti test, Rivermead mobility Index, Timed Up and Go test), as well as posture examination before and after the EMS course. Postural stability was assessed using the BioMera stabilography platform (BioMera LLC, Russia). The data were analyzed using repeated measures ANOVA (GraphPad Prism version 8). The significance level was at $\alpha=0.05$.

RESULTS

After the EMS course, EMS group patients showed an improvement in the results of balance function on the Tinetti scale by 0.96± 0.15 points ($p<0.0001$), as well as the walking function on the Tinetti scale by 0.54±0.12 points ($p=0.0002$). Thus, the total score on the Tinetti scale increased by 1.54± 0.23 points ($p<0.0001$). It is worth noting that in the EMS group, this total score was on average below 24 points, which indicated the presence of a risk of falls. But the EMS course allowed us to mitigate it. Rivermead Mobility Index also increased after the EMS course by 0.32±0.10 points ($p=0.0051$; Fig. 1A). The results of the Timed Up and Go Test are also noteworthy. Before the EMS course, the test performing time was 14.56±3.64 s, which indicated the presence of motor disorders and the risk of falls. The reduction in this parameter after the EMS course to 13.00±3.09 also demonstrated the mitigating of this risk and an increase in functional capacity. Thus, patients performed the Timed Up and Go Test 1.56±0.35 s faster ($p=0.0002$; Fig. 1A). Moreover, after the EMS course, the main parameters of postural stability also improved. For example, the area of the ellipse of the center of pressure (COP) displacement significantly decreased from 730.5±202.9 to 413.6±117.8 mm2 ($p=0.0331$) when closed eyes. In the control group, no significant changes were observed in similar tests and examinations (Fig. 1B). The results obtained allow us to confirm the hypothesis suggested, and also demonstrate the prospects of an approach based on the comparison of the influence of microgravity and aging.

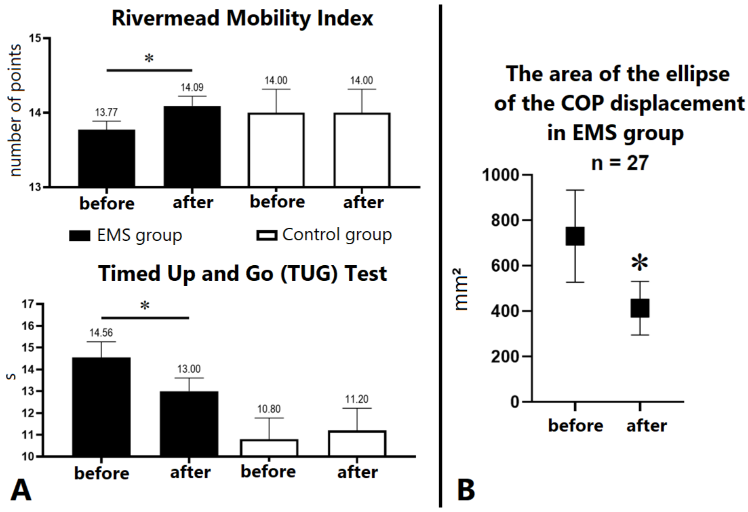


Figure 1. A - Results of Rivermead mobility index and Timed Up and Go (TUG) Test in the EMS group (black columns) and the control group (white columns). B - The area of the ellipse of the COP displacement while standing with eyes closed in the EMS group. before – initial data; after – data obtained after the EMS course. * – significant difference vs. initial data.

FUNDING

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

REFERENCES

Biolo G., Heer M., Narici M. and Strollo F., 2003, Curr Opin Clin Nutr Metab Care, 6(1), 31-40.

Demertzi A., Van Ombergen A., Tomilovskaya E. et al., 2016, Brain Struct. Funct, 221, 2873-2876.

Ozdemir R.A., Contreras-Vidal J.L. and Paloski W.H., 2018, Mech. Ageing Dev, 169, 19-31.

Kozlovskaya I.B., Sayenko I.V., Sayenko D.G., Miller T.F. et al., 2007, Acta Astronaut, 60, 285-294.

Scicchitano B.M., Rizzuto E. and Musaro A., 2009, Aging, 1, 451-457.

Vinciguerra M., Musaro A. and Rosenthal N., 2010, Adv Exp Med Biol, 694, 211-233.

Margot Winters – "Assessing the Resilience of Lab-On-Chip Devices with OoC-STRAT: A study on High-Radiation Effects Using Stratospheric Balloons"

Assessing the Resilience of Lab-On-Chip Devices with OoC-STRAT: A study on High-Radiation Effects Using Stratospheric Balloons

Margot Winters

TU Delft, Faculty of Aerospace Engineering [MSc]

margotwinters@hotmail.com

Space exploration presents a range of health challenges to astronauts that are not encountered on Earth (Aubert et al., [1]). Whilst the use of Organ-on-chip devices in space is a promising approach to better understand the effects of space-related conditions on human organs (Jiang et al., [2]), a crucial hurdle lies in assessing the vulnerability of these devices to space radiation. The Organ-On-Chip STRATospheric (OoC-STRAT) experiment is an innovative effort to assess the practicality of lab-on-chip devices by investigating the influence on their performance with stratospheric balloon flights.

By launching two stratospheric balloons with lab-on-chip samples near Krakow, Poland, ´ OoC-STRAT seeks to identify changes in functionality due to exposure to stratospheric conditions (Figure 1). As the middle stratosphere of Earth furthermore bears similarities to the surface conditions of Mars, the results of this study also provide valuable insights in the challenges to store lab-on-chips and perform experiments on the Red Planet (Kaplan, [3]). The expected outcomes of this experiment are the assessment of the impact of radiation on the devices, improvement of the devices for future space applications, deepening our understanding of how different devices could survive on Mars, and identifying key shielding factors that contribute to device survival in harsh extra-terrestrial environments.

The future of research in this area looks promising, as the need for radiation-resistant technologies will only become more critical when space exploration continues. Further studies can focus on identifying the mechanisms behind radiation-induced changes and how to harness these changes to create resilient devices that can thrive in space.

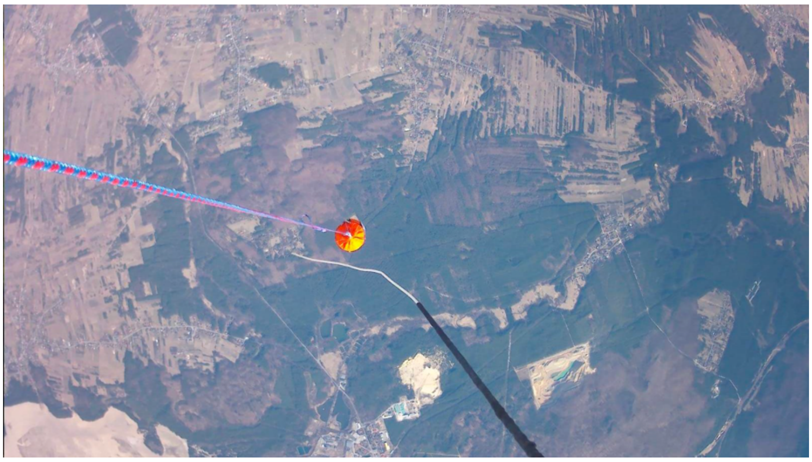


Figure 1: Past stratospheric balloon flight experiment at the AATC, Krakow, Poland in 2016.

[1] Aubert A et al. (2016) Towards human exploration of space: the THESEUS review series on cardiovascular, respiratory, and renal research priorities. Microgravity 2, 16031.

[2] Jiang L et al. (2022). Organ-On-A-Chip Database Revealed—Achieving the Human Avatar in Silicon. Bioengineering 9(11): 685.

[3] Kaplan DI (1988) Environment of Mars, 1988. NASA STI Technical Report N 89: 14187.

16:00 Inês Antunes – “ESA's SciSpacE team”

ESA's SciSpacE team

This presentation will concern the challenges faced by astronauts during long-duration space missions and how the European Space Agency (ESA) is supporting their health and performance through research on the impact of space conditions on the human body and the development of countermeasures and technologies to mitigate those consequences. The presentation will focus on ESA's SciSpacE team, which conducts research in ground analogues, suborbital platforms, and on the International Space Station (ISS), and how the research conducted in space can benefit terrestrial medicine. The process of ideation, call for proposals, review, selection, and implementation of research projects will also be outlined. The presentation aims to provide insights into how ESA is working to keep astronauts healthy and productive during long-term space missions and how these efforts can also benefit medical science on Earth.

16:15 Masahiro Terada – “The implementation of the educational programs for space medicine in Japan”

The implementation of the educational programs for space medicine in Japan

Masahiro Terada
Kyoto University, Unit of Synergetic Studies for Space, Shogoin Kawahara-cho, Sakyo-ku,
Kyoto 606-8507 Japan, masahiro.terada.2m@kyoto-u.ac.jp

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

This educational program consists of the lectures and the hands-on training. In the lectures, student can learn the physiological effects on the human body during the spaceflight. The lectures include many topics such as the muscle atrophy, bone loss, motion sickness, fluid shift, mental stress, space radiation and so on. In space, the microgravity induces the muscle atrophy and bone loss. Therefore, astronauts must perform the exercise for about 3 hours/day in International Space Station (ISS). By the understanding of these topics, students will learn the common factors of the physiological changes on ISS and the phenomena of aging on the earth. In the hands-on training, we will provide the opportunity of performing the bedrest study. In the bedrest, subjects will have various physiological changes in the reality. They may have the muscle atrophy and bone loss, cardiovascular effects, fluid shift and so on. In the bedrest study, student can learn the various physiological changes by the decreasing of the mechanical stress on the leg or body.

In our educational programs for space medicine, students can learn many necessary knowledges for future human space mission. Therefore, this program must be very important opportunity to inspire the students to be interested in space medicine.

16:30 Andrei Sapera – "New commercial applications for space physiology and human spaceflight"

New commercial applications for space physiology and human spaceflight

A. Sapera[1], H. Stenuit[2], L. Surdo[3] and M. Ferreira[4]
[1]Space Applications Services / ICE Cubes Service (andrei.sapera@spaceapplications.com)
[2]Space Applications Services / ICE Cubes Service (hilde.stenuit@spaceapplications.com)
[3]Space Applications Services / ICE Cubes Service (leonardo.surdo@spaceapplications.com)
[4]Space Applications Services / ICE Cubes Service (miguel.ferreira@spaceapplications.com)

As human spaceflight becomes more common, accelerated by commercial activities, missions like Axiom-1 and upcoming ones provide the opportunity to use, test and validate innovative solutions for health monitoring in space.

The adoption of Artificial Intelligence / Machine Learning (AI/ML) techniques in space can result in important steps forward for a wide range of human health research and applications, such as real-time Big Science Data processing, AI-powered assistants for medical care, research, evaluation and support of crew activities and performance, opening new portals into what future human spaceflight missions could look like.

Health apps

During the RAKIA mission on Axiom-1, the ICE Cubes service had the opportunity to support the implementation of several health apps solutions involving tablet applications used to perform a series of physiological and psychological tests on-board the ISS. (ICE Cubes Journal Ref.1)

One activity from the RAKIA mission had the objective to conduct an 'albumin to creatinine ratio' (ACR) urinalysis test in space, with the aim to help astronauts measure kidney function and get immediate diagnostic results. This involved the use of a tablet app running a special (space edition) Kidney Test protocol. While the topic of kidney function in space has already been researched, previous studies required urine collected in space to be returned frozen back to Earth for post-flight analysis. This new app, instead, enabled astronaut crews to obtain immediate medical diagnostic information in-situ, which is particularly relevant as missions to space become longer.

Living in space can also affect eyesight and cause neuro-ocular syndrome (SANS). The RAKIA mission also included the utilization of another tablet app that helped assess in real-time eye fitness before, during, and after a task, with the aim to investigate spaceflight effects on visual function, the early onset of spaceflight associated neuro-ocular syndrome, and the recovery of visual functions.

With increased human presence in space, and new space stations and habitats being developed to expand the scope of space missions, astronauts will need a variety of modern diagnostic tools to screen and monitor their health in the space environment. These tools and applications can be tested, validated and demonstrated on-board the ISS, and future space stations, in a fast-track and direct manner through the ICE Cubes Service, which also provides AI/ML capabilities and support for big data processing and analysis. (ICE Cubes Journal Ref.2)

AI for R&D

In modern scientific research, AI is already playing a crucial role in providing in-situ, fast, real-time processing of big data generated by a variety of experiments, measurements and activities, either in crewed or fully-automated spacecraft. One can therefore predict that the application of AI/ML assets will support scientists and operators in more efficiently understanding and monitoring the effects of the space environment and microgravity in all its facets, by deciphering models and patterns that are not easily observable. This opens new avenues for R&D in life sciences, biotech & pharma, agrifoodtech, and many applications relevant to the realm of human health monitoring and protection, providing a boost in computational capability for data analysis, problem solving or automation of complex tasks.

The effects of long-term exposure to spaceflight conditions manifest in many areas of (human) physiology: neurology, ophthalmology, cardiovascular, pulmonary, gastrointestinal, urinary, musculoskeletal, hematology, immunology, oncology, and psychological stress (N. M. Haney et al, 2020)

AI/ML techniques can enhance in-space research in any of these areas, at different scales and under different setups, by combining and analyzing data stemming from specific individual processes of sub-systems (e.g. organoids, spheroids, tissues, genomics via lab/organ-on-chip research, etc.) [ICE Cubes Journal Ref. 3, 4], or from larger systems and their processes (e.g. astronauts as health research subjects).

Future applications

Commercial spaceflight comes with a different approach to conducting activities in space, tapping into the solutions already available on different non-space/terrestrial markets. In recent years, the rapid development of technology on Earth offers a wide range of tools, applications and off-the-shelf solutions that can be quickly adapted for utilization in space. This not only allows for more advanced activities and processes, but also significantly shortens the time required for integration, implementation of R&D activities, data retrieval and analysis. Such solutions include wearable devices, biosensors, diagnostic imaging, augmented and virtual reality tools.

The empowerment of such applications with AI/ML capabilities can pave the way for the implementation of innovative health monitoring and evaluation solutions. The validation and demonstration of these solutions in space can then become part of the health check protocols and evaluation process of space crews, enabling for an automatic data analysis in orbit, while also providing real-time connection for data and results comparison and cross-checking by the medical staff on ground.

Combining research payloads and devices with edge computing capabilities in orbit offers the opportunity to test and benchmark new solutions and applications required by future long-term deep space exploration missions, where (near) real-time communication with ground (Earth) is not possible. Such solutions may include machine vision applications, in-situ processing and analysis of data via AI/ML powered visual inspection, development of smart devices, robotic assistants and habitats, as well as AR/VR-based applications for future space activities and operations (in-orbit and on-ground).

References

[1] 'Axiom-1 RAKIA – New health monitoring apps for human spaceflight', ICE Cubes Journal, 2022, accessible here: https://www.icecubesservice.com/journal/axiom-1-rakia-new-health-monitoring-apps-for-human-spaceflight/

[2] 'AI-Box for AI/ML on ISS', ICE Cubes Journal, 2022, accessible here: https://www.icecubesservice.com/journal/ai-box-for-ai-ml-on-iss/

[3] AI's role in deep space, Nora M. Haney, A. Urman, T. Waseem, Y. Cagle, José M. Morey, 2020, Journal of Medical Artificial Intelligence, accessible here: https://jmai.amegroups.com/article/view/5712/html

[4] 'Axiom-1 RAKIA – Stress and DNA damage response during spaceflight', ICE Cubes Journal, 2022, accessible here: https://www.icecubesservice.com/journal/axiom-1-rakia-stress-and-dna-damage-response-during-spaceflight/

[5] 'Axiom-1 RAKIA – Bone-forming and skeletal muscle cells under microgravity', ICE Cubes Journal, 2022, accessible here: https://www.icecubesservice.com/journal/axiom-1-rakia-bone-forming-and-skeletal-muscle-cells-under-microgravity/

16:45 Ryan T. Scott – “Open Science for Life in Space: Data Sharing, Standards, and Informatics for Reuse and Knowledge Discovery”

Open Science for Life in Space: Data Sharing, Standards, and Informatics for Reuse and Knowledge Discovery

Ryan T. Scott[1], Lauren M. Sanders[2], Jamie L. Bales[3], Ana E. Uriarte Acuna[1], Danielle K. Lopez[1], Samrawit G. Gebre[4], Sylvain V. Costes[4]
[1]KBR, Moffett Field, CA, USA; [2]Blue Marble Space Institute of Science, Moffett Field, CA, USA; [3]Alidyne Consulting, Melbourne, FL, USA; [4]Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA, USA

Health countermeasures and biomonitoring systems for space missions are required to counteract space health hazards and to support life to thrive in deep space (e.g., humans, animals, plants, crops; entire ecosystems within spacecrafts, habitats, and spacesuits). The development of these mission components are highly dependent on our understanding of basic biological and health responses to myriad space hazards (ionizing radiation, altered gravitational fields, altered day-night cycles, confined isolation, hostile-closed environments, distance-duration from Earth, planetary dust-regolith, and extreme temperatures/atmospheres).

The fast-growing array of space biological, space health and mission telemetry data, which in the past was simply archived without minimal processing, or formatting to ensure reusability, holds great potential for addressing these mission challenges once it is standardized and made public for re-analysis and re-use by the Open Science community. Organizing the data for such analysis is a challenge because of its multi-hierarchical, multimodal, and heterogenous nature (molecular, cellular, tissue, organ, whole organism, behavior, ecosystem, microbiome; tabular, omics, imaging, code, video, biospecimens, environmental physical-chemical telemetry).

The NASA “Open Science Data Repository” (OSDR; osdr.nasa.gov/bio) addresses these needs. Its design and development extends the successes and lessons learned from NASA GeneLab, a publicly available 'omics repository for spaceflight and space relevant life sciences data. OSDR includes GeneLab, but also NASA Ames Life Sciences Data Archive (ALSDA; repository of physiological-phenotypic-telemetry data), and NASA Biological Institutional Scientific Collection (NBISC; a collection of space-flown or space-relevant biospecimens). In 2021, a community of researchers rallied to form the ALSDA Analysis Working Group (AWG) and provided scientific consensus on physiological-phenotypic standards for both the sample-assay metadata as well as the raw or processed data themselves. OSDR also includes an Environmental Data Application (EDA) which allows users to visualize and analyze telemetry data (radiation, O2, CO2, relative humidity, temperature, acceleration) across missions both longitudinally and/or cumulatively.

Here we report efforts on making space biological and medical data FAIR (findable, accessible, interoperable, reusable), effective data ingestion/dissemination, Open Science collaborations, federation of data from non-NASA sources, analysis techniques, machine learning/knowledge graph/modeling methods, natural language processing, and data integration/discovery tools. We demonstrate that the OSDR user community and excitement around the OSDR system has already led to several data reuse studies, showing the value of mining OSDR using machine learning, knowledge graphs, and meta-analysis approaches. We further report efforts to integrate human 'omics and physiological-phenotypic data into OSDR collected from commercial missions such as Inspiration-4. OSDR implemented access controls for sensitive data and embrace a data governance continuum from closed to mediated to embargoed to open.

17:00 Anna Catharina Carstens – "Fluorescent live cell imaging on a centrifuge in space: FLUMIAS"

FLUORESCENT LIVE CELL IMAGING ON A CENTRIFUGE IN SPACE: FLUMIAS

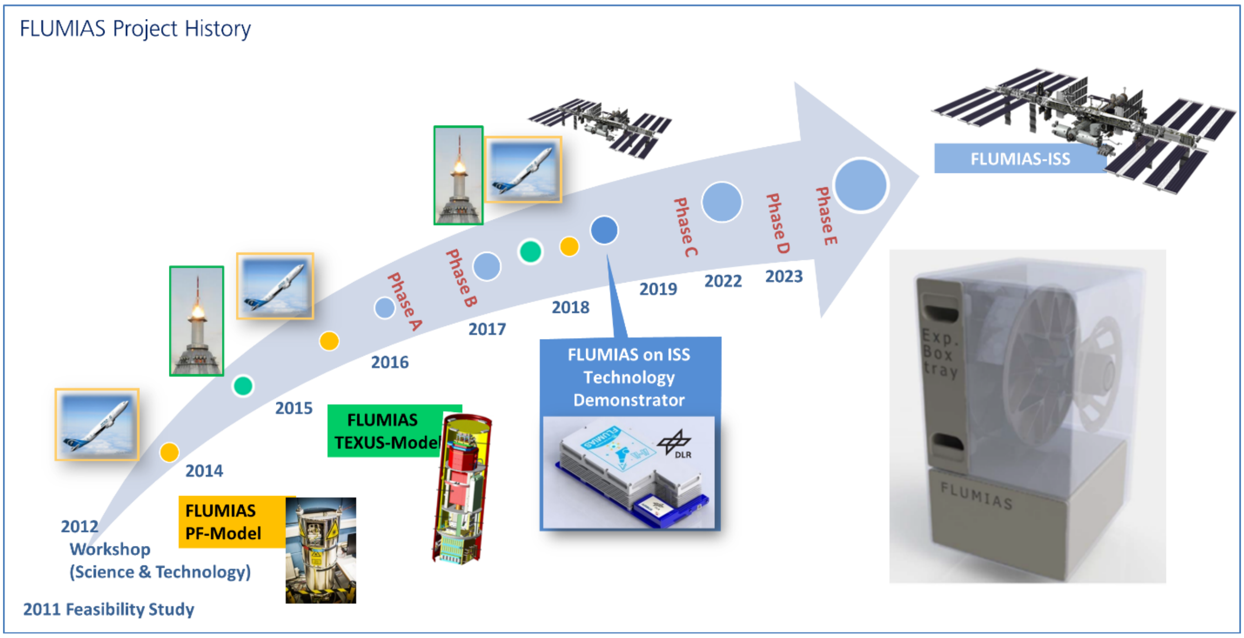
A.C. Carstens[1], M. Braun[1]
[1]German Space Agency at DLR, Königswintererstr. 522-524, 53227 Bonn, Germany, anna.carstens@dlr.de, m.braun@dlr.de

SUMMARY

FLUMIAS-ISS is a novel high-resolution microscopy facility for the ISS, developed by the German Space Agency at DLR as a national contribution to ESA´s SciSpace program. It will provide the opportunity to perform high-quality Live-Cell-Imaging on board the ISS. Automated performance, adaptable g-levels and experiment-specific life support systems were key design goals for this multi-user facility.

OVERVIEW

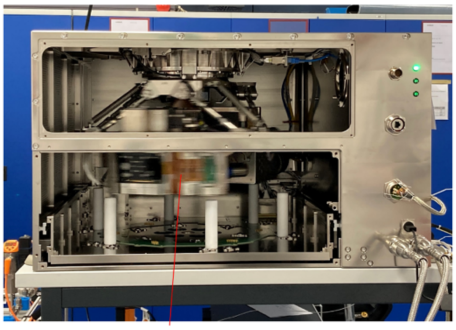
How do cells of mammals and plants react to changing gravity levels? What are the thresholds for gravity sensing of living organisms and what are the underlying molecular mechanisms? How long does it take for a living cell or tissue to adapt to reduced gravity? With crewed space missions and the exploration of our solar system in this decade and the ones to come, deciphering those questions will be key in ensuring the sustainable success of humankind in Space. Even though the International Space Station offers a variety of scientific opportunities, the research technologies that are available in Space are still limited in comparison to terrestrial labs. High-resolution fluorescence microscopy is a fundamental technology in many research fields such as biology, physiology, immunology and more. The FLUMIAS-program within the DLR Space Agency has enabled researchers in Germany to make use of such a microscope on parabolic flights and sounding rockets (Corydon et al. 2016, Thiel et al., 2019). To support research in LEO and beyond, it is now being adapted to the needs of doing science in Space: An integrated centrifuge for in-flight controls and establishing reduced gravity levels, adjustable life-support for long-term observation and exchangeable experiment blocks that can be adapted to the needs of a variety of experiments. The development of the facility is contracted by DLR Space Agency to Airbus Defense and Space, Friedrichshafen.



KEY FEATURES

The core of the facility is an innovative Structured-Illumination Microscope (by TILL I.D., Martinsried, Germany) that is so robust and compact that it can be rotated on a centrifuge, thereby offering acceleration levels between 0 g and 1g. The SIM technology produces images in high resolution, comparable in quality to a confocal microscope. Six exchangeable Experiment Blocks (EBs) each containing one experiment are accommodated in the facility's magazine. For imaging, the EBs are shifted from the magazine to the centrifuge by a transfer mechanism that is controlled from ground. Compressed images are downloaded during the experiment, so researchers can control and readjust their specimen.

FLUMIAS Facility – Engineering Model

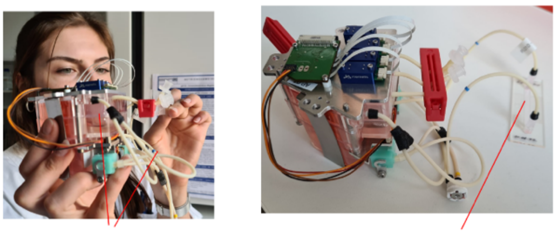


Experiment Block on rotating centrifuge

PROGRAMMATIC IMPLEMENTATION AND SCIENTIFIC USE

FLUMIAS-ISS has been developed by the German Space Agency at DLR and will be handed over to ESA as a national contribution to ESA´s SciSpace Program. 10 science proposals for a first round of experiments were selected via an international joint DLR/ESA announcement of opportunity. Selected experiments investigate a diverse set of organisms such as bacteria, yeast, muscle- and cancer cells. The rationale behind the development of this multi-user facility is to enable a broad range of experiments including but not limited to cell biology, microbiology, biotechnology, immunology and molecular physiology. The development is accompanied by ongoing scientific testing of all relevant hardware components.

Life Support System (Experiment Block insert, Prototype Test)



Laboratory-grade sterile Fluidic System by Ibidi, Germany

Mammalian cells cultured in off-the-shelf microscopy slide

OUTLOOK

FLUMIAS is scheduled for launch to the ISS in 2025. Here we will present a design overview of the hardware, science-relevant technical properties, a description of the currently selected experiments and an outlook to possible future applications for Live Cell Imaging in Space.

REFERENCES

Corydon, Thomas J., et al. "Alterations of the cytoskeleton in human cells in space proved by life-cell imaging." Scientific reports 6.1 (2016): 20043.

Thiel, Cora Sandra, et al. "Real-time 3D high-resolution microscopy of human cells on the international space station." International journal of molecular sciences 20.8 (2019): 2033.

17:15 Alamelu Sundaresan – "Development and Characterization of 3D culture of bone tissue as a physiological test bed for pharmaceutical formulations"

Development and Characterization of 3D culture of bone tissue as a physiological test bed for pharmaceutical formulations

Vivek Mann[1], Alamelu Sundaresan[1]*

[1] Osteoimmunology and Integrative Physiology Laboratory, Department of Biology, Texas Southern University, Cleburne, Houston, TX 77004, USA; vivekmann7@gmail.com

*Correspondence: Alamelu Sundaresan, alamelu.sundaresan@tsu.edu; Tel.: (+1-832-725-0940)

Received: date; Accepted: date; Published: date

Abstract

Contemporary technological advances in tissue engineering applied to medicine and regenerative medicine have a vast untapped potential for future space and terrestrial medical applications. Human cells form three-dimensional tissue constructs (e.g. cartilage, blood vessel intima constructs and others) when exposed to modeled microgravity (m-μg). Here we have discussed different modeled microgravity models using cells alone and cells plus scaffolds to make 3D bone constructs. We investigated human fetal osteoblast (hFOB 1.19) cells exposed to Rotary Cell Culture System (RCCS-a model of m-μg) for 21 days, with the aim of exploring the effects of m-μg on biological processes and to fabricate 3D bone constructs. RCCS exposure of the hFOB 1.19 cells induced modifications in cell adhesion and manifested a distinctive histological expression of collagen and the extra cellular matrix. After 21 days of RCCS exposure, the 3D constructs presented a bone-like morphology. In another experiment Human Mesenchymal stem cells grown on scaffolds were exposed to a Random Positioning Machine (RPM-a model of m-μg) for 14 days which induced a significantly altered release of the cytokines and bone biomarkers like tumor necrosis factor 1 alpha (TNF-1α), interleukin 6 (IL-6), Leptin, osteocalcin (OC), sclerostin (SOST) and osteoprotegerin (OPG). In conclusion, our preliminary data suggests that cells cultured in m-μg conditions embodies an advancement for tissue-engineering of bone constructs and can act as a test bed for pharmaceutical formulations.

Keywords: osteoblasts; bone tissue engineering; rotary cell culture system (RCCS); High aspect ratio vessel (HARV), cytoskeleton; collagen; scaffold.

17:30 Marina Cara Tuschen – "µIMMUNE - Development of automated microfluidic immune monitoring for spaceflight, a first report"

µIMMUNE – Development of automated microfluidic immune monitoring for spaceflight, a first report

M. C. Tuschen*[1], M. Geissler[2], L. Malic[2], C. Miville-Godin[2], D. Moser[1], A. Choukér[1] & T. Veres[2]
*marina.tuschen@med.uni-muenchen.de [1]Laboratory of Translational Research 'Stress and Immunity', Department of Anesthesiology, LMU Hospital, Ludwig-Maximilians-University, Munich, Germany [2]Life Sciences Division, National Research Council of Canada, Boucherville, Canada

THE HUMAN IMMUNE SYSTEM IS DRASTICALLY AFFECTED BY SPACEFLIGHT

It is established that the human immune system is highly responsive to exogenous factors, particularly physiological and psychological stress as revealed by studies on astronauts and overwintering crews in Antarctica (Crucian B. E. et al, 2016, Mehta S. K. et al, 2017, Buchheim J.-I. et al, 2020).
Influencing stress factors associated with spaceflight are microgravity, isolation, confinement, disrupted sleep and circadian rhythm, changes in nutrition and the human microbiome, lack of exposure to natural sunlight, increased exposure to radiation, and one of the most important, the psychological pressure of performing at the highest level of expectations, the risk of failure as well as the interpersonal relations between crew members.
Consequentially, the incidence of diseases or reactions (Crucian B. E. et al, 2016) as well as the reactivation of latent viruses can increase while in space (Mehta S. K. et al, 2017). These are in line with altered cytokine shedding and correlate with a shift towards an immune response profile normally associated with Th2-mediated immunity (Mehta S. K. et al, 2013).

The major methodological challenge is here that these alterations in immune function are only detected after missions are completed and that they can relate to several confounding factors.
Therefore, there is a need for close-meshed real-time monitoring of both innate and adaptive immunity, as it has been pointed out that these are not equally affected by the same conditions (Kaufmann, I. et al, 2012, Crucian B. E. et al, 2018).
This is especially important in regard of extremely long manned missions in the future where crew health maintenance is the major bottleneck that restricts the implementation of such endeavors.

µIMMUNE ADDRESSES THE LACK OF AUTOMATED REAL-TIME IMMUNE MONITORING DURING SPACEFLIGHT

The µIMMUNE project sets out to develop an automated microfluidics-based ex vivo approach for reliable analysis of immune-relevant analytes aboard the International Space Station or in Antarctic research stations in real-time in order to study the human immune system in space more closely.
Due to the design of custom-made microfluidic processing chips that are installed on the PowerBlade platform, only minimal sample volume, hands-on working time and expert resources will be required, thus facilitating the real-time monitoring. Additionally, the PowerBlade platform offers the possibility of remote control.

One focus of this project is the development of an automated whole blood incubation assay with subsequent cytokine detection.
The current challenge to overcome is the limited multiplexing of the detection of cytokines in plasma using a spatial indexing strategy. Promising proof-of-concept experiments showed that this strategy of multiplexing seems indeed suitable for this, as we did not observe any signal interference or cross-detection. We constructed chip elements (Fig. 1) that can be run manually to prove this concept and mimic the microfluidic flow that will eventually be controlled through centrifugation and pneumatic pumps on the PowerBlade. Despite this progress, another major obstacle is to develop this detection quantitatively by using calibrator samples.
Once these issues are resolved, the detection elements can be incorporated into custom-made chips that allow for automated running followed by fluorescence detection and, if technically possible, quantification.

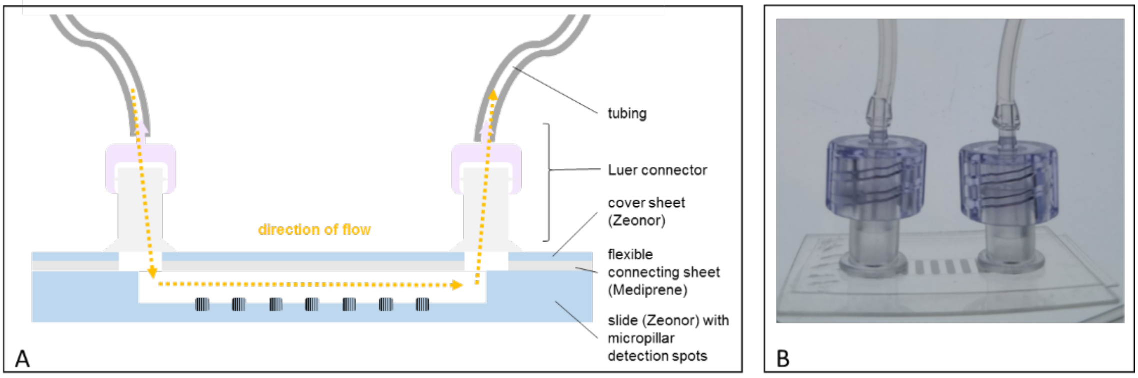


Fig. 1 (A) Cross-sectional view of a microfluidic detection element with multiple spatially separate micropillar scaffolds, each coated differently for multiplex cytokine detection. Intended for controlled running with electrical syringe pumps. **(B)** Photograph of a microfluidic detection element.

In parallel, whole blood incubation chips with different stimulating agents are in development and a validation with chip designs for manual processing (Fig. 2A) is ongoing with the attempt to downscale the volumes required for one individual sample as much as possible (Fig. 2B).

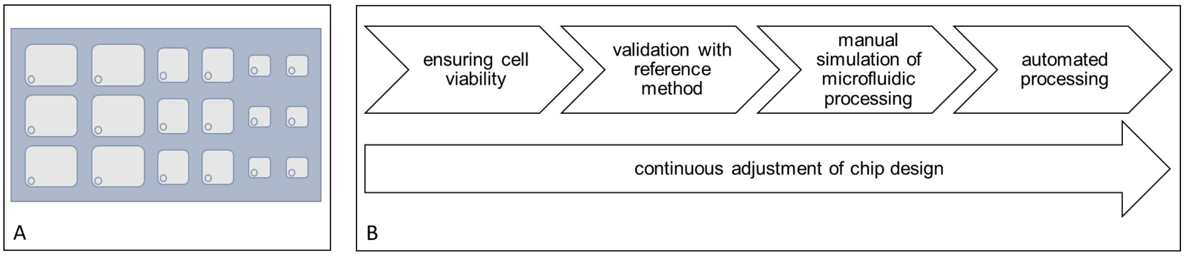


Fig. 2 (A) Design of a chip for manual processing of a cell incubation experiment with various chamber volumes. **(B)** Workflow of adaptation of a whole blood incubation assay process to PowerBlade chips.

The main objective after the establishment of these applications on the PowerBlade platform is the observation of changes in the human immune system, particularly cellular immunity, in response to the space exposome in real-time. Thus, the investigation of dynamic immune functions in astronauts during a long-term mission or in an overwintering crew in Antarctica are possible to serve as pilot studies using the PowerBlade µIMMUNE monitoring system.

REFERENCES

Crucian, B., Babiak-Vazquez, A., Johnston, S., Pierson, D. L., Ott, C. M., and Sams, C. (2016). Incidence of clinical symptoms during long-duration orbital spaceflight. International journal of general medicine, 383-391.

Mehta, S. K., Laudenslager, M. L., Stowe, R. P., Crucian, B. E., Feiveson, A. H., Sams, C. F. and Pierson, D. L. (2017). Latent virus reactivation in astronauts on the international space station. NPJ microgravity, 3(1), 11.

Buchheim, J. I., Billaud, J. N., Feuerecker, M., Strewe, C., Dangoisse, C., Osterman, A. and Choukér, A. (2020). Exploratory RNA-seq analysis in healthy subjects reveals vulnerability to viral infections during a 12-month period of isolation and confinement. Brain, Behavior, & Immunity-Health, 9, 100145.

Mehta, S. K., Crucian, B. E., Stowe, R. P., Simpson, R. J., Ott, C. M., Sams, C. F. and Pierson, D. L. (2013). Reactivation of latent viruses is associated with increased plasma cytokines in astronauts. Cytokine, 61(1), 205-209.

Kaufmann, I., Veres, T., Buchheim, J.-I. and Choukér, A. (2012). Considerations for Development and Application of Health Monitoring Tools in Space. In A. Choukér (Ed.). Stress Challenges and Immunity in Space (2, 407-420). Berlin: Springer.

Crucian, B. E., Choukér, A., Simpson, R. J., Mehta, S., Marshall, G., Smith, S. M. and Sams, C. (2018). Immune system dysregulation during spaceflight: potential countermeasures for deep space exploration missions. Frontiers in immunology, 9, 1437.

16:00 Nikita Shishkin – “Sensory Organization of Postural Control After Long Term Space Flight”

Sensory Organization Of Postural Control After Long Term Space Flight

Nikita Shishkin[1], Vladimir Kitov[1]*, Elena Tomilovskaya[1]*
[1]Laboratory of Gravitational Physiology of the Sensorimotor System, Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia
Correspondence: Nikita Shishkin, chachaturan@yandex.ru

Introduction

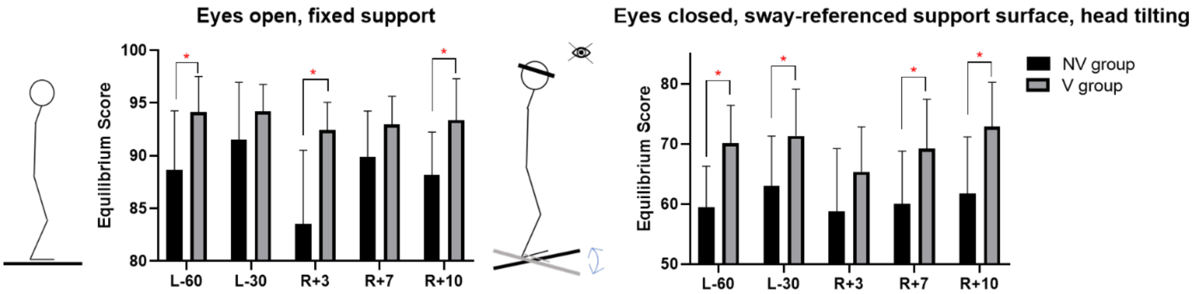
Postural stability deficit is an inevitable consequence of space flights (SF) of any duration (BLACK and PALOSKI 1998; Wood et al. 2015). In 1970 Nashner introduced a Computerized Dynamic Posturography (CDP), the development described in Black and Paloski, 1998. It is based on creating conditions when information from either visual, proprioceptive or vestibular inputs becomes unreliable or insufficient to determine the orientation of the body in relation to the gravitational vertical. Also, it has been shown that the hip strategy is engaged in complicated support surface conditions, whereas the ankle strategy is used in normal conditions (Horak and Nashner 1986). In this study we attempted to determine contributions of different sensory systems to postural control deficit in cosmonauts after long-term SF with the use of CDP and hip strategy involvement estimate.

Methods

The study involved 33 cosmonauts – crew members of expeditions to the ISS of 166-196 days duration. The studies were conducted twice before launch (L-30, L-60) and on the 3-4 (R+3), 7-8 (R+7) and 10-11 (R+10) days after landing. We used protocol from Wood et al., 2015, which include postural tests with sway referenced visual surround and support surface for disturbing visual and proprioceptive inputs and head tilting for disturbing the vestibular input. When processing the data, the scalar Equilibrium Score was calculated (Wood et al. 2015).
Postural control may rely differently on proprioceptive or visual sensory inputs in different individuals. We divided subjects into two groups, according to the sensory modality, which was determined using the Romberg coefficient (RC) – a ratio of the values of the stabilographic parameters obtained when standing with eyes closed and eyes open (Fujita et al. 2005): the group of subjects with RC>1, i.e. with the leading visual modality ("visual" or V group), and the group with RC<1, i.e. with the leading proprioceptive modality, (“non-visual” or NV group).
The cosmonauts were equipped with a system of infrared sensors (NDI OptoTrack, USA). Angles in the ankle, knee and hip joints were calculated. The average velocities of the angle fluctuations (VEL) and random mean square (RMS) of these fluctuations were analyzed.

Results

The majority of cosmonauts – 25 out of 33 – were included in V group. A separate examination of the V and NV groups revealed a better postural stability and a progressive recovery of post-flight values in V group in the tests with eyes open (EO), Sway Referenced Visual Surround, as well as with eyes closed (EC), Sway Referenced Support Surface.



In the test with EO and Fixed Support surface the significant changes in the VEL in the ankle joint were detected (from 0.13°/s to 0.17°/s, p = 0.014), while no changes were observed in the hip joint – the postural balance was provided by an ankle strategy.

In the test with EO, Sway Referenced Support Surface on the 3rd day after landing significant changes in the RMS in the hip joint were detected (from 0.12° to 0.21°, p = 0.03), as well as a significant difference from hip joint RMS in the simplest test with EO and Fixed Support Surface, conducted in the same post-flight session (from 0.12° to 0.21°, p= 0.02). These changes suggest the recruitment of hip strategy for maintaining postural balance in the conditions of disturbed proprioceptive input.

In the test with EC, Sway Referenced, Support Surface and head tilts significant changes in the VEL and RMS were found both in the ankle (from 1.23°/s to 2°/s, from 0.90° to 1.26°, p<0.001) and in the hip joints (from 0.47°/s to 0.86°/s, from 0.27° to 0.55°, p<0.001), which corresponds to 40% an increase in the median RMS value and a 44% increase in the 3rd quartile in the ankle joint, a 100% increase in the median RMS and a 135% increase in the 3rd quartile in the hip joint, which suggests an increase in the contribution of the hip strategy to balance maintenance (Kaminishi et al. 2021).

Conclusion

The changes in vestibular system activity play the leading role in reducing postural stability after a long-term space flight. The proprioceptive system is apparently the second in importance, and the visual system is of the least importance. Predominant sensory modality plays an important role in the efficacy of the postural control in sensory challenging conditions. Cosmonauts with a predominant reliance on the visual input demonstrate better stability than those who rely on non-visual sensory inputs. Long-term space flight leads to the change of postural strategy in the condition of unreliable proprioceptive input. The study was supported by the Russian Academy of Sciences (63.1).

REFERENCES

Black F., Paloski W., 1998, *Head and Neck Surgery* 118 (3), 45-51.

Fujita, T., Nakamura, S., Ohue M., Fujii, Y., Miyauchi A., Takagi Y., Tsugeno H., 2005, *Journal of bone and mineral metabolism* 23 (2), 152–156.

Horak F. B., Nashner L. M., 1986, *Journal of neurophysiology* 55 (6), 1369–1381.

Kaminishi K., Chiba R., Takakusaki K., Ota J, 2021, *Gait & posture,* 90, 67–72.

Wood S., Paloski, W., Clark J., 2015, *Aerospace medicine and human performance* 86 (12 Suppl), 45-53.

16:15 Kunihiko Tanaka – "Magnetic Vestibular Stimulation Amplifies Posture and Arterial Pressure Control"

Magnetic Vestibular Stimulation Amplifies Posture and Arterial Pressure Control

Kunihiko Tanaka, Akihiro Sugiura
Graduate School of Health and Medicine, Gifu University of Medical Science
795-1 Ichihiraga, Seki, Gifu, Japan 501-3892
ktanaka@u-gifu-ms.ac.jp

It is known that stochastic resonance phenomenon can be seen in vivo. According to the input, applying weak noise to the signal inputs improve the response output.

Nystagmus often occurs in the medical magnetic resonance imaging system with 1.5 or 3T, and the patient complains of dizziness. We hypothesized that a stochastic resonance may be caused by a weaker magnetic stimulus. In this study, we investigated the effects of lower magnetic vestibular stimulation on posture and arterial pressure (AP) control those are related to the vestibular system (Tanaka et al, 2009, 2012).

Twelve healthy subjects were recruited. Posturography was measured in eight subjects placed upright on a rubber foam with their eyes closed. AP change at the onset of head-up tilt (HUT), that is related to the vestibular function were measured in four subjects. A 0.4 T magnets (0.4T) or aluminum disks (0T) were attached behind the auricle. The average trajectory length, sway area, frequency components of the X and Y directions of posturography were significantly decreased with the magnets, compared to those with aluminum disks (Figure). Mean AP at the onset of HUT did not change without the magnet, but increased with the magnet.

Magnetic stimulation around the inner ear was suggested to amplifie the vestibular-related reflex.

REFERENCES

Tanaka K et al., 2012, *Autonomic Neuroscience* 166, 66-71.
Tanaka K et al., 2009, *Autonomic Neuroscience* 148, 90-96.

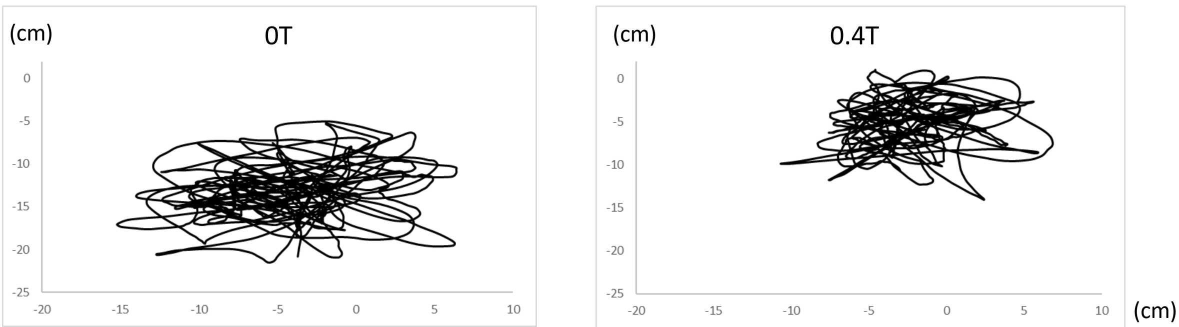


Figure: Typical responses of posturography with 0T and 0.4T of magnet

16:30 Maria Bekreneva – “Effect of 5-day Dry Immersion On Vertical Stability And Voluntary Walking in Woman”

Effect Of 5-day Dry Immersion On Vertical Stability And Voluntary Walking In Women

M.P. Bekreneva[1], A.A. Saveko[1], I.I. Ponomarev[1] and E.S. Tomilovskaya[1]
[1] Institute of Biomedical Problems of the Russian Academy of Sciences; mbekreneva@gmail.com

INTRODUCTION

According to Saralyn M. et al. (2014), during the transition to weightlessness, female astronauts had a higher incidence of space motion sickness compared to men. At the same time, during the return to Earth, male astronauts experienced motion sickness symptoms more often (47%) than their female colleagues (40%). It was also revealed that women experience more pronounced immediate post-flight orthostatic intolerance. This is probably due to lower vascular resistance in the lower extremities and greater plasma volume losses in women compared to men (Waters W.W. et al., 2002). Sex differences are observed in the structure of the brain, in the differentiation and development of neurons (Cahill L., 2006), in the perception of the vertical when the trunk is tilted (Darlington C.L. et al., 1998), in the pattern of muscle activation (Semmler J.G. et al., 1999) and biomechanics of movements (Kang J. et al., 2002).
The facts listed above make it necessary to further study the characteristics of the reactions of the female body to the factors of space flight, since at the moment the available information is insufficient to form final conclusions. Ground-based models of the key factors of space flight allow experiments to be carried out under controlled conditions and are necessary for solving such issues (Reynolds R.J. and Shelhamer M., 2020). So, until 2020, only male subjects participated in the conditions of Dry Immersion (DI) (Tomilovskaya E. et al., 2021). In 2022 at the SSC RF-IMBP RAS (Moscow, Russia) a complex experiment was conducted in a 5-day of DI with the participation of 16 women of reproductive age. Based on the data obtained in this experiment, we conducted a comparative analysis of the support reactions of the feet when performing postural and locomotor tests before and after DI exposure between groups of women and men.

METHODS

The study involved 25 volunteers: 15 female participants of the 5-day of DI, and 10 male participants of the 7-day of DI. The participants performed the test twice – before the start of DI exposure and within the first 3 hours after its completion. The experimental session included two consecutive tests: postural and locomotor. The postural test included recording the pressure exerted by the feet on the sensors of the sensory platform when the participant was asked to keep the center of pressure (COP) at a given point in a standing position for 20 seconds using biofeedback (image on the screen). Then, on command, the participant closed his eyes and continued to stand for 20 seconds, trying to remain in the same position. During the first session of the experiment, the position of the feet on the platform (the distance between the heels and toes) was fixed and repeated for each experimental session. The locomotor test included voluntary walking on the sensor platform 6 times (3 times back and forth). 2m High-End Footscan system (RSscan International, Belgium) was used for the registration of foot ground reaction forces (GRF) data during performing these tests. This system also allows a synchronous register of the main postural (the area of the ellipse of COP fluctuations and the velocity of COP displacement) and locomotor (walking speed, length and width of the step, the periods of the step cycle) characteristics. The data were analyzed using repeated measures ANOVA (GraphPad Prism version 8).

RESULTS

All tests were performed in full – we did not register any case of significant loss of balance and falling. The results obtained generally indicate the similarity of reactions to support unloading and their mechanisms in men and women, and also allow us to conclude that the DI effect on the female body is not accompanied by additional risks from locomotor and postural functions. At the same time, the results of the study allow us to identify some features of postural and locomotor control in women after DI exposure.
Thus, after DI, during standing with eyes closed, the area of the ellipse of COP increased in both women and men (Fig. 1A), but only men had a significant increase in the velocity of COP displacement (by 1.96± 0.46 mm per sec; p=0.0008; Fig. 1C) and only in women there was a significant decrease in GRF (by 175.4 ±62.56 N/m2; p=0.03). Similarly, after DI, a decrease in the velocity of voluntary walking was observed in two experimental groups (Fig. 1B), however, in the male group, the walking speed decreased due to a reduction in the length of steps and an

increase in their width. At the same time, changes in the time structure of the step were observed, which reduced the COP fluctuations when walking – a decrease in the swing time during an increase in the double support time (a postural component of the step). Interestingly the reverse changes occurred in the women group – an increase in the swing phase time (by 1.11±0.50%; p=0.03) with a reduction in the stance phase time (by 0.97±0.51%; p=0.07) and the double support time (by 2.04±0.79%; p=0.04; Fig. 1D). These features may be due to both gender differences in reactions to immersion exposure, and the different duration of DI exposure in groups.

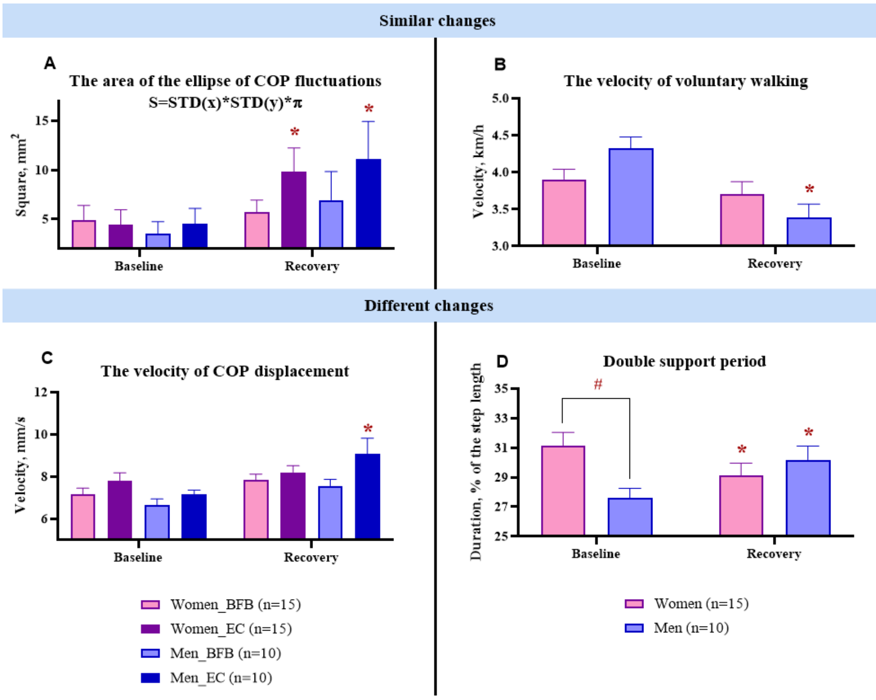


Figure 1. A – dynamics of changes in the area of the ellipse of COP fluctuations; B – dynamics of changes in the velocity of voluntary walking; C – dynamics of the velocity of COP displacement while maintaining a vertical posture (20 s); D – changes in the double support period. Baseline - before DI exposure; Recovery - the period within the first 3 hours after DI completion; BFB – measurement results with biofeedback; EC – eyes closed. * - significant difference from the initial data; # - significant difference between groups of women and men.

FUNDING

The study was supported by a grant from the Russian Science Foundation № 19-15-00435P, https://rscf.ru/en/project/19-15-00435/.

REFERENCES

Saralyn M., Scott G.B.I., Donoviel D.B. et al., 2014, Journal of Women's Health, 23(11).

Waters W.W., Ziegler M.G., and Meck J.V., 2002, Journal of Applied Physiology, 92(2), 586-594.

Cahill L., 2006, Nature reviews neuroscience, 7(6), 477-484.

Darlington C.L. and Smith P.F., 1998, Journal of Vestibular Research, 8(2), 151-153.

Semmler J.G., Kutzscher D.V. and Enoka R.M., 1999, Journal of Neurophysiology, 82(6), 3590-3593.

Kang J., Chaloupka E.C., Mastrangelo A.M. et al., 2002, Eur J Appl Physiol, 86, 503–508.

Reynolds R.J. and Shelhamer M., 2020, In Beyond LEO-Human Health Issues for Deep Space Exploration, IntechOpen.

Tomilovskaya E., Amirova L., Nosikova I. et al., 2021, Frontiers in Physiology, 12, 661959.

16:45 Olga Kuldavletova – "Comparison of postflight mission critical tests between astronauts and bilateral vestibular patients"

Comparison of postflight mission critical tests between astronauts and bilateral vestibular patients

O. Kuldavletova [1], G. Clément [1], T. Macaulay [2], S. Wood [3] and P. Denise [1]
[1] Université de Caen Normandie, INSERM, COMETE U1075, CYCERON, CHU de Caen, Normandie Univ, F-14000 Caen, France, olga.kuldavletova@unicaen.fr [2] KBR, Houston, TX 77058, USA, and [3] Neuroscience Laboratory, NASA Johnson Space Center, Houston, TX 77058, USA.

ABSTRACT

Astronauts returning from long-term missions report spatial disorientation and perceptual illusions; exhibit postural instability, impaired locomotion and manual control; and are affected by re-entry motion sickness and orthostatic intolerance (Moore et al., 2019; Mulavara et al., 2018; Reschke et al., 2017). These symptoms result from the deconditioning of the vestibular, somatosensory, muscular, and autonomic nervous systems by weightlessness. Before an adaptation takes place, astronauts' performance is impaired immediately after landing following spaceflight. These impairments occur during a critical part of the mission, because landing and egressing the spacecraft require taking control, making decisions, and acting rapidly and accurately. Functional, mission-critical tests such as tandem walk, sit-to-stand, and walk-and-turn have shown that astronauts performance during these tests are altered for up to one week after landing following long-duration stays on board the International Space Station (Clément et al., 2022).

On Earth, patients with bilateral vestibular loss (BVL) also exhibit spatial disorientation, postural instability, and gait disturbances. In these patients, the absence of vestibular input is compensated by a stronger reliance on visual and somatosensory inputs. Therefore, comparing the results of the astronauts and BVL patients can help to disentangle the role of the vestibular and somatosensory systems in the performance of these tests by astronauts. We administered three functional tests to astronauts (tandem walk and sit-to-stand: n=22, 14M/8F, mean age 48.1; walk-and-turn: n = 11, 7M/4F; mean age 42.4), BVL patients (n = 31, 14M/17F; mean age 61.4) and control participants (n = 32, 14M, 18 F; mean age 38.6). The preliminary results indicate similar impairment in performance in astronauts just after landing and BVL patients during the tests that involve challenging locomotion tasks, such as tandem walk and walk-and-turn. They suggest that the alteration of vestibular inputs is mostly responsible for the impairment of performance during these tasks.

REFERENCES

Moore, S. T., Dilda, V., Morris, T. R., Yungher, D. A., MacDougall, H. G., & Wood, S. J. (2019). Long-duration spaceflight adversely affects post-landing operator proficiency. Scientific Reports, 9(1), 2677.

Mulavara, A. P., Peters, B. T., Miller, C. A., Kofman, I. S., Reschke, M. F., Taylor, L. C., Lawrence, E. L., Wood, S. J., Laurie, S. S., Lee, S. M. C., Buxton, R. E., May-Phillips, T. R., Stenger, M. B., Ploutz-Snyder, L. L., Ryder, J. W., Feiveson, A. H., & Bloomberg, J. J. (2018). Physiological and Functional Alterations after Spaceflight and Bed Rest. Medicine & Science in Sports & Exercise, 50(9), 1961-1980.

Reschke, M. F., Good, E. F., & Clément, G. R. (2017). Neurovestibular Symptoms in Astronauts Immediately after Space Shuttle and International Space Station Missions. OTO Open, 1(4), 2473974X1773876.

Clément, G., Moudy, S. C., Macaulay, T. R., Bishop, M. O., & Wood, S. J. (2022). Mission-critical tasks for assessing risks from vestibular and sensorimotor adaptation during space exploration. Frontiers in Physiology, 13, 1029161.

17:00 Philippe Lefèvre – "A haptic illusion created by gravity"

A haptic illusion created by gravity

P. Lefèvre[1,2*], B. P. Delhaye[1,2], V. Théate[1,2], J-L. Thonnard[1,2] and L. Opsomer [1,2*]
[1]Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium, [2] Institute of Information and Communication Technologies, Electronics and Applied Mathematics, Louvain-la-Neuve, Belgium.
*philippe.lefevre@uclouvain.be
**laurent.opsomer@uclouvain.be

ABSTRACT

Human dexterity requires very fine and efficient control of fingertip forces, which relies on the integration of cutaneous and proprioceptive feedback. Here, we examined the influence of gravity on fingertip force control in the context of a force reproduction task. We trained participants to reproduce vertical forces (load forces, LF), applied isometrically by the thumb and index fingertips, in a normal gravitational environment, and tested them during parabolic maneuvers creating phases of micro- and hypergravity, thereby strongly influencing the proprioceptive feedback. We found that gravity creates the illusion that upward forces are larger than downward forces of the same magnitude. The illusion increased under hypergravity and was abolished under microgravity (Fig. 1). The illusion also affected the control of the grip force (GF) employed to secure the grasp, suggesting that it fooled the sensorimotor system. These findings suggest that gravity-dependent proprioceptive feedback contributes to the haptic estimation of forces, and have strong implications for the design of haptic devices or prostheses incorporating force feedback.

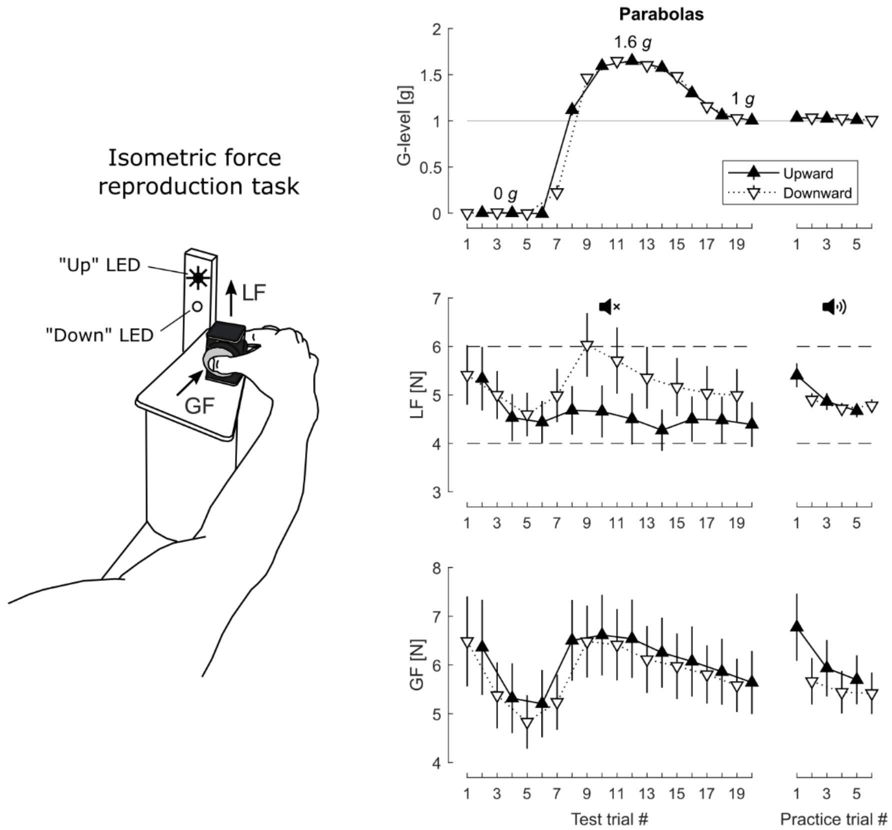


Figure 9 – Task illustration and main results. Left: experimental setup. Participants were asked to reproduce a memorized isometric LF, produced vertically (upward or downward) on a static dynamometer. Right: average Load Force (LF) and Grip Force (GF) produced during parabolic maneuvers creating phases of hyper- (>1.5g) and micro- (0 g) gravity. During the test trials, no explicit feedback was given to the participants regarding the forces they were producing. During the practice trials (always performed at 1 g), explicit feedback was provided via a beep, emitted whenever the produced LF was within the target zone ([4N, 6N]).

17:15 Michele Tagliabue – "The Role of Gravity in Eye-Hand Coordination: Effects of Short-Term and Long-Term Head-Gravity Misalignment"

The Role of Gravity in Eye-Hand Coordination: Effects of Short-Term and Long-Term Head-Gravity Misalignment

M. Tagliabuer[1], M. Beraneck[2], and J. McInytre[3]
[1]*Université Paris Cité, CNRS, Integrative Neuroscience and Cognition Center, Paris, France,* michele.tagliabue@u-paris.fr
[2]*Université Paris Cité, CNRS, Integrative Neuroscience and Cognition Center, Paris, France* mathieu.beraneck@u-paris.fr
[3]*Université Paris Cité, CNRS, Integrative Neuroscience and Cognition Center, Paris, France; Ikerbasque Science Foundation, Bilbao, Spain*, and *TECNALIA, Basque Research and Technology Alliance (BRTA), San Sebastian, Spain,* joe.mcintyre@u-paris.fr

INTRODUCTION

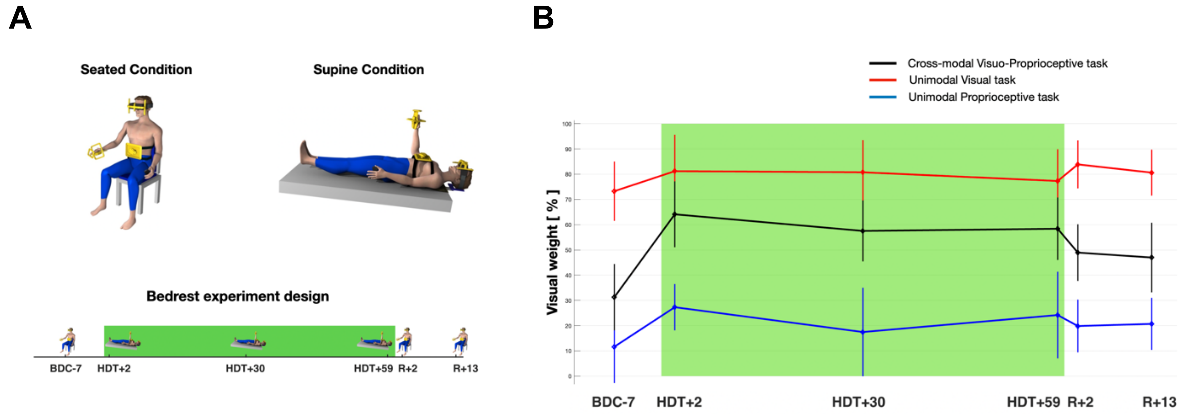
To correctly position the hand when reaching/grasping an object, visual information about the target position/orientation and proprioceptive information from the hand must be compared. Since visual and proprioceptive sensory modalities are inherently encoded in a retinal and musculoskeletal reference frame, respectively, this comparison requires cross-modal sensory transformations. Previous studies have shown that lateral tilts of the head interfere with the visuo-proprioceptive transformations (Burns and Blohm 2010, Tagliabue and McIntyre 2011, Tagliabue et al. 2013). It was not clear, however, whether this phenomenon was due to the neck flexion or the head inclination with respect to gravity. To disentangle these two hypotheses, we performed a set of experiments in which the subject performed reach-to-grasp tasks to virtual objects involving lateral head movements both in a seated and in a supine posture.

ACUTE EFFECT OF HEAD-GRAVITY MISALIGNMENT

We were able to show that when supine, the errors made by the subjects increased significantly in the tasks requiring cross-modal transformations, but not in the unimodal tasks. These modulations of the precision were accompanied by clear changes in the relative weight given to visual and proprioceptive sensory information. When interpreted in the frame of the statistical model of optimal sensory integration, these results suggest that the head misalignment with respect to gravity strongly affected the subjects' ability to perform cross-modal transformations (Bernard-Espina et al. 2022).

ADAPTATION TO HEAD-GRAVITY MISALIGNMENT (BEDREST STUDY)

We then investigated whether the subjects could adapt to a long-term misalignment with respect to gravity. To this end, we analyzed the same type of tasks in 20 subjects participating to a two months bedrest study. The results show that initial increase in response error gradually reduced during the two months suggesting a possible adaptation. The alteration of the sensory weighting, however, never went back to the pre-bedrest values, suggesting that a new multi-sensory integration strategy was learned by the subjects to optimally adapt to the horizontal posture.



A) The upper figures show the postural conditions in which the subjects were tested. The bottom scheme represents the temporal organization of the bedrest study. The subjects were tested in the seated condition during the baseline data collection (BDC) before the head down tilt (HDT) and 2 days and 13 days after the end of the HDT period. The subjects were tested in the supine posture the 2^{nd} the 30^{th} and the 59^{th} day of HDT. **B**) Value of the relative weight associated to the visual information at the six experimental sessions for the cross-modal task and for the two unimodal tasks.

CONCLUSION

These results about the effect of short-term and long-term head-gravity misalignment represent a clear evidence of the theorized central role of gravity in spatial perception and movement control (Paillard 1991). More precisely, otolithic signals appear to contribute to reciprocally align the reference frames in which the available visual and proprioceptive sensory information can be encoded.

REFERENCES

Bernard-Espina J, Dal Canto D, Beraneck M, McIntyre J and Tagliabue M, 2022, Front. Integr. Neurosci. 16:788905

Burns J.K and Blohm G., 2010, Front. Hum. Neurosci. 4:221.

Paillard J., 1991, in Brain And Space, ed. J. Paillard, Oxford: Oxford University Press, 461–481.

Tagliabue M. and McIntyre J., 2011, J. Neurosci. 31, 1397–1409.

Tagliabue M., Arnoux L., and McIntyre J, 2013, Neuroscience 248, 88–94.

17:30 Deborah Cecilia Navarro Morales – "Duration judgement in astronauts and patients with idiopathic bilateral vestibular loss"

Duration judgment in astronauts and patients with idiopathic bilateral vestibular loss

D. C. Navarro Morales[1], O. Kuldavletova[1], G. Quarck[1], P. Denise[1] and G. Clément[1]
[1] UNICAEN-INSERM U1075 COMETE, University of Caen Normandy, Esplanade de la Paix, 14032 Caen, France
(deborah.navarro-morales@unicaen.fr)

INTRODUCTION

While in space, astronauts feel the time run faster. They under-produce the one-minute duration (Navarro Morales et al., 2023) and intervals ranging from 2 to 38s (in single task or with a concurrent dual-task) (Kuldavletova et al.2023). Weightlessness decrease the tonic vestibular inputs, which might alter duration judging (Denise et al., 2022; Utegaliyev et al., 2022). We hypothesize that patients with idiopathic bilateral vestibular loss under-produce time intervals as astronauts do during spaceflights.

METHODS

15 patients with idiopathic bilateral vestibular loss according to the criteria of the classification committee of the barany society (10 females, 5 males; age M = 60.1, SD = 13.2) (Strupp et al., 2017) and 15 healthy subjects (6 females, 9 males; age M = 43.2, SD = 18.8) participated in this study. It should be noted that patients have kept a residual cVEMP reflex. The experimental methodology employed in this study was identical to that of Kuldavletova et al. (2023), and Navarro Morales (2023). We use three production of duration tasks : production of time intervals ranging from 2 to 38s in two conditions, a counting condition (single-task) and a concurrent reading condition (dual-task) and the one-minute test.

RESULTS

Bilateral vestibular loss under-produced intervals during the production single-task ($p<0.001$), the production dual-task ($p<0.001$) and the production of one-minute ($p=0.007$) compared to the control subjects.

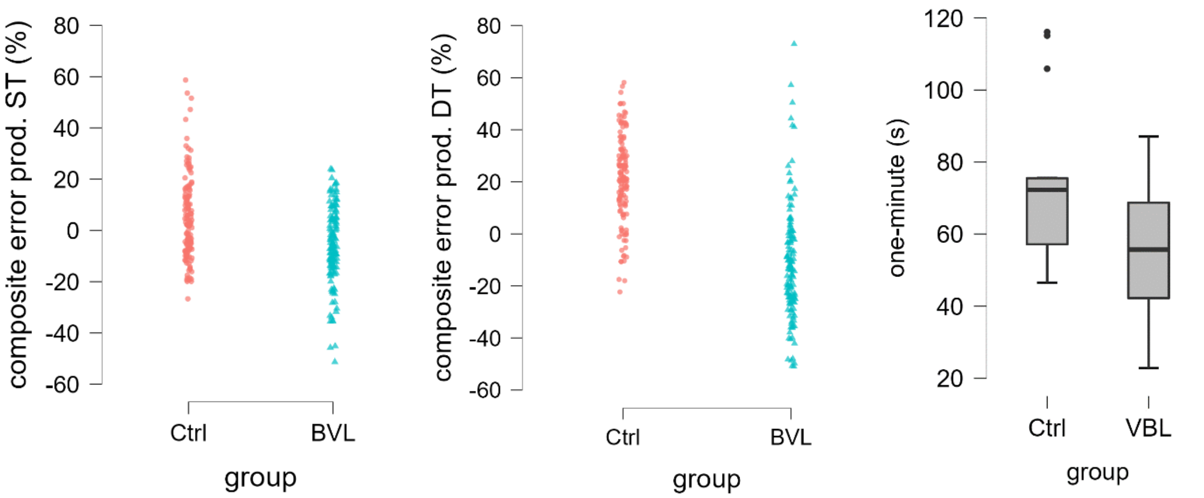


Figure 10: Production tasks of bilateral vestibular loss compared to control subjects. In the left panel production single-task, in the central panel the production dual-task. Analysis of these two tasks were done with the composite error (%). In the right panel the production of one-minute test.

CONCLUSIONS

This study suggests that alteration in vestibular system affects time perception.

REFERENCES

Denise, P., Harris, L. R., & Clément, G. (2022). Editorial : Role of the vestibular system in the perception of time and space. Frontiers in Integrative Neuroscience, 16, 1067425. https://doi.org/10.3389/fnint.2022.1067425

Kuldavletova O, Navarro Morales DC, Quarck G, Denise P, Clément G (2023) Spaceflight alters reaction time and duration judgment of astronauts. Frontiers in Physiology (in press)

Navarro Morales DC, Kuldavletova O, Quarck G, Denise P, Clément G (2023) Time perception in astronauts on board the International Space Station. Nature Microgravity 9: 6; doi: 10.1038/s41526-023-00250-x

Strupp, M., Kim, J.-S., Murofushi, T., Straumann, D., Jen, J. C., Rosengren, S. M., Della Santina, C. C., & Kingma, H. (2017). Bilateral vestibulopathy : Diagnostic criteria Consensus document of the Classification Committee of the Bárány Society1. Journal of Vestibular Research, 27(4), 177-189. https://doi.org/10.3233/VES-170619

Utegaliyev, N., von Castell, C., & Hecht, H. (2022). Vestibular Stimulation Causes Contraction of Subjective Time. Frontiers in Integrative Neuroscience, 16, 831059. https://doi.org/10.3389/fnint.2022.831059

11:30 Peter Fernandez – "Preliminary insights into bone adaptation and differences in energy metabolism between males and females under (DI) conditions"

Preliminary Insights Into Bone Adaptation and Differences In Energy Metabolism Between Males and Females Under (DI) Conditions

P. Fernandez[1], M. Linossier[1], L. Peurière[1], C. Bonneau[2], M. Bareille[3], A. Beck[3], G. Gauquelin-Koch[4], A. Ombergen[5], L.Vico[1]

[1]Université Jean Monnet Saint-Étienne, Mines Saint Etienne, INSERM, SAINBIOSE U1059, F-42023, Saint-Etienne, France, peter.fernandez@kcl.ac.uk, linossier@univ-st-etienne.fr, peuriere.laura@gmail.com, vico@univ-st-etienne.fr
[2]Université Jean Monnet Saint-Étienne, CHU Saint-Étienne, Department of Biochemical Analysis, F-42023, Saint-Etienne, France, christine.bonneau@chu-st-etienne.fr
[3]Institute of Space Physiology and Medicine (MEDES), Toulouse, France, marie-pierre.bareille@medes.fr, arnaud.beck@medes.fr
[4] French Space Agency (CNES), Paris, France, guillemette.gauquelinkoch@cnes.fr
[5]European Space Agency (ESA), angelique.van.ombergen@esa.int

INTRODUCTION

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

METHODS

A total of 36 healthy participants (eighteen males 33.61yrs±5.52) and (eighteen females 29yrs±4.76) underwent five days of DI (Fig.1), which included a 48hr post-DI recovery period. All participants underwent a comprehensive medical assessment and a thorough physical examination which included a VO2max assessment prior to enrolment. All participants were deemed medically fit by a certified physician and were free from acute and chronic illnesses. All female participants had regular menstrual cycles and were not on the combined oral contraceptive pill. All antecubital venous blood samples were collected at fasting and at the same time (approximately 07:30 AM) for the duration of the study. Samples were taken at baseline (BDC-24h), after 24h-(DI-24h), 48h-(DI-48h), 120h-(DI-120h) immersion, and finally 48h after the return to loading conditions (R+2)

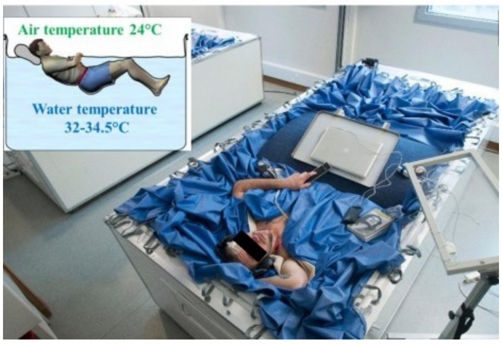


Figure 11:Participant under DI conditions. Image adapted from (Linossier et al.,2017)

C-terminal crosslinked telopeptide of type I collagen [CTx], procollagen type I N-terminal propeptide [P1NP], bone alkaline phosphatase [bAP], intact and N-mid osteocalcin fragment [OC] and insulin-like growth factor 1 [IGF1] were determined by automated chemiluminescence immunoassay (IDS-iSYS automated analyzer, Boldon, UK). Enzyme-immunoassay (EIA) kits measured the following parameters: tartrate-resistant acid phosphatase isoform 5b [TRAP5b] (Microvue Bone, Quidel Corporation, San Diego, CA, USA); undercarboxylated [Glu-OC] and carboxylated [Gla-OC] OC (Takara Bio, Inc., Otsu, Japan); a secretory form of nicotinamide phosphoribosyl-transferase [visfatin] (Adipogen AG, Liestal, Switzerland); periostin (Biomedica Medizinprodukte GmbH, Wien, Austria). Serum intact parathyroid hormone [1-84 PTH], calcium and phosphorus were measured using electrochemiluminescence immunoassay (Cobas®8000 modular analyzer, Roche Diagnostics Ltd., Rotkreuz, Switzerland).

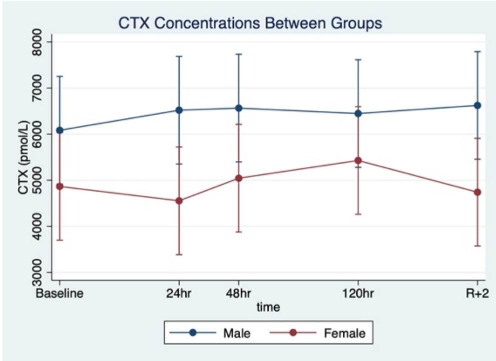


Figure 2: CTX concentrations between groups

RESULTS & CONCLUSION

Five days of DI highlighted differences in adaptation between groups, particularly in females. When looking at bone metabolism, bone formation markers exhibited higher concentrations in males than females. Interestingly, higher amounts of undercarboxylated OC were observed in females throughout all timepoints, with peak concentrations as early as 48hrs in both groups. Furthermore, CTX (Fig.2) a resorption marker, was lower in females and reached peak concentrations later than in males. In addition, osteocyte function was significantly more impaired in females throughout the DI period and recovery period (Fig.3). Taken together, the DI-induced changes in bone remodeling markers demonstrated an uncoupling effect favoring bone resorption during DI and the recovery phase in both groups with more pronounced changes in females.

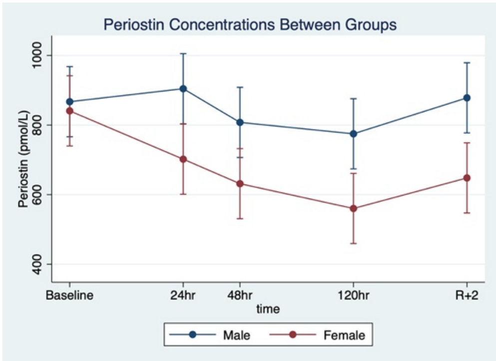


Figure 3: Periostin concentrations between groups

REFERENCES

Linossier M-T., (2017), PLoS One 12, e0182970.

Linossier M-T., (2022), Frontiers in Physiology, 801448.

11:45 Florian Pfeiffer – "Inspired by Space: E-Nose technology provides diagnostic tool for SARS-CoV-2 infection by breath analysis"

Inspired by Space: E-Nose technology provides diagnostic tool for SARS-CoV-2 infection by breath analysis

Florian Pfeiffer[1] for the E-NOSE study group*
[1]Laboratory of Translational Research 'Stress and Immunity', Department of Anesthesiology, LMU Hospital, Ludwig-Maximilians-University, Munich, Germany.

BACKGROUND

E-Nose Technology was applied in Space to investigate microbial contamination on the ISS. During the SARS-CoV-2 pandemic, the need for a fast, cheap and easy-to-use diagnostic tool for respiratory diseases has been apparent. Volatile organic compounds (VOC) in exhaled breath could provide this diagnostic potential, but current gold-standard mass-spectrometry analysis is neither fast nor inexpensive. In contrast, electronic nose (E-Nose) technology could be made widely available as it does not need lab support, and measurements are completed within ten minutes. This study aimed to establish a diagnostic tool for SARS-CoV-2 infection using E-Nose technology.

METHODS

Subjects with (n=63) and without (n=63) SARS-CoV-2 infection were included in this study. For breath collection, the ReCIVA® Breath Sampler was used in combination with Tenax® sorption tubes for enrichment of VOCs. Samples were analyzed using an Enrichment and Desorption unit (EDU) and a Portable Electronic Nose (PEN). Sorption tubes were heated by the EDU to detach stored VOC and samples were then injected into the PEN equipped with an array of ten metal oxide semiconductor sensors. Changes in conductivity were recorded. Three independent groups were tasked with analyzing the resulting data. Artificial Intelligence algorithms were applied and included variations of classifiers, their hyperparameters, training mode, and subsets of training data.

FINDINGS

The applied sampling method generated robust data for reliable E-Nose measurements. Statistical analysis showed that random forest and gradient boosting decision tree models achieved the best classification accuracy. Successful discrimination between SARS-CoV-2 infected and uninfected subjects was achieved using this newly established breath sampling protocol. Including information of all ten sensors lead to a misclassification error (ME) of 0.079. Training the AI model to use only data from the most relevant sensor further improved on this performance with ME of 0.062.

INTERPRETATION

In this study, VOC enrichment and E-Nose technology combined with machine learning algorithms to detect SARS-CoV-2 yielded classification results comparable to PCR analysis and superior to POC antigen testing. Using only the most relevant of the 10 sensors may be sufficient for future commercial POC testing. Studies on other types of pulmonary infection are warranted to establish rapid diagnostic approaches, especially for application in remote locations with where medical infrastructure is scarce, on Earth and in Space.

FUNDING

This study was supported by the German Aerospace Center (DLR) and the Federal Ministry of Economic Affairs and Technology (grant #50RP1920, to A Choukér).

*E-NOSE-Study Group
Maximilian Mandl[2], Wolfgang Sobtzick[4], Jörg Heitzer[3], Alisa Krstova[3], Luzie Kamm[1], Matthias Feuerecker[1], Dominique Moser[1], Viktor Fetter[3], Matthias Klein[6], Benedikt Aulinger[5], Michael Dolch[1], Anne-Laure Boulesteix[2], Daniel Lanz[4], Alexander Choukér[1], Tobias Woehrle[1;]

[2]Institute for Medical Information Processing, Biometry and Epidemiology, Faculty of Medicine, LMU Munich, Munich, Germany.[3]Airbus Defence and Space GmbH, Claude-Dornier-Straße, 88090 Immenstaad, Germany.
[4]LANZ GmbH, Am Branderhof 6. 51429 Bergisch Gladbach, Germany.
[5]Department of Medicine II, LMU Hospital, Ludwig-Maximilians-University, Munich, Germany.
[6]Emergency Department, LMU Hospital, Ludwig-Maximilians-University, Munich, Germany

12:00 Federico D'Amico – "Immune cell activation state in 5-day Dry Immersion: a comparative study between a female and male cohort"

Immune cell activation state in 5-day Dry Immersion: a comparative study between a female and a male cohort

Federico D´AMICO[1], Alexander CHOUKÉR[1] and Dominique MOSER[1] for the VIVALDI STUDY TEAM*

[1]Laboratory of Translational Research 'Stress and Immunity', Department of Anesthesiology, LMU Hospital, Ludwig-Maximilian-University, Munich, Germany. * Dominique.Moser@med.uni-muenchen.de

*VIVALDI STUDY TEAM: Angelique Van Ombergen, Adrien Robin, Audrey Bergouignan, Laurence Vico; Claire Laurens; David Green; Elisabeth Rosnet; Guillaume Py; Hervé Normand; Michael Tipton; Anne Pavy - Le Traon; Pierre Denise; Stephane Blanc; Angèle Chopard; Nastassia.Navasiolava; Marc-Antoine Custaud; Inês Antunes; Marie-Pierre Bareille; Rebecca Billette de Villemeur

ABSTRACT

Immune system dysregulations can occur both during and after spaceflight and pose a serious threat to long-duration manned missions (Crucian et al., 2008). Spaceflight-related immune impairment can result in reactivation of latent viruses such as HSV (Mehta SK et al., 2014), increase in allergic reactions or newfound allergies, worsening or slatentization of autoimmune diseases, and more (Crucian et al., 2018). An array of factors can affect the immune system in such conditions, including microgravity, physical and psychological stress, physical activity, radiation, alteration of cyrcadian rhytms and nutrition (Crucian et al., 2018), although the extent and details of such alterations is still subject of ongoing investigations. Given the peculiar logistic of conducting investigations in a spaceflight setting, ground analogues are often adopted as a surrogate to simulate one or more of spaceflight-associated conditions like isolation and weightlessness. Most commonly used models for microgravity include head-out water immersion (WI), Dry Immersion (DI), horizontal bed rest (HBR) and head-down bedrest (HDBR). WI was first proposed to investigate rapid-onset microgravity effects (Beckman et al., 1961) until DI was developed for long-duration studies (Shulzhenko et al., 1976). In DI, subjects are immersed in a water bath covered by a folded waterproof fabric, allowing the participant freedom of movement. This model provides hypokinesia and a greater degree of mechanical unloading when compared to other analogues, such HDBR.

While the effects of DI on muscoloskeletal, cardiovascular and sensori-motor systems have been thoroughly investigated (Tomilovskaya et al., 2019), few studies have assessed the immune system in this model (Ponomarev et al., 2013). Moreover, until very recently (Tomilovskaya et al, 2021), DI studies have been performed only on male subjects. As of December 2022, roughly 12% of all-time astronauts have been female, a percentage quickly destined to increase – thus, the need for a systematic approach to sex difference in spaceflight-related health effects becomes clear, as well as for comparative studies in ground models.

In the ESA-sponsored study at MEDES Space Clinic (Toulouse, France) a team of experts investigated the effects of dry immersion on many physiological systems for 5 days in females and males. In this consortial effort our team has evaluated innate and adaptive immune cell activation states over the course of two separate 5-days Dry Immersion studies. 20 healthy females and 20 healthy males aged between 20 and 40 were separately enrolled in each study. Peripheral blood was drawn at multiple timepoints during the study and analyzed for monocytes, granulocytes and lymphocytes expressing HLA-DR, TLR2, TLR4, CD4, CD8, CD11b, CD14, CD16, CD28, CD40, CD62L, CD66b, CD69 and CD86 receptors.

Activation markers on monocytes (CD14+) were not significantly affected by DI in both sexes, although males showed a higher pre-existing activation, as demonstrated by higher level of activation markers such as CD40, TLR2 and CD69. Monocytes subsets were, instead, significantly affected by DI in males: classical monocytes (CD14++, CD16-) decreased with a corresponding increase of intermediate (CD14+, CD16+) and non-classical (CD14dim, CD16+) monocytes, whereas in females no such trend was observed.

Granulocyte activation markers were, as well, not significantly affected by DI, with males showing again a pre-existing higher activation state compared to females, particularly with CD62L and CD66b levels.

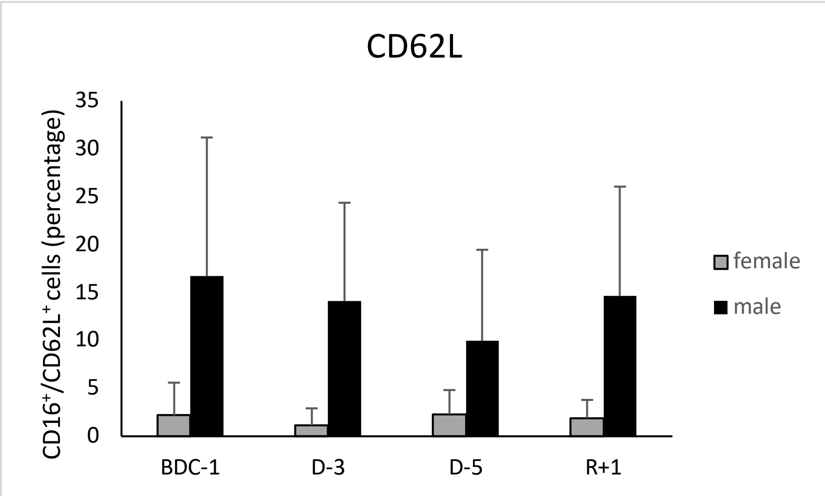


Figure 1. Comparison of CD62L expression on granulocytes ($CD16^+$) between males and females at four timepoints (BDC-1 = baseline, D-3 = DI day 3, D-5 = DI day 5, R+1 = first day after DI).

DI did not significantly affect T-cell activation state in both sexes. A significant difference in activation markers was present between sexes, where males displayed a lower CD28 expression and a higher CD69 expression than females on both CD4+ and CD8+ T cells.

This study is, to our knowledge, among the firsts to include an all-female cohort in a DI protocol, and the first to compare immunity markers between males and females in this setting. These preliminary data, which will be further complemented by humoral factors - such as cytokine panels and assessment of circulating soluble markers - show that DI does not significantly affect immune cell activation state, while highlighting pre-existing differences between sexes. Although the biological sex plays a considerable role in infectious (Goble et al., 1973) and autoimmune diseases (Whitacre et al., 2001), few studies tried to assess sex-based differences in immunity during spaceflight, partly due to reduced sample sizes (Kennedy et al., 2014). Given the known differences in immune responses and the higher likelihood of mixed crews in the near future, it is important to assess if ground-based models are adequate to investigate particular aspects of immunity. Here, a deeper immune function analyses of these VIVALDI cohorts in samples available to us will verify these findings and help integrating these observations into other organ changes assessed by the VIVALDI study group. The results of such an integrative view will pave future studies and mission designs, respectively.

REFERENCES

Beckman E. L., Koburn K. R., Chambers R. M., Deforest, Re, Augerson WS, Benson V. G., 1961, Physiologic changes observed in human subjects during zero G-simulation by immersion in water up to neck level. Aerospace Med. 32 1031–1041
Crucian BE, Stowe RP, Pierson DL, Sams CF, 2008, Immune system dysregulation following short- vs long-duration spaceflight. Aviat Space Environ Med. Sep;79(9):835-43.
Crucian BE, Choukèr A, Simpson RJ, Mehta S, Marshall G, Smith SM, Zwart SR, Heer M, Ponomarev S, Whitmire A, Frippiat JP, Douglas GL, Lorenzi H, Buchheim J-I, Makedonas G, Ginsburg GS, Ott CM, Pierson DL, Krieger SS, Baecker N and Sams C, 2018, Immune System Dysregulation During Spaceflight: Potential Countermeasures for Deep Space Exploration Missions. Front. Immunol. 9:1437
Kennedy AR, Crucian BE, Huff JL, 2014, Effects of Sex and Gender on Adaptation to Space: Immune System. J Females's Health 23(11):956–8. 10.1089/jwh.2014.4913
Mehta SK, Laudenslager ML, Stowe RP, Crucian BE, Sams CF, Pierson DL, 2014, Multiple latent viruses reactivate in astronauts during Space Shuttle missions. Brain Behav Immun. 2014 Oct; 41:210-7.
Navasiolava NM, Custaud M A, Tomilovskaya ES, Larina IM, Mano T, Gauquelin-Koch G, Gharib C, Kozlovskaya IB, 2011, Long-term dry immersion: review and prospects. Eur. J. Appl. Physiol. Jul; 111 (7):1235-60 (Review).
Ponomarev, S.A., Rykova, M.P., Antropova, E.N. et al., 2013, Human innate immunity under the conditions of five-day dry immersion. Hum Physiol 39, 780–786
Shulzhenko E. B., Vill-Villiams I. F., 1976, The opportunity to conduct long-term water immersion method Dry Immersion. Russ. Biol. Aerospace Med. 9 82–84
Tomilovskaya E, Shigueva T, Sayenko D, Rukavishnikov I and Kozlovskaya , 2019, Dry Immersion as a Ground-Based Model of Microgravity Physiological Effects, Front. Physiol. 10:284
Tomilovskaya E, Amirova L, Nosikova I, Rukavishnikov I, Chernogorov R, Lebedeva S, Saveko A, Ermakov I, Ponomarev I, Zelenskaya I, Shigueva T, Shishkin N, Kitov V, Riabova A, Brykov V, Abu Sheli N, Vassilieva G, Orlov O., 2021, The First Female Dry Immersion (NAIAD-2020): Design and Specifics of a 3-Day Study. Front Physiol. Jun 14; 12:661959
Whitacre CC, 2001, Sex differences in autoimmune disease. Nat Immunol. Sep;2(9):777-80. doi: 10.1038/ni0901-777.

12:15 Angela Kubik – "Defining CDKN1a/p21-induced changes to the functional and regenerative capabilities of bone-marrow derived stem cells in models of aging, loading, and spaceflight"

Defining CDKN1a/p21-induced Changes to the Functional and Regenerative Capabilities of Bone-Marrow Derived Stem Cells in Models of Aging, Loading, and Spaceflight

Angela Kubik[1], Margareth Cheng-Campbell[2], Noah Allen[1], Meghan Pendyala[1], Hera Song[1], Jasmine Joshi[1], Harshitha Narahari[1], Yang Bai[1], Cassandra Juran[2,3], Eduardo Almeida[4], Deva Chan[5], and Elizabeth Blaber[1,4]
[1]Department of Biomedical Engineering, Center for Biotechnology & Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA [2] Blue Marble Institute of Science, NASA Ames Research Center, Moffett Field, CA, 94035 [3] College of Arts & Sciences, Embry-Riddle Aeronautical University, Daytona Beach, FL, 32114, USA [4]The Space Bioscience Division at NASA Ames Research Center, Moffett Field, CA, 94035, USA [5]Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN, 47907, USA

INTRODUCTION

Over the past decade, growth of the private space sector and advancements in space technology have lowered launch costs by almost ninety five percent, dramatically increasing human presence in outer space. Once a domain led by a few spacefaring nations, the cosmos is now accessible to civilian missions, student experiments, and commercial launches. This highlights the need to understand the physiological effects of prolonged exposure to spaceflight factors. The documented effects of sustained unloading and heavy ion (HZE) radiation are widespread, encompassing the musculoskeletal system, immune system, cardiovascular system, and more. Recently, studies have demonstrated that extended exposure to the spaceflight environment resembles that of physiological aging on Earth, through increased oxidative stress (Buettmann, E.G. et al., 2022), mitochondrial dysfunction (Nguyen, H.P. et al., 2021), and decreased wound healing (Buchheim, J.I. et al., 2019). In addition to these pathological overlaps between spaceflight and aging, recent findings suggest that spaceflight activates premature senescence via upregulation of CDKN1a/p21 (Blaber, E.A. et al., 2013) in a load-dependent manner (Juran, C.M., et al., 2021) and ultimately, impairs stem cell-based tissue regeneration; another hallmark of cellular aging. Although phenotypic correlations have been drawn between spaceflight- and age-induced effects, there is little insight into the mechanistic changes. We therefore aimed to leverage next generation sequencing, senolytic mouse models, and ex vivo culturing techniques to demarcate the molecular mechanisms driving load- and age-related degenerative function. Here, we present a comparative analysis of multiple studies, which together, interrogate the age-related, senescence-associated, and load-induced state of mesenchymal and hematopoietic precursors derived from the bone marrow milieu on a functional and transcriptomic level.

METHODS

(1) We first conducted a 30-day hindlimb unloading (HU) study comprised of 16-week-old wildtype (WT) and CDKN1a/p21 knockout (KO) female mice. Cohorts of the WT and KO mice were subjected to various loading interventions, such as a treadmill-facilitated exercise regimen three times weekly and/or a 14-day recovery period following HU. Control groups were normally ambulated, not exercised, and not allowed to recover. Mice were euthanized by CO_2 asphyxiation followed by cervical dislocation. Whole right hindquarters were dissected and preserved in 4% PFA to assess microarchitectural changes to cortical and trabecular bone via high resolution microCT (Bruker Skyscan 1272). Femurs and tibiae from the left hindlimb were separated and cleaned. Bone marrow from femurs were flushed via centrifugation for scRNA-seq, while tibiae were preserved in PBS-soaked gauze to capture mechanical changes via 3-point bending and physiochemical changes via Raman Spectroscopy (WITec Alpha 300R). **(2)** Next, we performed a complementary study involving an aged model of the WT and KO mice described in the previous experiment. Mice from both genotypes were aged to 11 weeks, 18 weeks, 12 months, 18 months, and 24 months. At the defined age, mice were dissected, and bone marrow was syringe-flushed from tibiae and femurs. Bone marrow cells were plated and fed with osteoblastic differentiation media every three days. After 30 days, wells were preserved to assess mineralization via von Kossa staining, senescence fraction via beta-galactosidase staining, and RNA gene expression. A portion of cells were then replated and grown under osteoblastic media for a subsequent 30 days. To test regenerative potential, the same endpoints were assessed on the replated cells. All scRNA-seq analysis was performed in R using the packages Seurat and Monocle3, while all statistical analyses were performed in R or GraphPad Prism.

RESULTS

According to our findings, there are no significant differences in tibial stiffness or Young's modulus between groups. However, an interaction between mechanical loading and exercise in tibial stiffness may suggest exercise recovers stiffness in HU groups that exhibit slightly decreased stiffness without exercise. Our data also show the maximum load tibiae withstand increases with exercise and decreases in KO mice. Interestingly, there is also an interaction between load and exercise post yield displacement suggesting exercise mitigates the increased ductility of HU tibiae. Gene expression (scRNA-seq) data reveal similar shifts in immune progenitor populations after both loss of mechanical signaling and aging. Additionally, trajectory analysis and GO enrichment analysis show the progression of certain hematopoietic lineages (i.e. B Cells) respond more sensitively to exercise-facilitated loading upon inhibition of senescence-associated molecules, such as those seen to increase with age and spaceflight (**Fig. 1a-c**). Lastly, *ex vivo* culture of bone marrow-derived stem cells under osteoblastic induction suggest that senescence propagates with age, even in CDKN1a/p21$^{-/-}$ mice. However, mineralization and regenerative potential is enhanced in KO cultures across all age groups (**Fig. 1d**). Overall, these results highlight the commonalities in mechanisms of senescence-associated cellular aging and spaceflight-induced tissue degeneration.

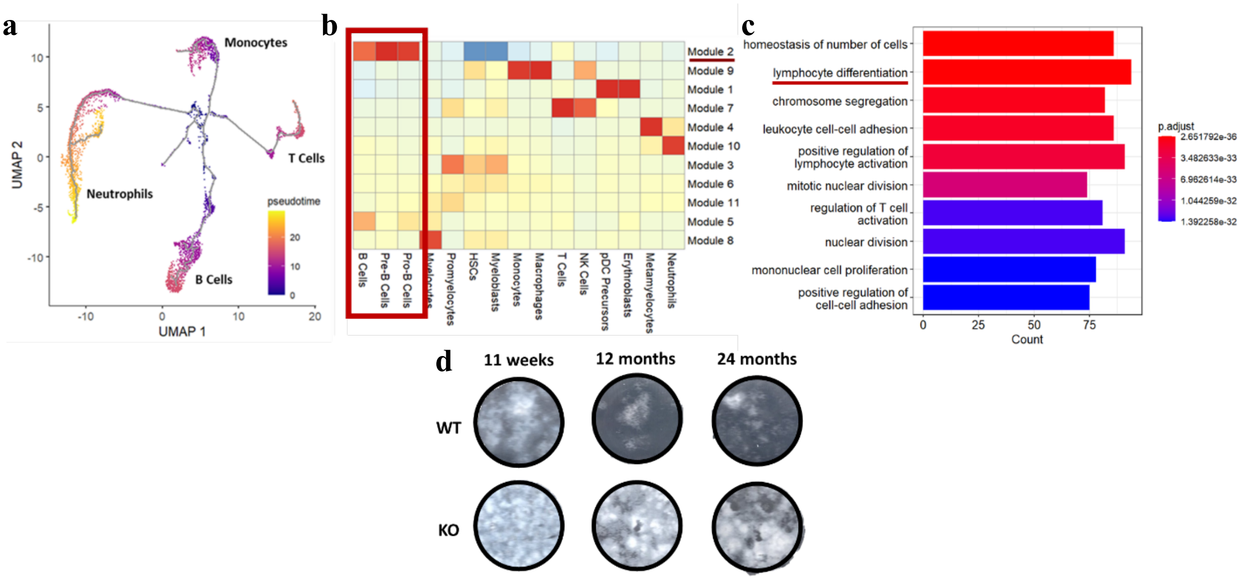


Figure 1. (a) UMAP with overlayed pseudotime trajectory analysis, (b) pseudotime-dependent co-expressed gene modules significantly altered with exercise and HU, (c) top enriched GO terms with exercise and HU, (d) von Kossa staining of osteoblast mineralization from aged WT and KO mice.

REFERENCES

Buettmann, E.B., …, and Donahue, H.J., 2022, *Journal of Bone and Mineral Research*, 37, 1417-1434. Nguyen, H.P., …, and Yang, S.G., 2021, *npj Microgravity*, 44. Buchheim, J.I., …, and Chokèr, A., 2019, *Frontiers in Physiology*, 10. Blaber, E.A., …, and Almeida, E.A., 2013, *PLoS ONE*, 8. Juran, C.M., …, and Almeida, E.A., 2021, *Stem Cell Research*, 56.

Katharina Block – "Project NEMUCO - The neuromuscular junction-like structure in vitro to study muscle and nerve cellcell communication in space"

Project NEMUCO - The neuromuscular junction-like structure *in vitro* to study muscle and nerve cell-cell communication in space

K. Block[1,2], G. Trautmann[1], A. Bosutti[3], A. Nori[4], S. Furlan[4], M. Gutsmann[1], P. Lorenzon[3], P. Volpe[5], D. Blottner[1,2], M. Salanova[1,2]

[1] Charité–Universitätsmedizin Berlin, Institute of Integrative Neuroanatomie, Berlin, Germany, [2] Center of Space Medicine Berlin, Berlin, Germany; [3] University of Trieste, Department of Life Sciences, Trieste, Italy, [4] University of Padova, Department of Biomedical Sciences, Padova, Italy, [5] CNR-Neuroscience Institute, Section of Padua, Padova, Italy.

ABSTRACT

Introduction

Chronic muscle unloading in different neuromuscular diseases, myopathies, denervation, aging and/or long term bedrest or spaceflight missions result in significant decline in neuromuscular junction (NMJ) structure and function, with impact on muscle mass and thus, fine motor performance control. Here we established a 3D co-culture experimental model that allows for generating NMJ-like structures in vitro to investigate muscle and nerve cell-cell communication in space.

Methods

Murine C2C12 myoblast and NSC-34 neuronal cells were co-cultured on both synthetic and biological 3D scaffold coated with extracellular matrix in presence of neural agrin. Cells were differentiated according to an established experimental protocol, and thereafter processed, by immunocytochemistry, for several NMJ-specific molecular markers, in combination with muscle- and nerve-cells specific differentiation markers. Digitized z-stacks images were acquired at different time points by a high-resolution confocal microscope, and further processed with the Leica Application Suite X (LAS X) software. Western blot experiments have been carried out to assess the expression pattern of proteins of interest related to the myogenic maturation program and the developing NMJ.

Results

In our 3D co-culture system, specialized cell-cell contacts were present suggestive of NMJ-like structures. Triple immunostaining indicated that neurofilament-positive axonal nerve endings approaching α-bungarotoxin-positive clustered acetylcholine receptors (AChRs) in desmin- and myosin-positive myotubes, suggesting pre-assembly of NMJ-like structure in vitro. 3D reconstruction of z-stack images/morphometry analysis measuring the distance between the axon terminal and the acetylcholine receptor positive region showed a distance of approx. 88 nm in the synthetic scaffold and a distance of approx. 50 nm in the biological scaffold. These results are comparable to in vivo synaptic cleft sizes. In addition, biochemical analysis of protein expression showed higher expression of muscle proteins in the co-culture than in the myotubes alone.

Conclusions

NMJ-like structures are present in our in vitro 3D co-culture system suggesting the reliability of our experimental protocol to generate specialized cell-cell communications/contacts in vitro. Patch clamp experiments will allow us to assess the electric cell-cell communication and thus, confirm the establishment of functional synapses. The spatial and temporal expression of different NMJ molecular players on earth (1g) vs space (microgravity) is of particular interest to further understand the molecular and cellular mechanisms leading to NMJ imbalance in different extreme environmental conditions and to further develop reliable countermeasure interventions.

Sponsors

German Aerospace Center (DLR), Cologne; BMWI/DLR e.V. (#50WB2116), and Charité Universitätsmedizin Berlin, Berlin, Germany

Kyung-Ju Shin – "Microgravity induces oxidative stress and mitochondrial dysfunction which is mittigated by TPP-niacin in retinal epithelial cells"

MICROGRAVITY INDUCES OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION WHICH IS MITTIGATED BY TPP-NIACIN IN RETINAL EPITHELIAL CELLS

Kyung-Ju Shin[1,2,], Seungheon Shin[3], Phuong Hoa Tran[1,2], Ngoc Thuan Truong[1,2], Steve K. Cho[3,4] Su-Geun Yang [1,2], and Kyu-Sung Kim[1,]

[1]Inha Institute of Aerospace Medicine, and [2]Department of Biomedical Science, BK21 FOUR program in Biomedical Science and Engineering, Inha University College of Medicine, Incheon 22332, Republic of Korea

[3]Department of Biomedical Science and Engineering, Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea.

[4]School of Life Sciences, Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea

(Kyung-Ju Shin : kjshin123@gmail.com, *Su-Geun Yang: Sugeun.Yang@inha.ac.kr)

ABSTRACT

Microgravity and space radiation after space travel influences on the human body. One of the most sensitive organs affected by them is the eye, particularly the retina. The conditions that astronauts suffer, such as visual acuity, hyperopic refractive error shifts, and nerve fiber layer infarcts, is collectively called a spaceflight-associated neuro-ocular syndrome (SANS). However, the underlying molecular mechanism of the microgravity-induced ocular pathogenesis is not clearly understood. The current study explored how microgravity affects the retina function in ARPE19 cells in vitro under the simulated microgravity (μG) generated by clinostat. We found multicellular spheroid (MCS) formation and a significantly decreased cell migration potency under μG conditions compared to 1G in ARPE19 cells. We also observed that μG increases intracellular reactive oxygen species (ROS) and causes mitochondrial dysfunction in ARPE19 cells. Subsequently, we showed that μG activates autophagic pathways. Furthermore, we demonstrated that mitophagy activation is triggered via the mTOR-ULK1-BNIP3 signaling axis. Finally, we validated the effectiveness of TPP-Niacin in mitigating μG-induced oxidative stress and mitochondrial dysfunction in vitro, which provides the first experimental evidence for TPP-Niacin as a potential therapeutic agent to ameliorate the cellular phenotypes caused by μG in ARPE19 cells. Further investigations are, however, required to determine its physiological functions and biological efficacies in primary human retinal cells, in vivo models, and target identification.

REFERENCES

Nguyen, H.P., et al., The effects of real and simulated microgravity on cellular mitochondrial function. 2021, NPJ Microgravity, 7(1): p. 44.

Cialdai, F., et al., Effect of space flight on the behavior of human retinal pigment epithelial ARPE-19 cells and evaluation of coenzyme Q10 treatment. 2021, Cell Mol Life Sci, 78(23): p. 7795-7812.

Lee, A.G., et al., Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. 2020, NPJ Microgravity, 6: p.7.

Darshan Chandramowli – “Targeted proteomic analysis of S. cerevisiae in microgravity conditions in response to antifungal stress”

Targeted proteomic analysis of *S. cerevisiae* in microgravity conditions in response to antifungal stress

D. Chandramowli[1], and B. Devreese[1]
[1]Protein Research Unit, Laboratory of Microbiology, Ghent University (Darshan.Chandramowli@UGent.be, Bart.Devreese@UGent.be)

The search for life in extraterrestrial environments has always been one of the most appealing aspects of astrobiology. However, one common hurdle facing astronauts during long-term space missions in this regard is the drop in immunity. This is in part due to microgravity, which has been shown to have an effect on human cells after prolonged exposure. Further, since astronauts do not have the luxury of the atmosphere to protect against radiation, they receive doses of ionising radiation several times that they would normally be exposed to on the Earth (Blaber et al., 2010). These stress conditions result in increased susceptibility to bacterial and fungal infections. Additionally, microbes present in the astronauts' environments are also subject to these adverse conditions, and the resulting adaptation is usually an increase in antimicrobial resistance (Klaus and Howard, 2006).

The constant co-evolution between microbes and humans in these conditions limits effective treatment options, thereby necessitating the timely development of suitable therapy. In order to do so, it becomes important to understand what cellular processes are affected in pathogens upon exposure to antibiotic/antifungal agents. Here, we examine a list of proteins from the model organism S. cerevisiae that are known to be involved in a range of functions (cell death, cell division and cycle regulation, response to antifungals, etc.) to see how the levels are altered in response to different antifungals in conditions of simulated microgravity (sim-µg). This is accomplished using targeted proteomics (mass spectrometry) and fluorescence microscopy.

(results in the form of charts and tables to be added if accepted)

REFERENCES

1. Blaber, E., Marçal, H. and Burns, B.P., 2010. Bioastronautics: the influence of microgravity on astronaut health. *Astrobiology*, *10*(5), pp.463-473.
2. Klaus, D.M. and Howard, H.N., 2006. Antibiotic efficacy and microbial virulence during space flight. *Trends in biotechnology*, *24*(3), pp.131-136.

Ivan Vasilev – "Study of vascular hemodynamics in healthy men undergoing 21-day antiorthostatic hypokinesia"

Study of vascular hemodynamics in healthy men undergoing 21-day antiorthostatic hypokinesia

I.M. Vasilev [1,2], A.V. Polyakov [1], G.Yu. Vassilieva [1], O.I. Orlov [1]
1- Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russia
2 – Phlebology Center of the V.V. Veresaev Moscow State Clinical Hospital, Moscow, Russia

The patterns of regulation and functioning of human circulatory system under space flight factors are among the urgent problems of modern space medicine. The key factors influencing vascular hemodynamics in space are microgravity, the G-loads at the launch and descent stages (up to 7g) and the mission duration (from several days to the record-braking 437 days).

There is an assumption that (in microgravity conditions) transmural pressure decreases below the hydrostatic indifference point and increases above it, which is apparently reflected in the restructuring of the vascular hemodynamics regulation [Egorov A.D., 2007].

The anti-orthostatic hypokinesia (or HDT – head-down tilt) is an important method of experimental exposure that allows to study the influence of space flight factors on the human body in terrestrial conditions, as well as physiological processes.

Previous studies on volunteers in HDT has revealed certain changes in the vascular system. In particular, when male volunteers stayed in a 60-day HDT at -6 0, an increase in the diameters of the inferior vena cava, portal vein, superior mesenteric and splenic veins was observed, accompanied by increased pulsation in all epigastric vessels [Afonin B. et al., 1999]. Similarly, ultrasound signs of venous stagnation in the splanchnic blood flow were observed (HDT at -15 0 for 12 hours) [Afonin B. et al., 2007]; a 20% decrease in the wall thickness of the leg artery was shown after 3 weeks in HDT [Platts et al., 2009.]; the "internal remodeling" of the femoral artery was revealed in HDT at -6 0 for 5-week [Palombo et al. 2015.]. Changes in the vascular hemodynamics were also detected in experiments with "dry" immersion. In particular, the results obtained from the study of the vascular bed in women who stayed in dry immersion for 3 days showed a statistically significant decrease in the diameters of the inferior vena cava, common, external, and internal iliac veins at different examination periods ($p<0.05$) [Vasilev I. et al., 2021]. Moreover a statistically significant increase was revealed in the diameter of the inferior vena cava, as well as in the medial-lateral diameter of the right internal jugular vein during the aftereffect ($p<0.05$) in men who stayed in 7-day dry immersion [Vasilev I. et al., 2022].

Thus, the studies of vascular hemodynamics in healthy volunteers in analog experiments demonstrate the presence of sufficiently pronounced changes in the vascular bed.

It is worth to add that the venous system in human lower limbs has a number of anatomical features: a large absolute capacity of the venous bed; the presence of two independent, interconnected systems (superficial and deep ones); a complex multi-channel structure of the bed in the joint area; the presence of diverse connecting vessels; the characteristic structure of the vein wall and the presence of backup and duplicating mechanisms that ensure relative stability of the venous blood return [Shvalb P.G., Ukhov Yu.I., 2009.]. At the same time, a number of researchers recognize the existence of an interdependent continuous process of remodeling in the bed of the inferior vena cava system. But it should be noted that most of these studies are scattered, and there have been practically no systematic research on the state of the vessels of the inferior vena cava system in HDT.

The aim of our work was to obtain data on the state of the vascular bed In men under 21-day antiorthostatic hypokinesia (head-tilted bed rest) with the effect of normobaric hyperoxygenation in the early period of adaptation (5 men), and without countermeasures (7 men).

To assess venous hemodynamics in 12 healthy men without bad habits, we performed ultrasound scanning of the inferior vena cava, aorta, common femoral artery, external iliac artery, as well as neck vessels (internal jugular vein and common carotid artery) according to the clinical protocol. The measurements were taken 7 days before the start of HDT, on the 7th, 14th and 20th days of the experiment, and on the 6th-7th days of the recovery period (LogiqE ultrasound scanner, GE, USA). The primary data was processed using the software "Statistica for Windows v. 12.0" (StatSoft, Inc.).

We registered a statistically significant decrease in the diameter of the inferior vena cava during the time the volunteers were in antiorthostasis, and subsequent recovery to background values in the aftereffect, as well as an increase in the medial-lateral diameter of the right internal jugular vein during the aftereffect ($p<0.05$). Interestingly, there was a statistically significant decrease in the diameter of the right common femoral vein on days 7 and 14 in comparison with background data ($p<0.05$).

Along with this, significant changes in the diameters of the aorta and the common femoral artery ($p<0.05$) were noted during the subjects' stay in HDT.

These changes confirm the need for an in-depth study of venous hemodynamics and possible mechanisms of thrombosis at different stages of a space flight.

Hendrik Bouwman – "Effects of sustained hypergravity on growth and reproduction in earthworms"

Effects of sustained hypergravity on growth and reproduction in earthworms

C-M van der Merwe[1], M Maboeta[1], C Weldon[1], C Bezuidenhout, H Bouwman[1*]
[1]Research Unit: Environmental Sciences and Management, North-West University, Potchefstroom, South Africa, henk.bouwman@nwu.ac.za

INTRODUCTION

It is likely that humans will establish colonies on Mars and Moon (Rappaport and Szocik, 2021). For this to happen, we believe that earthworms will play a crucial role in ameliorating regolith into arable soils by composting and incorporating organic waste into the regolith (Duri et al., 2020). To the best of our knowledge, no studies have been performed on earthworms in micro- or hypergravity to determine if they will survive prolonged hypergravity.

We conducted experiments on the effects of hypergravity on the earthworm Eisenia andrei, a common compost earthworm (Fig. 1A; Neuhauser et al., 1988). Concurrently, we ran four gravity treatments (3 G, 5 G, and 6.5 G) to determine survival, change in biomass, and reproduction over 30 days at 25ºC. We used a 3D printed adaptor for a centrifuge that has four compartments per gravity treatment (6 worms per container, for 48 worms per treatment). The reference group was 1 G, and not spun at all. The growth medium we used was cow manure that is able to sustain growth (Mitchell, 1997.), wetted to an appropriate consistency, and within Ih the worms were originally cultured. After 30 days, cocoons were collected and weighed. Histology of the worms were conducted to measure changes in cross-sectional and longitudinal morphology (Fig. 1B).

RESULTS AND DISCUSSION

The initial mass of young worms added at day 1 did not differ statistically between treatments, nor did the final masses of the worms at day 30, between any treatment (Fig. 1C). Surprisingly therefore, worms increased their biomass to reach equivalent biomass at the day 30, after continuous gravity treatments. This 'insensitivity' to gravity, we believe is due to the subterranean nature of the earthworms where that are able to resist the mass of the overlying materials. As far as we are aware, this aspect has not been researched before.

For thickness of the epidermis, longitudinal muscle thickness, and circular muscle thickness (Figs. 1D-E), there were differences per treatment with a peculiar trend. The thinnest thickness was always at 3 G. At higher gravities, the thicknesses seem to increase to such an extent that there were no differences between 1 G and 6 G. We have no explanation for this trend. Reproduction, measured at numbers of cocoons produced after 30 days, showed the same peculiar trend, with the least number produced at 3 G, and then increasing again, afterwards (Fig. 1F). Again, we have no explanation for this trend that reflects the morphometrics. Interestingly, the mass of the cocoons did not differ between treatments (Fig. 1G).

Stress responses are a universal occurrence in several different organisms from microbes to vertebrates, organisms have evolved to cope and adapt to a broad range of physical and biotic threats (Taborsky et al., 2022). Stress responses can occur at cellular, tissue, or at the organismal level. Taborsky et al. (2022) suggested that stress responses have beneficial effects, particularly over short periods. This statement may be applicable to the findings of this study, although 30 days is not a 'short' period. The results of the morphometric measurements of the 6.5 G exposure earthworms suggest that the earthworms may have adapted towards chronic centrifugation as a result of optimal stress response, but this does not explain the patterns observed at lower gravity treatments.

Variable changes in body mass have been reported for rats and mice under long-term hypergravity (e.g. Ronca et al., 2000; Oyama and Zeitman, 1967). However, we could not find any reports on the effects of hypergravity on biomass of soil-dwelling organisms, and very little on invertebrates in general. Effects of hypergravity on soil dwelling animals are important to know, as the weight of the overlaying medium adds to the pressure experienced by the animal. Compaction may also play a role. Both factors would compress the medium and hinder movement, feeding, growth, and reproduction. We have seen some of these effects.

We showed that earthworms tolerate and accommodate hypergravity up to at least 6.5 G for 30 days while increasing biomass, apparently able to adjust muscular performance requirements, as was the case with fruit flies

(Schilder and Raynor, 2017). This study points the way towards more experiments, with longer duration with more worms to confirm these finding, as well as to determine effects on multiple generations. The earthworm is therefore a good model to investigate the effects of hyper- and microgravity on terrestrial animals. This model also allows for investigations of other soil biota such as nematodes, mites, and microorganisms.

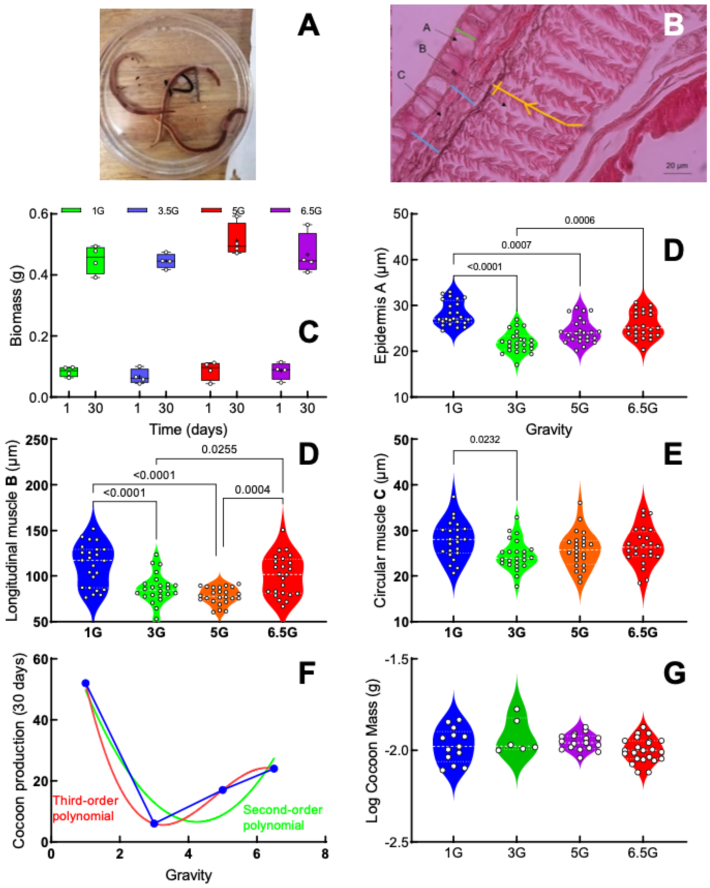


Figure 1. A Earthworms. B Cross section, showing the morphometric measured. C Biomass at start and finish of 30 days. C Thickness of epidermis. D Thickness of Longitudinal muscle. E Thickness of Circular muscle. F Cocoon production. G Cocoon mass.

REFERENCES

Duri LG et al. (2020) Mars regolith simulant ameliorated by compost as in situ cultivation substrate improves lettuce growth and nutritional aspects. Plants 9: http://dx.doi.org/10.3390/plants9050628

Mitchell, A., 1997. Production of Eisenia fetida and vermicompost from feed-lot cattle manure. Soil biology and biochemistry, 29(3-4), pp.763-766.

Neuhauser EF (1988). The potential of earthworms for managing sewage sludge. C.A. Edwards, E.F. Neuhauser (Eds.), Earthworms in Waste and Environmental Management, SPB Academic Publishing BV, The Hague, pp. 9-20 Oyama J and Zeitman B (1967) Tissue composition of rats exposed to chronic centrifugation. American journal of physiology. 213:1305-1310.

Rappaport MB & Szocik K (2021) The human factor in the settlement of the Moon. Springer Nature, Switzerland.

Ronca AE et al. (2000) Body mass, food and water intake, and activity of pregnant and lactating rat dams during 1.5-g centrifugation. Journal of gravitational physiology 7:131–132.

Schilder RJ and Raynor M (2021) Molecular plasticity and functional enhancements of leg muscles in response to hypergravity in the fruit fly Drosophila melanogaster. Journal of experimental biology. 220:3508.

Taborsky, B., Kuijper, B., Fawcett, T.W., English, S., Leimar, O., McNamara, J.M. & Ruuskanen, S. 2022. An evolutionary perspective on stress responses, damage, and repair. Hormones and Behavior, 142:105-180.

Laurence Stevens – “Chronic recording of EMG activity by telemetry in mice skeletal hindlimb muscles during long-term inactivity”

Chronic recording of EMG activity by telemetry in mice skeletal hindlimb muscles during long-term inactivity

L. Stevens[1], V. Montel[1], L. Cochon[1], J. Dereumetz[1], V. Orea[2], D. Guyonnet[2], J.C. Lloret[3], and E. Girard[2]
[1]UREPSSS, Physical Activity, Muscle and Health, Eurasport, University of Lille, France
[2] UAR 3453, SFR Santé Lyon-Est, ANIPHY Plateforme, University of Lyon 1, France
[3] CNES, Toulouse, France

The aim of the present study was to record electromyogram (EMG) activity in mice hindlimb muscles before and after 30 days of inactivity by hindlimb suspension.

Indeed, modifications of the level of global electrical activity of rat muscle have been reported during an episode of microgravity simulated by HU. Thus, for the soleus muscle, the EMG activity, and therefore its motor activity, was shown to be decreased as soon as rats has been unloaded (Ohira et al., 2002; De-Doncker et al., 2005). The EMG activity remained approximately 60% of the control level during the first week of HU. Then, it gradually increased and, from the 9th day, recovered identical values as pre-HU level (Ohira et al., 2002; De-Doncker et al., 2005). The characteristics of the EMG were also modified by HU since it changed from a tonic to a phasic activity. Here, chronic recording of EMG activity was performed thanks to a telemetry method that may be obtained in freely moving animals without interference with the experimenter.

Under general anesthesia, ETA-F10 transmitters from DSI (Data Science International) were implanted subcutaneously in the backside of 16 C57BL6J mice. Extending from the silicone housing of the transmitter are two flexible leads, i.e. bipolar stainless steel electrodes, that were subcutaneously guided to hindpaw and implanted into gastrocnemius muscle. ETA-F10 transmitters from DSI are able to measure biopotential (ECG, EEG or EMG), temperature and activity data in mice (1.6 g weight and a battery life duration of 2 months). A nearby receiver (RPC-1) collected the signal and the extracted data (EMG, temperature and signal strength) were saved using the Dataquest acquisition system (DSI).

After one week of surgery recovery, data recording was performed during the second week, and afterwards, two groups of implanted mice were constituted: a control (n = 10) one and a second that was submitted to hindlimb Unloading (HU) (n = 10) during 30 days. EMG, temperature and signal strength in both groups were chronically recorded (for 24 h) in parallel during 30 days.

Then, thanks to a homemade script derived from Spike 2 analysis device, the following parameters of the EMG signal were determined: total duration(s), maximum (mV), mean (mV), area (mV*s), number of bursts/min, burst mean duration (s), bursts area (mV) and activity rate (%).

These results, for the first time described on mice muscles, will be discussed in terms of the effects of a 30 day-inactivity period on muscle motor system as it is the case during long-term spaceflight missions

Many acknowledgments to Guillemette Gauquelin-Koch and fundings from French Spatial Agency (CNES).

Danielle Greaves – “Cervical intervertebral distance measured using 3D ultrasound after dry immersion and 6 month spaceflight”

Cervical Intervertebral Distance Measured Using 3D Ultrasound after Dry Immersion and 6 Month Spaceflight

Danielle Greaves[1], Lonnie Petersen[2], Philippe Arbeille[3]
[1]dgreaves@uwaterloo.ca, Faculty of Health, University of Waterloo, Canada, [2] lgpeters@mit.edu, Department of Aeronautics and Astronautics, Massachusetts Institute of Technology, Cambridge, USA, and [3]arbeille@med.univ-tours.fr, CERCOM-UMPS, Faculté de Médecine Université de Tours, France

Background

Cervical Intervertebral distance may be possible to measure with ultrasound during routine common carotid artery scans to measure intima media thickness. The recently launched 3D motorized ultrasound (ESA ECHO), scan all the targets below the probe head and the scientist can select the Carotid view with the intima media visible on which the cervical spine (C4-C5) will appear and their distance measured. In this study, identical hardware was used during experiments onboard the ISS as well as during a dry immersion (DI) analog study. Following the same scanning procedures and analysis, we measured and compared changes in cervical intervertebral distance before and at the end of Dry immersion (Medes experiment) and before and at the end of 6 month mission (Vascular Echo experiment)

Methods

Neck scans were completed using a 3D motorized probe (17 MHz) prior to and at the end of four days DI in 18 male participants with the subjects still in the bath as well as on 8 astronauts prior and at flight day 150 of 6M ISS flights. Intervertebral distance was measured from the resulting 3D neck scan videos.

Results

At day 4 in DI, intervertebral distance was reduced by 8.7% (pre DI: 5.40 +/-0.51 mm; day 4 DI: 4.93 +/-0.35 mm). This change was consistent in all subjects. Conversely after 150 days in microgravity there was no significant change in the intervertebral distance (pre 4,36+/-1,12 mm; flight day 150: 4,38+/-0,84 mm).

Discussion

The narrow spread in data points pre and post DI, indicate a high degree of reliability of the ultrasound method. Moreover, ultrasound provided a feasible method of data collection allowing the subject to remain in the analogue environment of DI or during spaceflight. The absence of significant change after 150 day in microgravity is confirmed by postflight ultrasound and is consistent with postflight MRI data. Previous data has indicated feasibility of in-flight US-based disk height evaluation but also significant challenges relative to probe angle. (Harrison paper 2018). Our data and implementation of long-axis scans and 3D reconstruction of images provides a novel, more reliable, method of data collection.

The reduction In the cervical vertebral distance In DI Is Indicative of Earth 1G gravitational forces acting on the neck 24h a day during DI while during spaceflight the gravity vectors are abolished. One may note that there was no clear tendency between the 8 astronauts investigated which confirm that the effect of microgravity (if any) on the cervical vertebra distance is not uniform among the population of astronauts. The present results provide proof-of-concept that the possibility exists to estimate cervical intervertebral distance changes from a standard neck view which can be easily performed by any novice in ultrasound. Moreover, this long axis view using the motorized scan head is easily repeated, which allows a good reproducibility of the measurement The 3D software allows the user to select the best image after collection is complete. In this way, the carotid/vertebra scans are optimized even ifa novice operator made incorrect probe orientation.

- Harrison MF, Garcia KM, Sargsyan AE, Ebert D, Riascos-Castaneda RF, Dulchavsky SA. Preflight, in-flight, and postflight imaging of the cervical and lumbar spine in astronauts. Aerosp Med Hum Perform. 2018; 89(1):32-40.

Adriana Salatino – "Motor awareness in microgravity: action self-monitoring during parabolic flights"

Motor awareness in microgravity: action self-monitoring during parabolic flights

Adriana Salatino[1,2,3], Julien Lambert[1], Roberto Gammeri[2], Emanuele Cirillo[2], Dominika Sulcova[1], Benvenuto Jacob[1], Stefano T. Chiadò[4], Mark S. George[5,6], Donna R. Roberts[7], André Mouraux[1], Anna Berti[2], Raffaella Ricci[2]

1. Institute of Neuroscience (IoNS), Université Catholique de Louvain, 1200 Brussels, Belgium ; 2. Department of Psychology, University of Turin, Turin, Italy; 3. Department of Life Sciences, Royal Military Academy, 1000, Brussels, Belgium; 4. Vastalla S.r.l., Turin, Italy; 5. Department of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC, USA; 6. Ralph H. Johnson VA Medical Center, Charleston, SC, USA; 7. Department of Radiology and Radiological Science, Medical University of South Carolina, Charleston, SC, USA

BACKGROUND

Having a conscious control of one's actions is a crucial aspect for coherent purposeful behavior. However, during space exploration missions the effects of microgravity on motor awareness remain to be clarified. In the present study, we took advantage of short periods of zero gravity (0g) during Parabolic Flights (PF) to investigate the acute effects of changes in gravitational input on Conscious Monitoring of Motor Performance (Fourneret & Jeannerod, 1998).

METHODS

We used a motor task that required subjects to draw a straight line to a target with their (hidden) hand. During the task, participants received visual feedback about their action on a computer screen. In most trials, the line trajectories shown on the screen was deviated by a variable angle. Therefore, in order to trace straight trajectories, subjects had to deviate in the opposite direction. At the end of each trial, participants had to indicate whether the line shown on the screen was the line actually drawn. To control for the presence of psychological stress and/or arousal that might affect task performance, we also performed physiological measurements of Electrodermal Activity (EDA). Eighteen participants were tested during three ESA parabolic flight campaigns. Participants performed the motor task under four conditions: Pre-flight (PRE) at 1g on the ground, on board during the flight at 0g (0G) and 1g (1G), and post-flight (POST) at 1g on the ground. A repeated-measure ANOVA was performed to compare the effect of condition (PRE, 0G, 1G, POST) on the angle at which participants became aware of the deviation.

RESULTS

Consistent with previous results1, participants did not recognize the deviation until a certain degree of angle in PRE, POST, and 1G conditions (M=8.28°). Importantly, in 0G, the amplitude of the angle at which participants became aware of the correction of their trajectory significantly increased (M=9.71°; $p<.001$) compared to PRE, 1G, and POST. The EDA results showed that there were no significant changes in the arousal levels between conditions.

DISCUSSION

These results indicate that the reduction of gravitational input worsens motor awareness, as indicated by the increase, in the amplitude of the angle at which participants became aware of the correction of their trajectory at 0g. The unweighting of otolith inputs in microgravity may have increased the weight of vision, requiring a higher degree of trajectory perturbation for participants to become aware of the signals generated by their own movements. The finding that motor awareness decreased in microgravity provides further evidence for the role of the vestibular system in motor control and intention (Lopez, 2013; Dora ET AL., 2008). The findings that vestibular input plays a crucial role in the action self-monitoring have important implications for the identification of countermeasures to be applied in altered gravity conditions and, on Earth, for the implementation of rehabilitation treatment in patients with vestibular disorders.

REFERENCES

Fourneret, P., & Jeannerod, M. (1998). Limited conscious monitoring of motor performance in normal subjects. Neuropsychologia, 36(11), 1133–1140.

Lopez, C. (2013). A neuroscientific account of how vestibular disorders impair bodily self-consciousness. Frontiers in Integrative Neuroscience, 7.

Dora E. Angelaki, Kathleen E. Cullen (2008).Vestibular System: The Many Facets of a Multimodal Sense. Annu. Rev. Neurosci. 2008. 31:125–150.Author A.B and Author C.D., 2022, Journal of publication, 123, 356-789.

Sarah Schunk – "Astrocyte reactivity can be modulated by altered gravity"

Astrocyte Reactivity can be Modulated by Altered Gravity

Yannick Lichterfeld[1], Sarah Schunk[1], Laura Kalinski[1], Theresa Schmakeit[1], Zoe Meerholz[1], Maximilian Sturm[1], Patrick Lau[1], Sebastian Feles[1], Timo Frett[1], Ruth Hemmersbach[1] and Christian Liemersdorf[1]
1 German Aerospace Center, Institute of Aerospace Medicine, Linder Hoehe, D-51147 Cologne, Germany
Yannick.Lichterfeld@dlr.de; Sarah.Schunk@dlr.de; Laura.Kalinski@dlr.de; Theresa.Schmakeit@dlr.de; Zoe.Meerholz@dlr.de; Maximilian.Sturm@dlr.de; Patrick.Lau@dlr.de; Sebastian.Feles@dlr.de; Timo.Frett@dlr.de; Ruth.Hemmersbach@dlr.de ; Christian.Liemersdorf@dlr.de

Neurodegenerative disorders caused by various diseases and neuronal injuries are among the highest investigated but still unresolved topics in modern neuroscience. Key regulatory mechanisms in neuronal dysfunction, regenerative processes and tissue homeostasis are astrocytes. Astrocytes are glial cells that are able to shift towards a reactive state as an initial response to environmental stimuli, such as e.g. neuronal injuries or inflammation. During neurodegeneration progression in vivo astrocytes mediate the formation of the glial scar. On the one hand reactive astrocytes prevent the widespread of inflammation, but on the other hand they create an inhibitory environment constraining neuronal regeneration. The inhibitory potential of reactive astrocytes is caused by a complex phenotypic shift upon reactivity induction including increased migratory behaviour, polarization, hyperproliferation, elevated levels of GFAP and other gene expression changes. Thus, reactive astrogliosis in vivo results in long-lasting restrictions in patients.

The aim of our project NeuroSpace is to investigate the impact of altered gravity on mechanosensitive pathways regulated in astrocytes to identify the underlying mechanisms of astrocyte reactivity induction. For the assessment of altered gravity conditions of both simulated micro- and hypergravity we utilize ground-based facilities at the DLR Institute of Aerospace Medicine in Cologne, Germany.

We used primary murine astrocytes from C57BL/6J mouse embryos, as these cells are closely related to human astrocytes in vivo in neuronal tissues, can be cultured up to several months, and serve as an ideal model system for human glial cell function and astrogliosis in vitro. We exposed them to hypergravity conditions in the physiologically relevant range of 2g for various durations up to weeks using the DLR Multi Sample Incubator Centrifuge (MuSIC) and the Hyperscope, a fully automated fluorescence live-cell imaging microscope on the DLR human centrifuge. For simulation of microgravity (s-µg), we utilized DLR custom-designed 2D-clinostats. We then compared key cellular characteristics in both experimental approaches to normal gravity (1g) controls.

The DLR ground-based facilities allowed us to gain insights into cellular dynamics and stimulus responses due to altered gravity conditions. We conclude that increased mechanical loading by hypergravity attenuates various traits of reactive astrogliosis. In contrast, clinorotation induced gene expression changes related to reactive astrogliosis in primary astrocytes in vitro.

Identifying the underlying mechanisms and target genes or pathways involved in these regulatory processes might aid to the development of pharmacological treatments which shall balance and modulate reactive astrogliosis and thus enhance neuronal regeneration in vitro and in future also in vivo in patients.

Reference

Lichterfeld, Y.; Kalinski, L.; Schunk, S.; Schmakeit, T.; Feles, S.; Frett, T.; Herrmann, H.; Hemmersbach, R.; Liemersdorf, C. Hypergravity Attenuates Reactivity in Primary Murine Astrocytes. Biomedicines 2022, 10, 1966. https://doi.org/10.3390/biomedicines10081966.

Human Postural Responses To Artificial Gravity Training

A. A. Saveko[1], M. I. Koloteva[1], and E. S. Tomilovskaya[1]
[1] Institute of Biomedical Problems of the Russian Academy of Sciences; asaveko@gmail.com

INTRODUCTION

Short-radius centrifugation (SRC) is a promising countermeasure against the adverse effects of space flight (SF). Centrifugation in the range from 0.6 to 4 G prevents cardiovascular deconditioning and has a positive effect on bones and muscles. The artificial gravity from 0.22-0.5 G is sufficient for a subjective feeling of vertical position. The main difficulty of using this method is the potential occurrence of undesirable orthostatic and vestibular reactions – syncope, cross-coupled illusion, and associated motion sickness. Moreover, tolerance to high G along the longitudinal body axis is degraded during SF (Kotovskaya A.R. et al., 1980; Clément G. and Pavy-Le Traon A., 2004). Developments in adapting humans to artificial gravity conditions are discussed as a way of maintaining sensory-motor and structural integrity in missions involving transitions between different gravity environments. Recent studies using SRC and Human Centric Rotator Device suggest that there exists a potential for any individual to tolerably vestibular acclimate to a given spin rate of operational importance (Bretl K.N. and Clark T. K., 2020, 2022). There is also evidence that artificial gravity training may improve orthostatic tolerance (Stenger M.B et al., 2007). Based on the above, and also taking into account the presence of cardio-postural interactions and muscle-pump baroreflex (Verma A.K. et al., 2017; Goswami N. et al., 2021) and their activation during short-arm centrifugation (Blaber A. et al., 2014), we hypothesized that repeated SRC would also improve postural tolerance. The work aimed to study the postural characteristics after five consecutive SRC sessions in the interval training mode with angular velocities from 22 to 28 rpm.

METHODS

Six healthy male volunteers aged from 34 to 45 have been observed before and immediately after each of the five consecutive SRC sessions with a rotation radius of 235 cm. The interval between SRC was at least 3 days and averaged 6.11±2.17 days. The first SRC session was the first experience for all participants. All SRC sessions were the same and lasted 60 min. Gravity gradient (ΔG) = 74.5%. The subject's head was at a distance of 60 cm from the axis of rotation. See more details in Fig. 1.

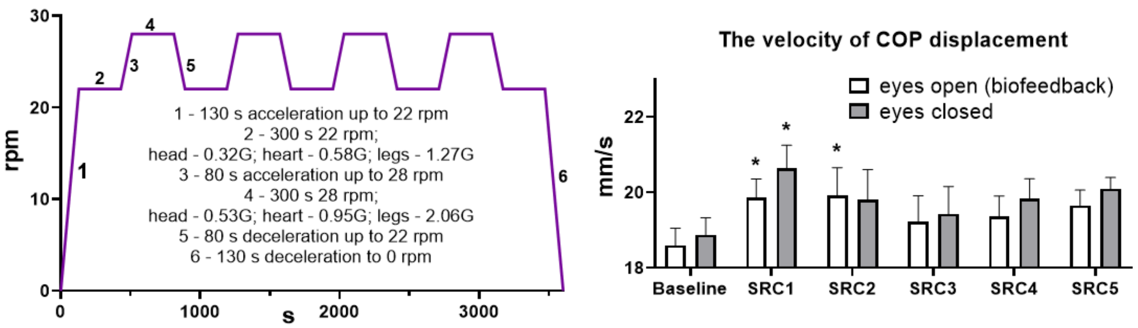


Figure 1. Scheme of SRC mode (left); and right – dynamics of the velocity of COP displacement while maintaining a vertical posture (20 s) with visual feedback (columns without fill) and immediately after its elimination (grey columns) before SRC exposure (Baseline) and after each of five consecutive SRC session (SRC1-5). * - significant difference from the initial data.

Entry Level Footscan system 0.5 m (RSscan International, Belgium) was used for the registration of postural characteristics and ground reaction forces (GRF) data. The test included recording the pressure exerted by the feet on the sensors of the sensory platform when the participant was asked to keep the center of pressure (COP) at a given point in a standing position for 20 seconds using biofeedback (image on the screen). Then, on command, the participant closed his eyes and continued to stand for 20 seconds, trying to remain in the same position. During the first session of the experiment, the position of the feet on the platform (the distance between the heels and toes) was fixed and repeated for each experimental session. Since no trends and significant differences were found between all sessions before SRC, we averaged data from 5 sessions held before SRC to indicate the baseline of values. Thus, there was no effect of sensorimotor learning to perform testing, and the duration of the breaks between SRC was sufficient to restore the studied parameters. The data were analyzed using repeated measures ANOVA (GraphPad Prism version 8).

RESULTS

After the first SRC session, there was a deterioration in postural stability, accompanied by a significant increase in the area of the ellipse of the COP displacement with eyes closed - by 5.68 ± 1.50 mm2 ($p = 0.005$) and in the velocity of COP displacement with eyes open and closed - by 1.40±0.51 ($p=0.05$) and 1.90±0.51 mm/s ($p=0.006$), respectively (Fig. 1). Apparently, this phenomenon led to the more active participation of the feet in maintaining balance - GRF and the area of the used surface of the feet increased. The results obtained demonstrate the SRC training effect which is already noticeable during the second SRC session. During the third SRC session, almost no changes in the studied characteristics were observed (Fig. 1). It should be noted that repeated SRC sessions in this mode did not lead to any significant deformation of the feet morphology. The results of the study confirm the proposed hypothesis and expand the understanding of the capability of sensorimotor adaptation to artificial gravity.

FUNDING

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

REFERENCES

Kotovskaya A.R, Illyin E.A., Korolkov V.I. and Shipov A.A., 1980, *Physiologist*, 23, 27-29.

Clément G. and Pavy-Le Traon A., 2004, *Eur J Appl Physiol*, 92, 235-248.

Bretl K.N. and Clark T.K., 2020, *npj Microgravity*, 6(1), 22.

Bretl K.N. and Clark T.K., 2022, *Journal of Vestibular Research,* 32(4), 305-316.

Stenger M.B., Evans J.M., Patwardhan A.R., Moore F.B. et al., 2007, *Acta Astronautica*, 60(4-7), 267-272.

Verma A.K., Garg A., Xu D., Bruner M. et al., 2017, *Scientific reports*, 7(1), 45301.

Goswami N., White O., Blaber A., Evans J. et al., 2021, *Acta astronautica*, 189, 216-221.

Blaber A., Goswami N., Xu D. and Laurin A., 2014, *40th COSPAR Scientific Assembly*, 40, F5-3.

Alterations in gravity influences conscious experiences

J.F.Nottage[1], M. R. Longo [2], and E. R. Ferre[3]
[1]Department of Psychological Sciences, Birkbeck, University of London, London, UK, j.nottage@bbk.ac.uk,
[2] Department of Psychological Sciences, Birkbeck, University of London, London, UK, m.longo@bbk.ac.uk
and [3] Department of Psychological Sciences, Birkbeck, University of London, London, UK, elisa.ferre@bbk.ac.uk

BACKGROUND

Gravity is one of the pivotal aspects of human life. On Earth, gravity is always there: it is stable, it is permanent, it is unchanging. The vestibular otoliths – sophisticated receptors inside the inner ear – constantly detect the direction of gravitational acceleration. Gravity is therefore the most persistent sensory signal in the brain and it may pervasively influence human behaviour.

Exposure to weightlessness leads to dramatic structural and functional changes in human physiology. Since the early Apollo missions, astronauts reported flashes or streaks of light that seemed to come out of nowhere, depersonalisation/derealisation-like sensations and temporarily altered states of consciousness. Such experiences may be caused by a mismatch between the information signaled by the vestibular receptors and the lifelong experience with the terrestrial gravity prior. When such conflict occurs, the boundary between “reality” and “unreality” fades away.

No systematic research has considered gravity as a perceptual signal for describing the world and our interaction within it. Here we used a novel interdisciplinary approach to tackle this challenge. Alterations in gravity were simulated using floatation methods on Earth. Healthy participants were immersed for an hour in a floatation tank filled with approximately 10 inches of water containing enough dissolved Epsom salt to reduce the physical – and therefore the perceived – gravity. Altered states of consciousness have been frequently reported during and after floatation. Critically, we focused specifically on alterations of vestibular perceptions. We hypothesized that a sensory mismatch between the ‘non-terrestrial’ gravity signals transmitted by vestibular organs and the terrestrial gravity prior would induce illusory and nonveridical vestibular sensations.

METHODS

Thirty-four healthy participants took part in this experiment. Experiments were conducted at Yue Float Center in London, UK (https://yuefloat.com/about). During floatation participants were lying for 60 minutes in a supine position inside a quiet and dark float tank, filled with salt water maintained at skin temperature. The tank (manufactured by Float Spa, Hungary) is made from thermo-moulded antibacterial sanitary acrylic and is 2.5m in length and 1.77m wide. The tank contains 750l of water and 500kg of Epsom salt (magnesium sulfate) which increases the water’s density and ensures that participants are able to float safely and without any effort. A heating system creates a water temperature of 34-35°C. During floatation, the lights in the tank and room were turned off. Participants completed the Expected Deviation from Normality (EDN) scale immediately after floatation. This 36-itemsvisual analogue scale has been developed to quantify subjective experiences in the flotation tank pods (Kjellgren, 2008; Kjellgren and Hanne Buhrkall, 2010). Critically, we added 7 items to quantify vestibular sensations (see Table 1).

To control for non-specific effects of flotation, a control study was performed in which participant lay on their backs for a hour in a quiet, dimly lit room.

Table 1

1.	I felt lightheaded
2.	It felt as if I was spinning or rotating.
3.	It felt as if I was moving, although I was actually staying in the same place.
4.	It felt as if I was rising upwards or sinking downwards.
5.	I felt dizzy.
6.	It felt as if I was rocking or swaying.
7.	It felt that my body kept tilting to one side or another.

RESULTS

The percentage of "Yes" responses to EDN items and vestibular items after flotation condition were compared with those after control condition using direct t-test comparison. Bonferroni correction for multiple comparison was applied. Four out of the seven vestibular items had significant greater percentage of "Yes" responses after flotation condition compared to control condition. In particular, the items "It felt as if I was spinning or rotating" scored 66.2% "Yes" responses during flotation compared to 6.4% during control ($p<0.0001$); "It felt as if I was rocking or swaying" scored 59.8% "Yes" responses during flotation compared to 6.2% during control ($p<0.0001$); "It felt as if I was rising upwards or sinking downwards" scored 50.2% "Yes" responses during flotation compared to 4.0% during control ($p<0.0001$); and "I felt dizzy" scored 17.5% "Yes" responses during flotation compared to 1.8% during control ($p<0.0001$).

CONCLUSIONS

On Earth, when the head moves with respect to gravity, the vestibular otoliths shift with the direction of gravitational acceleration, moving the hair cell receptors and signaling to the brain the gravitational acceleration. Thus, when the vestibular system works efficiently, the pull of gravity generates a constant sensory flow from early foetal life until death. Here we demonstrated that alterations in gravity signaling induces altered conscious experiences, and in particular illusory vestibular sensation. How does gravity underpin conscious experiences? Most recent neurocognitive models are founded on predictable sensory experiences that make the organism feel anchored to the physical world. On Earth, gravity provides a strong, stable and reliable prior reference. When actual signals detected by the vestibular otoliths cannot be aligned with the terrestrial gravity prior – as during spaceflight – a conflict occurs. One possible consequence of this conflict is the sensory hallucinations and alterations in the state of consciousness described by astronauts.

REFERENCES

A. Kjellgren, F. a. (2008). Sensory Isolation in Flotation Tanks: Altered States of Consciousness and Effects on Well-being. *The Qualitative Report, 13*, 636-656.

Buhrkall, A. K. (2010). A comparison of the restorative effect of a natural environment with that of. *Journal of Environmental Psychology, 30*, 464–472.

The Influence of Spaceflight Experience on Structural Brain Changes

Pinja Kääriäinen[1,2], Steven Jillings[1], Peter zu Eulenburg[3], and Floris Wuyts[1]
[1] The Lab for Equilibrium Investigations and Aerospace, University of Antwerp, Belgium;
[2]Leiden University, Leiden, The Netherlands; [3]Institute for Neuroradiology, LMU Munich, Munich, Germany
E-mail: Pinja Kääriäinen p.a.kaariainen@umail.leidenuniv.nl, Steven Jillings steven.jillings@uantwerpen.be

INTRODUCTION

Long-duration spaceflight has widespread impact on the human brain, inducing both structural and functional brain changes. Structural brain changes include brain position shifts, [1] cerebrospinal fluid (CSF) redistribution, [2,3] and ventricular enlargement. [4] Recently, ventricular enlargement has been associated with mission duration [5] and the number of previous missions has been shown to correlate with smaller increases in right lateral ventricle size in astronauts. [6] It is important to properly understand the impact of the space environment on the brain, as well as the different influencing factors, particularly because of the increasing interest towards crewed spaceflight and future mission plans [7,8] that require longer stays in space. This research investigates how spaceflight related characteristics like previous number of missions and time spent in space influence the structural brain changes seen in cosmonauts after spaceflight.

METHODS

We conducted a voxel-based multiple regression analysis to examine the association between total time spent in space (days) and gray matter (GM) and cerebrospinal fluid (CSF) volume on T1-weighted MRI scans of cosmonauts. In total, 24 male Roscosmos cosmonauts were scanned as part of the BRAIN-DTI project prior, shortly after and on average 8 months after spaceflight. The scans were obtained with two 3.0 T MRI systems at the Federal Center for Treatment and Rehabilitation in Moscow, Russia (Discovery MR750; GE Healthcare) and at the Research Center of Neurology in Moscow, Russia (Prisma; Siemens). We preprocessed the data with SPM12/CAT12 toolbox [9, 10] using a processing pipeline for longitudinal data. Images were spatially normalized using Geodesic Shooting with 1 mm shooting template and the smoothing was done with an isotropic 8-mm FWHM Gaussian kernel. After preprocessing we built a multiple regression model including the preprocessed preflight (n=85) and follow-up (n=41) scans of all cosmonauts to explore the relationship between total time spent in space (days) and the volumes of GM and CSF. The model was controlled for age, and we corrected for total intracranial volume in the model to account for individual differences in head-size. Furthermore, we extracted CSF volumes of the ventricles and calculated the sum of white matter hyperintensities (WMH) to use in a linear mixed models analysis. Statistical analysis was carried out in JMP Pro® 16 using subject as a random effect and categorical time from preflight to postflight as a fixed effect for each model. We investigated the effects of previous missions, previous time in space (days) and mission duration (days) on the volumes of 3rd, 4th, right and left lateral ventricles, the combined volume of 3rd ventricle and both lateral ventricles, and on the sum of WMHs over time.

RESULTS AND CONCLUSION

The Voxel-based multiple regression analysis showed associations between GM and CSF volumes and total time spent in space (days). There was a positive correlation between total time spent in space and increased GM volume at the top and increased CSF volume at the base of the skull. In addition, there was a negative correlation between total time spent in space and decreased GM volume at the bottom and negative correlations in CSF volume at the central sulcus and towards the anterior frontal lobe. The results were corrected for multiple comparisons after threshold-free cluster enhancement (TFCE) with family-wise error rate ($p<0.05$).

We found no significant effects of previous missions, previous time in space (days) or mission duration (days) on investigated structural changes in the linear mixed models. Time had a significant effect ($p < 0.0001$) on the volumes of 3rd and both lateral ventricles, and the combined volume of these ventricles, however not on the volume of the 4th ventricle ($p = 0.4362$). The mean change in the combined ventricular volume for the 3rd ventricle and both lateral ventricles was 12,97 % (SE ±1,91 %) from preflight to postflight for the first flight that was included in the dataset (Figure 1). Regarding white matter hyperintensities (Figure 2), time had a significant effect ($p < 0.05$) on the sum and the mean change in the sum was 13,39 % (SE ±5,58 %).

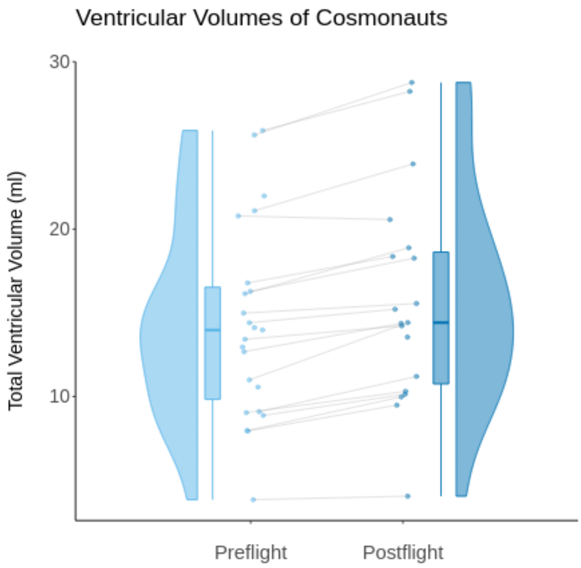


Figure 1. Ventricular Volumes of Cosmonauts. The mean change in the total ventricular volume of cosmonauts (n=18) from preflight to postflight was 12,97 % (SE ±1.91 %) for the first flight in the study. Total ventricular volume is the combined volume of 3rd ventricle and both lateral ventricles.

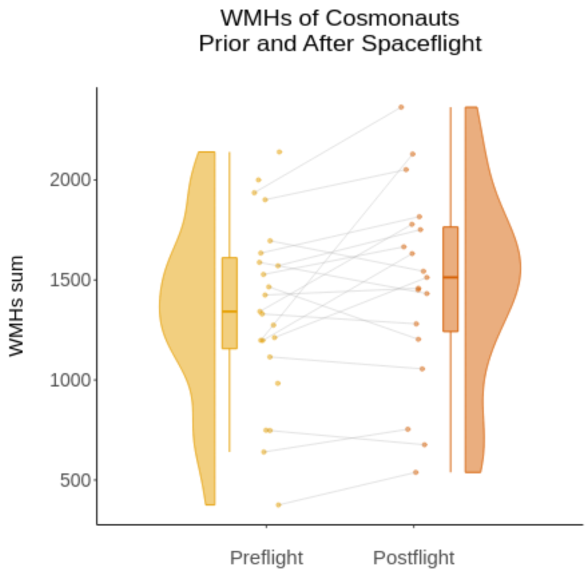


Figure 2. White matter hyperintensities (WMHs) of cosmonauts prior and after spaceflight. The mean change in the sum of WMHs of cosmonauts (n=18) from preflight to postflight was 13,39 % (SE ±5.58 %) for the first flight in the study. The sum was calculated as mean intensity of WMHs multiplied by the number of WMHs.

We have shown that there is a relationship between total time spent in space and certain structural brain changes, though such effect was not demonstrated in the ventricles. Continuing the investigation into the role of previous spaceflights and mission duration will be crucial to further monitor brain structural changes for future spaceflights.

REFERENCES

[1] Roberts, D.R. et al. (2017). Effects of Spaceflight on Astronaut Brain Structure as Indicated on MRI. N. Engl. J. Med., 377(18), 1746–1753.

[2] Jillings, S. et al. (2020). Macro- and microstructural changes in cosmonauts' brains after long-duration spaceflight. Sci. Adv., 6(36).

[3] Van Ombergen, A. et al. (2018). Brain Tissue–Volume Changes in Cosmonauts. N. Engl. J. Med., 379(17), 1678–1680.

[4] Van Ombergen, A. et al. (2019). Brain ventricular volume changes induced by long-duration spaceflight. Proc. Natl. Acad. Sci., 116(21), 10531–10536.

[5] Hupfeld, K. E. et al. (2020). The Impact of 6 and 12 Months in Space on Human Brain Structure and Intracranial Fluid Shifts. Cereb Cortex Commun., 1(1).

[6] McGregor, H. R. et al. (2023). Impacts of spaceflight experience on human brain structure. Sci. Rep., 13(1), 7878.

[7] NASA's Moon to Mars Strategy and Objectives Development. (2023). National Aeronautics and Space Administration.

[8] Li, K., & Mayer, M. (2023). China's Bifurcated Space Diplomacy and Institutional Density, The Hague Journal of Diplomacy, 18(2-3), 253-281.

[9] Ashburner, J., & Friston, K. J. (2000). Voxel-Based Morphometry—The Methods. Neuroimage, 11(6), 805–821.

[10] Gaser, G. et al. (2022). CAT – A Computational Anatomy Toolbox for the Analysis of Structural MRI Data. BioRxiv, 2022.06.11.495736.

DENTAL CARE IN SPACE AS AN INTERDISCIPLINARY PROCEDURE

The field of dental science encompasses a vast range of applications. Over time, it has seen the development of numerous specialized disciplines, one of which is Aeronautical Dentistry. This particular discipline focuses on addressing the unique health concerns of astronauts in the demanding environment of outer space. The challenges faced in space exploration are not only limited to physical factors but also extend to psychological aspects.

One of the most significant challenges in space is the effect of microgravity and cosmic rays on an astronaut's overall health. The human body is intricately adapted to the conditions present on earth including the force of gravity. In the microgravity environment of space, however, the body experiences a range of physiological changes. These changes can have profound effects on various bodily systems, including the head and neck region. Understanding the effects of space environments on the head and neck area is crucial for ensuring the well-being of astronauts. Dental professionals play a vital role in this regard. Each branch of dentistry, such as orthodontics, periodontics, and oral surgery contributes to the comprehensive dental care required for astronauts. Periodontics for example, focuses on the health of the supporting structures of the teeth, including the gums and bones. The microgravity environment may disrupt the delicate balance of these structures, leading to periodontal issues. Oral surgery plays a critical role in addressing any dental emergencies or complex procedures that may arise during space missions. Prosthetics on the other hand is responsible for the selection of appropriate materials and procedures in restorative dentistry. Considering the immense challenges posed by the space environment, dentistry is an indispensable factor in ensuring the overall health and well-being of astronauts. By addressing the specific dental needs of astronauts and mitigating the adverse effects of space on their oral health, dental science contributes to the general success and safety of space missions.

This poster aims to highlight the impact of space environments on the head and neck area, specifically focusing on dental health. It explores the contributions of various branches of dentistry in addressing the challenges faced by astronauts. Understanding dental health in space is crucial for maintaining astronauts' overall well-being and can have practical applications on Earth. By investigating the effects and involving dentistry, we can develop effective strategies to support astronaut health during space missions.

REFERENCES

1. di Prampero PE, Narici MV (2003) Muscles in microgravity: from fibres to human motion. J Biomech 36, 403-412.
2. McCarthy I, Goodship A, Herzog R, Oganov V, Stussi E, Vahlensieck M (2000) Investigation of bone changes in microgravity during long and short duration space flight: comparison of techniques. Eur J Clin Invest 30, 1044-1054.
3. Stevens M, Keyhan SO, Ghasemi S, Fallahi HR, Akbarnia S, Dashti M, Akbar Boojar FM, Zadeh MA, Cheshmi B (2020). Does microgravity effect on oral and maxillofacial region? International Journal of Astrobiology 1–7.
4. Das K, Dogra M, Gaur J, Dwivedi N. Dentistry in Air. Int J Oral Care Res 2017;5(1):1-3.
5. Bohra A, Vengal M, Patil N, Udawat V. Maxillofacial aeronautics: An evolving entity. SRM J Res Dent Sci 2016;7:205-7.
6. B. Reitemeier, G. Reitemeier, A. Schmidt, at all (2002) Evaluation of a device for attenuation of electron release from dental restorations in a therapeutic radiation field. J Prosthet Dent; 87(3), 323-327.
7. T. V. Tso, M. Hurwitz, D N. Margalit, (2019) Radiation dose enhancement associated with contemporary dental materials J Prosthet Dent; 121(4), 703-707.
8. I. Veronese , G. Guzzi, A. Giussani, et all Determination of dose rates from natural radionuclides in dental materials Journal of Environmental Radioactivity 91(1–2), 15-26

NAME OF AUTHOR

Dr. Dirk Neefs

MSD, ddcare and B-dent, Antwerp, Belgium, ESA Topical Team moderator

NAME OF CO-AUTHORS

1. Dr. Edward Kijak
   Assistant professor and head in the Department of Prosthetic Dentistry, Wroclaw Medical University
2. Dr. Ishita Singhal
   BDS, MDS in Oral Pathology & Microbiology, and Forensic Odontology, ESA Topical Team member
3. Dr. Małgorzata Kulesa- Mrowiecka
   PT, MSc, PhD; Department of Rehabilitation in Internal Diseases, Institute of Physiotherapy, Faculty of Health Sciences Jagiellonian University Medical College in Krakow, Poland
4. Dr. Victoria Sampson
   BDS MFDS RCS Ed PgDip

14:00 Mathieu Horeau – "Simulated microgravity differentially affects the iron metabolism in male and female rats"

Simulated microgravity differentially affects the iron metabolism in male and female rats.

Mathieu Horeau[1,2], Brice Martin[2], Luz Orfila[2], Ropert Martine[1], Patricia Leroyer[1], Olivier Loreal[1], Frédéric Derbré[2]
[1]INSERM, University of Rennes, INRAE, UMR 1317, AEM2 platform, Nutrition Metabolisms and Cancer (NuMeCan) institute, Rennes, France. [2]Laboratory "Movement Sport and Health Sciences" EA7470, University of Rennes/ENS Rennes, France

ABSTRACT

Objective

Data that we found in humans suggests that extreme physical inactivity affects differentially the iron metabolism between sexes. Our objective was to better understand the mechanisms involved in rats from both males and females exposed to hindlimb unloading (HU), an experimental model mimicking extreme physical inactivity reported in astronauts and bedridden patients.

Materials and Methods

8-week-old male and female Wistar rats (n=12/group) underwent 7 days of HU and were compared to control males and females. Serum, liver, spleen, and soleus muscle were collected. ICP-MS analyses were used to quantify iron concentration and analyze iron distribution. Iron metabolism gene and protein expressions were analyzed by RT-qPCR and Western Blot.

Results

Control females exhibit higher serum, hepatic and spleen iron concentrations (HIC and SIC) and transferrin saturation (TS) than control males, contrasting with previous observations in Humans. In HU males, serum iron and TS levels remain unaffected, whereas serum hepcidin level, SIC and HIC increase compared to their respective controls ($p=0.001$; $p=0.023$; $p=0.003$, respectively, **Fig. 1**). Moreover, spleen and liver ferritin protein levels increase in HU animals (+60.9% and +134%, respectively; $p<0.05$, **Fig. 1**), whereas the TfR1 protein and mRNA levels decrease (-50%; -35% respectively, $p<0.05$, **Fig. 1**). In HU females, serum hepcidin level, LIC, SIC remain unchanged, as well as TfR1 and ferritin protein levels in spleen and liver. In HU males, heme oxygenase-1 mRNA level, a marker of RBC phagocytosis, increases concomitantly to spleen iron accumulation ($p<0.001$, **Fig. 1**). Paradoxically, this marker also increases in HU females ($p<0.001$, **Fig. 1**). Soleus muscle atrophy reported in HU animals is associated to an increase of iron concentrations in both sexes ($p<0.001$, **Fig. 2**), but ferritin protein levels remain unexpectedly unchanged. HU reduces TfR1 protein and mRNA ($p<0.001$, **Fig. 2**) levels in soleus muscle, but increases myoglobin protein and heme exporter FLVCR1 mRNA levels ($p<0.001$, **Fig. 2**).

Conclusion

All our data support 1) that the distribution of iron seems to be different in basal state between rats and humans 2) that simulated microgravity impacts differently the redistribution of iron in both sexes 3) that erythrophagocytosis is the main candidate to this iron misdistribution 4) that in soleus from both sexes the higher iron concentration contrasts with a decoupling between the decrease of TFR1 mRNA level and the stability of ferritin protein levels. Specificity of muscle iron regulation must be clarified.

Figures

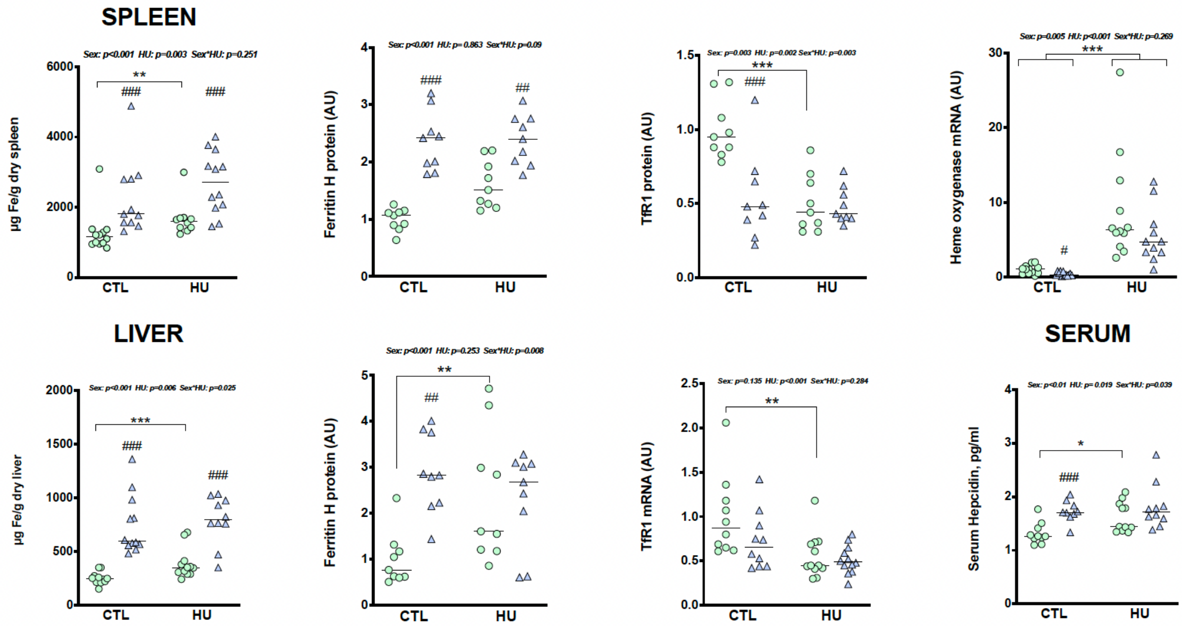


Figure 1. Effects of 7 days of HU on iron metabolism parameters in spleen, liver and serum. Green and blue plots represent respectively male and female rats.

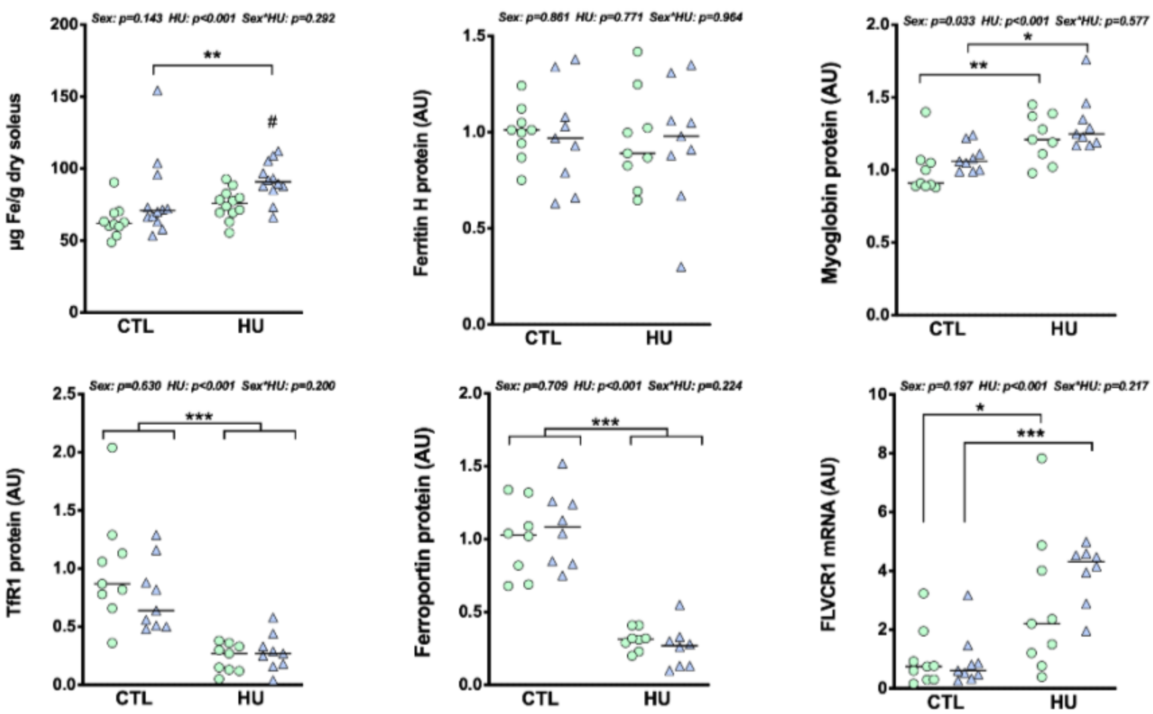


Figure 2. Effects of 7 days of HU on iron metabolism parameters in soleus muscle. Green and blue plots represent respectively male and female rats.

14:15 Frédéric Derbré – "Iron metabolism regulation in women and men exposed to simulated microgravity: results from the randomized trial AGBRESA"

Iron metabolism regulation in women and men exposed to simulated microgravity: results from the randomized trial AGBRESA

M. Horeau[1,2], M. Ropert[2,3], E. Mulder[4], J. Tank[4], P. Frings-Meuthen[4], G. Armbrecht[5], O. Loréal[2#], F. Derbré[1#]
[1]Laboratory "Movement Sport and Health Sciences" EA7470, University of Rennes/ENS Rennes, France
[2]INSERM, University of Rennes, INRAE, UMR 1317, AEM2 platform, Nutrition Metabolisms and Cancer (NuMeCan) institute, Rennes, France
[3]Department of Biochemistry, CHU Rennes, France
[4]Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany.
[5]Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Radiology, Berlin, Germany.

ABSTRACT

Background and objective

Iron metabolism imbalance could contribute to physical deconditioning experienced by astronauts due to its essential role in energy metabolism, cellular respiration, and oxygen transport. In this clinical pilot study, we wanted to determine whether short- and long-term extreme physical inactivity modulated iron metabolism, red blood cell indices, and body lean mass in healthy men and women participants exposed to head-down tilt (HDT) bed rest, the reference ground-based model of microgravity.

Methods

We recruited 8 healthy women and 16 healthy men who were all exposed to HDT bed rest for 60 days (i.e. AGBRESA study). Blood sample collection, DXA and CO rebreathing method were performed before and in different times points in short- and long-term of bed rest to determine red blood cell indices, iron status and body lean mass.

Results

We found that both serum iron concentrations (+31.3%, P = 0.027, Fig. 1) and transferrin saturation levels (+28.4%, P = 0.009, Fig. 1) increased in men after 6 days of HDT bed rest, as well as serum hepcidin concentrations (+36.9%, P = 0.005, Fig. 1). The increase of transferrin saturation levels persisted after 57 days of HDT bed rest (+13.5%, P = 0.026, Fig. 1), suggesting that long-term exposure to microgravity sustainably increases serum iron availability in men, and consequently the risk of iron excess or misdistribution. In women, 6 and 57 days of HDT bed rest did not significantly change serum iron, transferrin saturation, and hepcidin levels (Fig. 2). Paradoxically we observed an early decreased of hemoglobin mass (HDT5: -13.1%, P = 0.039, Fig. 2) and relative total lean mass (HDT30: −3.36%, P = 0.011, Fig. 2) in women than men (HDT21: −6.89%, P < 0.001; HDT45: −2.86%, P = 0.013, Fig. 1), both compartments storing most of the body iron.

Conclusions

The data from this pilot study suggest that iron metabolism parameters, especially iron availability for cells, are significantly increased in men, but not in women, exposed to long-term simulated microgravity. Because of the small sample size of women, we nevertheless must be cautious before concluding that iron metabolism could differently respond to microgravity in women. Finally, future studies must be conducted to elucidate the origin of this misdistribution with a specific focus on hemoglobin mass decreased and muscle atrophy.

FIGURES

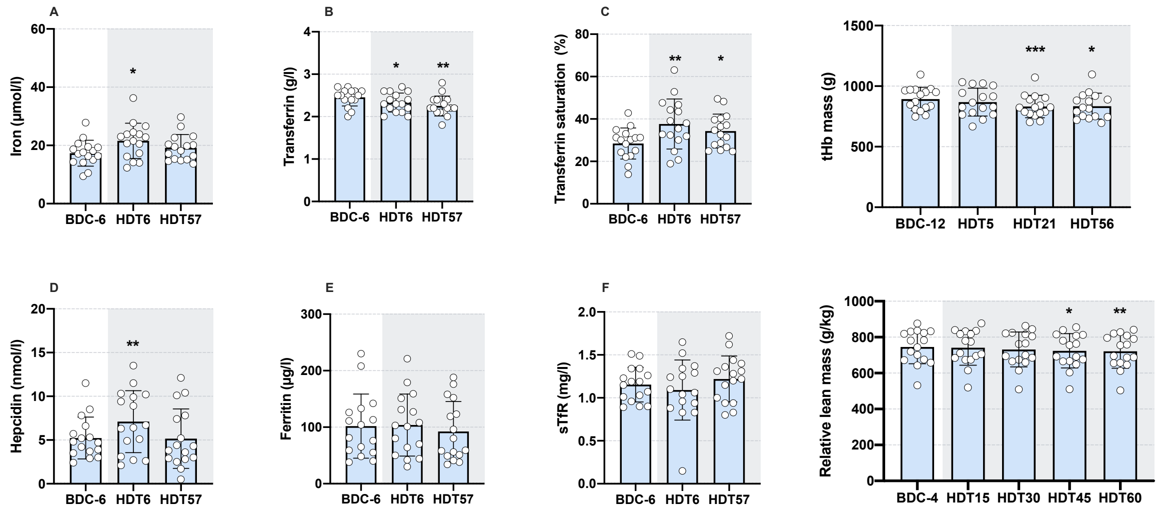


Figure 1. Effects of short- and long-term exposure to HDT bed rest on iron status, total hemoglobin mass and relative lean mass in men.

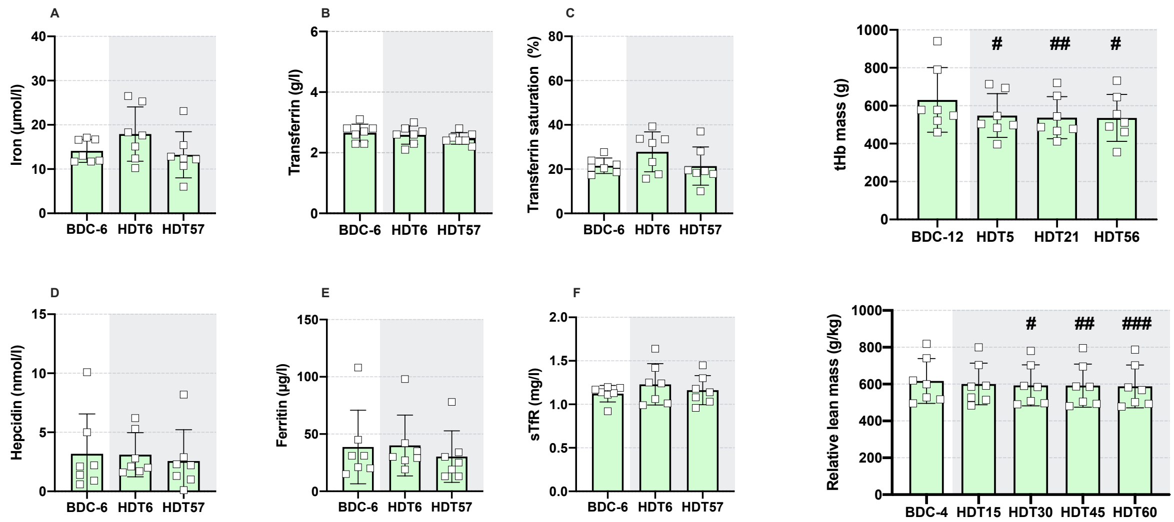


Figure 2. Effects of short- and long-term exposure to HDT bed rest on iron status, total hemoglobin mass and relative lean mass in women.

14:30 Martina Heer – "Glucose tolerance onboard the International Space Station: First results from an Oral Glucose Tolerance Test"

Glucose tolerance onboard the International Space Station: First results from an Oral Glucose Tolerance Test

M. Heer[1], D.K. Greaves[2], and R.L. Hughson[2]
[1] IU International University of Applied Sciences, Erfurt, Germany & University of Bonn, Bonn, Germany, Martina.Heer@iu.org, [2] Schlegel-University of Waterloo Research Institute for Aging, Waterloo, ON, Canada., dgreaves@uwaterloo.ca and Hughson@uwaterloo.ca

ABSTRACT STYLE GUIDE

Introduction

Analyses of serum glucose and insulin concentrations before and after the Apollo missions suggested reduced glucose tolerance during spaceflight with a 32% increase in insulin- and a 10% increase in serum glucose concentrations (Leach & Alexander, 1975). Since then no further studies focusing on glucose metabolism in microgravity were published, until recently Hughson et al. measured fasting plasma glucose- and serum insulin concentrations in the last third of 4-6 month space missions, between day 99 and 159. Similar to the Apollo results, there were significant, about 30% increases in serum insulin- while overall plasma glucose concentrations did not change. However, there were sex-specific changes in the latter, being lower in women than men at the end of the mission (Hughson et al., 2016). The homeostatic model assessment of insulin resistance (HOMA-IR), an index reflecting liver insulin resistance, was significantly increased in microgravity. Concomitantly, glycated albumin tended to increase too, demonstrating that there are most likely longer periods of higher glucose concentrations supporting the oxidation of albumin in the blood (Hughson et al., 2016).

Stroud et al. used metabolomic analyses to investigate the metabolites of complex physiological alterations (Stroud et al., 2022). They analyzed 355 blood samples from 40 male and 11 female astronauts in 4-6 month missions, respectively and have shown, beside others, sex-specific changes in energy metabolism. Here the top one or two altered pathways affected throughout the entire mission in males were glycolysis and gluconeogenesis by downregulation of glucose. In females these pathways were also commonly affected through the timepoints. Based on these results we hypothesized that whole body glucose tolerance decreases in microgravity and tested that by an oral glucose tolerance test (OGTT).

Material and methods

The OGTT is part of an the overall Vascular Aging experiment with a sample size of nine astronauts. We are presenting here results of the first five astronauts (male; mean age at launch 50.4 +7.1yrs:mean body weight 76.2 + 6.4 kg; mean height: 178.6 + 7.9 cm) who finalized the study. Each astronaut took part in five OGTTs, one preflight, 90-60 days before launch, one during the first four weeks (early inflight) and one during the last 4 weeks (late inflight) onboard the International Space Station (ISS), one ten days (R+10) and one 180 days after landing (R+180). A commercial of-the-shelf OGTT drink (Trutol®, Thermo Scientific) containing 75 g of glucose was consumed. The meal the evening before the OGTT was identical within a crewmember. Each astronaut selected a meal from the NASA space food menu prior to the preflight OGTT and then consumed this meal the evening before each of their OGTT to avoid any second meal effect (Wolever et al., 1988). Venous blood samples were drawn fasted. In parallel arterialized blood was also collected from the finger and analyzed using a glucometer (Ascensia Diabetes Care, Contour next one). During µG sessions, venous and finger sample timepoints were: 0, 30, 60, 90 and 120 mins after the OGTT consumption. During 0G sessions, venous sample timepoints were: 0, 120 mins while finger sample timepoints were as for ground. Fasting serum samples were analyzed for glucose, insulin, C-peptide and glycated albumin. The area under the curve (AUC) for concentrations of glucose and insulin were calculated, as well as the HOMA-IR (Matthews et al., 1985) and the Matsuda-Index (Matsuda & DeFronzo, 1999), reflecting liver and overall insulin resistance, respectively.

Results

The results of the 5 astronauts demonstrate a huge individual variation. Fasting serum glucose concentration and the glucose AUC from all OGTT sessions did not change. Fasting insulin and C-peptide concentrations of all OGTT sessions and AUC of insulin from the pre- and two postflight sessions were not different. HOMA-IR tended to be even lower inflight compared to preflight and R+10. Whole body insulin sensitivity calculated by the Matsuda-Index did not change early post- compared to preflight but tended to be higher at R+180.

Discussion

Based on the first OGTT carried out during spaceflight in these five astronauts there seems to be no strong evidence for glucose intolerance or insulin resistance. However, it needs to be considered that there is a huge interindividual variation between subjects. The extent of impact of dietary habits or body mass has changed within these astronauts, or what the individual daily exercise regime looked like is not known at this stage. Reduction in fat mass or higher exercise workload could improve glucose tolerance and thereby impact these results (Craig et al., 1989; Ryan & Yockey, 2017).

References

Craig, B. W., Everhart, J., & Brown, R. (1989). The influence of high-resistance training on glucose tolerance in young and elderly subjects. Mech Ageing Dev, 49(2), 147-157. https://doi.org/10.1016/0047-6374(89)90098-5

Hughson, R. L., Robertson, A. D., Arbeille, P., Shoemaker, J. K., Rush, J. W., Fraser, K. S., & Greaves, D. K. (2016). Increased postflight carotid artery stiffness and inflight insulin resistance resulting from 6-mo spaceflight in male and female astronauts. Am. J. Physiol Heart Circ. Physiol, 310(5), H628-H638. https://doi.org/ajpheart.00802.2015 [pii];10.1152/ajpheart.00802.2015 [doi]

Leach, C. S., & Alexander, W. C. (1975). Endocrine, electrolyte, and fluid volume changes associated with Apollo missions. In R. S. Johnston, L. F. Dietlein, & M. D. Berry (Eds.), Biomedical Results of Apollo (1 ed., pp. 163-184). National Aeronautics and Space Administration.

Matsuda, M., & DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care, 22(9), 1462-1470. http://www.ncbi.nlm.nih.gov/pubmed/10480510

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28(7), 412-419. https://doi.org/10.1007/BF00280883

Ryan, D. H., & Yockey, S. R. (2017). Weight Loss and Improvement in Comorbidity: Differences at 5%, 10%, 15%, and Over. Current Obesity Reports, 6(2), 187-194. https://doi.org/10.1007/s13679-017-0262-y

Stroud, J. E., Gale, M. S., Zwart, S. R., Heer, M., Smith, S. M., Montina, T., & Metz, G. A. S. (2022). Longitudinal metabolomic profiles reveal sex-specific adjustments to long-duration spaceflight and return to Earth. Cellular and Molecular Life Sciences, 79(11). https://doi.org/10.1007/s00018-022-04566-x

Wolever, T. M., Jenkins, D. J., Ocana, A. M., Rao, V. A., & Collier, G. R. (1988). Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. Am. J Clin. Nutr, 48(4), 1041-1047. http://www.ncbi.nlm.nih.gov/pubmed/2844076

14:45 Dominique Moser – "Preconditioning with mild hypergravity mitigates simulated microgravity-induced T cell dysfunctions"

Preconditioning with mild hypergravity mitigates simulated microgravity-induced T cell dysfunctions

Dominique Moser[1*], Katharina Biere[1], Christian Liemersdorf[2], Ruth Hemmersbach[2], Alexander Choukér[1]
[1]Laboratory of Translational Research 'Stress and Immunity', Department of Anesthesiology, LMU Hospital, Ludwig-Maximilian-University, Munich, Germany. *Dominique.Moser@med.uni-muenchen.de
[2]Gravitational Biology, Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany.

BACKGROUND

Artificial gravity encompasses both the restoration of Earth-like conditions in microgravity (µg) and the induction of hypergravity (hyper-g) in order to counteract µg-induced deconditioning in space. Hyper-g was shown to have beneficial effects on the musculoskeletal and cardiovascular system both as a countermeasure option for µg-related deconditioning and as "gravitational therapy" on Earth. However, the impact on the human immune system, which can be severely affected by µg was yet only sparsely explored. We investigated in a short-time in vitro approach, if an application of "mild" hyper-g (2.8g) is capable to counteract simulated (s-)µg-induced dysregulations in monocytes and T cells.

METHODS

To elaborate the single impact of the different g-grades used, whole blood of eight volunteers was incubated for six hours with the immunogenic antigens heat-killed listeria monocytogenes (HKLM) and Pokeweed mitogen (PWM) in either 1g, s-µg and hyper-g (2.8g). Alterations in immune responses were determined by flow-cytometric assessment of surface activation marker expression on T cells and monocytes and quantification of secreted cytokines. Three different sequences for subsequent hyper-g countermeasure approaches were designed: one preconditioning setting, where hyper-g (two hours) was applied before s-µg exposure (four hours) and two therapeutic approaches in which hyper-g was set either intermediately or at the end of s-µg.

RESULTS

In monocytes single g-grade exposure experiments revealed an increased pro-inflammatory state after antigen incubation in s-µg and an attenuated cytokine secretion in hyper-g. T cells displayed reduced surface expression of the activation marker CD69 as well as IFNγ secretion when antigen incubation was performed in s-µg. All three countermeasure approaches applying hyper-g didn´t alleviate the increased pro-inflammatory potential of monocytes. In T cells the preconditioning approach, in which samples were exposed to hyper-g before the s-µg phase, restored antigen-induced CD69 expression and IFNγ secretion to levels similar to 1g controls.

CONCLUSION AND OUTLOOK

This *in vitro* study demonstrates a proof of concept that mild hyper-g represents a gravitational preconditioning option to avoid T cell dysfunctions which are induced by s-µg.
Future approaches will focus on mechanistic insights as well as long-term effects and a more detailed resolution of the duration of hyper-g application.

FUNDING

This work was funded by ESA Ground based facility program [grant no. ESA-CORA-GBF-2018-002]. Support was granted by the Federal Ministry of Economics and Technology/Climate Action [BMWi/K; DLR grant 50WB1622 and 50WB1931] to DM, KB, MT and AC.

15:00 Silvana Miranda – "Gravity's Effect on T-Cell Activation: Unraveling the Mechanisms of Immune System Dysregulation in Space"

Gravity's Effect on T-Cell Activation: Unraveling the Mechanisms of Immune System Dysregulation in Space

S. Miranda [1,2], R. Vermeesen [1], S. Baatout [1, 2], B. Baselet [1]
[1] Radiobiology Unit, Belgian Nuclear Research Centre SCK CEN, Mol, Belgium.
[2] Faculty of Bioscience Engineering, Department of Biotechnology, Ghent University, Ghent, Belgium.
S. Miranda - *sfdsmira@sckcen.be*; R. Vermeesen – rvermeesen@sckcen.be; S. Baatout – sbaatout@sckcen.be; B. Baselet – bbaselet@sckcen.be

The space environment consists of a series of stress factors which are physical (cosmic radiation and altered gravity) and psychological in nature (stress). Space-related dysfunction of the human immune system has been shown during and after spaceflight, indicating a persistent nature. Dysregulation of the activation profiles of T-cells is present in the form of altered cytokine profiles, cytoskeleton alterations and gene expression dysregulation. However, the exact underlying molecular causes are not yet identified. Moreover, the knowledge on the effects of partial gravity levels contributed to other planetary bodies, such as the Moon or Mars, are still unknown. In order to enable long-duration space missions foreseen in the future, and human activities on the surface of the Moon and Mars, it is necessary to understand these mechanisms of space-induced immune dysfunction and develop successful countermeasure strategies.

In our project, activated Jurkat cells were exposed to simulated space conditions, alone or in different combinations, i.e. ionizing radiation (1 Gy carbon ions or X-rays), hydrocortisone (1µM), and 3 different gravity levels – Microgravity (~0.001g), Moon gravity levels(~0.167g) and Mars gravity levels(~0.333g). To assess cellular function, IL-2 levels were analyzed in the supernatant and cells were collected for quantitative polymerase chain reaction (qPCR) analysis. Results show a significant shift in IL-2 expression and secretion upon exposure to simulated space stressors. A significant reduction in IL-2 levels upon exposure to either 1µM of hydrocortisone or 1 Gy of radiation (carbon ions or X-rays) was observed. When hydrocortisone and ionizing radiation exposure were combined, a significant additional decrease was observed in IL-2 levels, suggesting an agonistic effect. When comparing the different gravity levels the cells were exposed to, we found that microgravity has the most prominent effect in the IL-2 levels, but an decrease proportional to the g value was not observed (Figure 1).

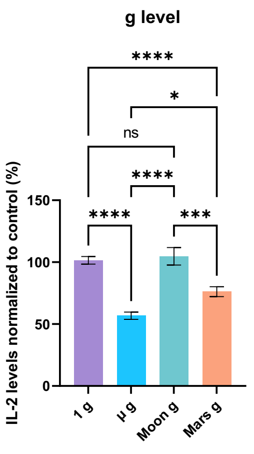


Figure 12 – Comparison of the effects of different gravity levels on the IL-2 concentration of Jurkat cells after 24h exposure. µ g = ~0.001g; Moon g = ~0.167g; Mars g= ~0.333g.
n=10; mixed effects analysis model on Prism 9 for Windows, version 9.0.1 (151); **** p<0.000

Future work will focus on donor-derived human CD4+ T-cells. For this, blood has been collected from healthy volunteers. Cells will be exposed to the aforementioned stressors and investigated using transcriptomic, proteomic, flow-cytometric and cytokine-profiling approaches to identify underlying mechanisms of space-induced CD4+ T cell dysfunction. An understanding of the molecular mechanisms underlying alterations of the T-cell population when exposed to the combined space environment can potentially enable the development and implementation of space-induced immune dysfunction countermeasures and also prove valuable input for the treatment of known immune-related pathologies occurring on earth.

Acknowledgements

This work was funded by ESA/BELSPO/Prodex IMPULSE-2 contract (PEA 4000109861). SM is supported by a doctoral SCK CEN/UGhent grant.

15:15 Judith-Irina Buchheim – "Role of individual immune profiles for wound healing under chronic stress during long-term space flight"

Role of individual immune profiles for wound healing under chronic stress during long-term space flight

J. I. Buchheim[1], K. Biere[2], S. Matzel[3], M. Hoerl[4], M. Feuerecker[5], D. Moser[6], M. Monici[7*], and A. Choukér[8*]
[1]Laboratory for Translational Research "Stress & Immunity", Dpt. of Anesthesiology, LMU University Hospital, Marchioninistr. 15, 81377 Munich, Germany, (Judith-irina.buchheim@med.uni-muenchen.de) , [2]Laboratory for Translational Research Stress & Immunity, Dpt. of Anesthesiology, LMU University Hospital Munich, Germany (katharina.biere@med.uni-muenchen.de), [3]Laboratory for Translational Research Stress & Immunity, Dpt. of Anesthesiology, LMU University Hospital Munich, Germany (sandra.matzel@med.uni-muenchen.de), [4]Laboratory for Translational Research Stress & Immunity, Dpt. of Anesthesiology, LMU University Hospital Munich, Germany (marion.hoerl@med.uni-muenchen.de), [5]Laboratory for Translational Research Stress & Immunity, Dpt. of Anesthesiology, LMU University Hospital Munich, Germany (matthias.feuerecker@med.uni-muenchen.de), [6]Laboratory for Translational Research Stress & Immunity, Dpt. of Anesthesiology, LMU University Hospital Munich, Germany (dominique.moser@med.uni-muenchen.de), [7]ASAcampus Joint Laboratory, ASA Res Div., Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy (monica.monici@unifi.it), [8]Laboratory for Translational Research Stress & Immunity, Dpt. of Anesthesiology, LMU University Hospital Munich, Germany, (alexander.chouker@med.uni-muenchen.de), *shared authorship

BACKGROUND

Wound healing is a multistep process that requires the coordinated interplay of different cell types and the efficient recruitment of immune cells to the site of the damage. Long-term stress (allostatic load) can interfere with wound repair and can result in infections or extensive scar tissue. In the health sector non-healing wounds are an enormous economic burden and are estimated to cause about $50 billion per year in the US alone. Among the patient groups at risk are people that suffer from high stress. Astronauts are subjected to high workload and other chronic stressors such as microgravity, isolation, sleep disruption and immune dysregulation placing them at risk for wound healing problems (Monici M., et al, 2022).

METHODS

In the frame of the ESA funded MAP project Wound Healing In Space: problems and PErspectives for tissue Regeneration and engineering (WHISPER) (Contract Number 4000130928/20/NL/PG/pt), data derived from the IMMUNO2 study (funded by ESA:ELIPS 3 and 4 and SciSpaceE programs and the German Space Agency (DLR) on behalf of the Federal Ministry of Economics and Technology/Energy (50WB0919, 50WB1319 and 50WB1622) was analyzed to evaluate and focus on stress load and wound healing problems associated with individual immune alterations in 7 Russian cosmonauts staying at least 5 months aboard the International Space Station (ISS). Perception of wound healing problems was measured by completion of the NASA wound healing survey at least once pre- in and postflight for each subject. We evaluated allostatic load employing questionnaires such as the current stress test (CST), profile of mood states (POMS), post-traumatic-symptom scale-10 (PTSS-10) and a numeric analogue scale for lower backpain. Additionally, the physical stress response was monitored in saliva, blood and hair samples, respectively. The functional immune status was assessed by immune cell counts as well as the cytokine response 48 hours after in vitro stimulation of whole blood. Furthermore, whole transcriptome analysis was performed using whole blood RNAseq data of 4 subjects at pre- and postflight timepoints. All studies were carried out according to the ethical code of the World Medical Association (Declaration of Helsinki). Ethical approval was obtained from the institutional review board of the Ludwig-Maximilians-University (LMU) Munich, Germany, and the medical board of the European Space agency (ESA) and the Russian Space Agency (Roscosmos).

RESULTS AND DISCUSSION

A strong 85% of participants estimated wound healing to be altered and 67% of those estimated the process to be delayed. 86% of the subjects believed that applying ointment on the wound would help improving the process. Perception of stress by cosmonauts seemed generally low suggesting that resilience and coping strategies in this cohort appear to work. Lower backpain was reported in some individuals after return from space, especially on day R+7 after return. Levels of the stress hormone cortisol, driven by the hypothalamic-pituitary-adrenal (HPA) axis and released under acute and chronic stress, as well as leukocyte subsets confirmed some of the data already seen in a previous long-term spaceflight study (Buchheim J.I., et al 2019) but showed also a lasting significant increase in monocytes on R+1 and R+7 after return. Stimulation assays revealed individually different trajectories of cytokine amounts after stimulation with lipopolysaccharide (LSP). Interestingly, two subgroups mirrored the results from the wound healing survey. Two individuals that perceived wound healing not to be impaired inflight also showed preserved interleukin 1b (IL-1b) levels, a key cytokine for wound healing, after stimulation with LPS compared to the lower IL-1b concentration detected in the other subjects. RNAseq data of cosmonauts again confirmed the individual genetic disposition or response to the spaceflight environment. In a pre- to post comparison two subjects (subject 1 compared to subject 4) showed a very distinct RNA expression pattern compared to each other. Interestingly the "antagonistic" patterns in these two subjects reflected again the results from the individual stimulation assays. One subject was from the group that showed higher IL-1b levels after stimulation, the other was from the group that showed a lower concentration compared to preflight. Our data show that individual immune responses to the very specific spaceflight environment render some participants more prone to wound healing problems than others despite similar training and preparation for the mission. Individual data analysis can help to develop personalized prevention strategies for future interplanetary missions.

REFERENCES

Monici M, van Loon J, Choukér A et al., 2022, *Front Bioeng Biotechnol*; 10:1078986.

Buchheim JI, Matzel S, Rykova M, et al., 2019, *Front Physiol*;10:85.

14:00 Ge Tang – "Establishing a link between brain and eye in long-duration spaceflight"

Establishing a link between brain and eye in long-duration spaceflight

Peter zu Eulenburg,[1,2] Ge Tang,[1]* Steven Jillings,[3] Ben Jeurissen,[4] Elena Tomilovskaya,[5] Alena Rumshiskaya,[6] Liudmila Litvinova,[6] Inna Nosikova,[5] Ekaterina Pechenkova,[7] Inessa B. Kozlovskaya,[5] Viktor Petrovichev,[6] Ilya Rukavishnikov,[5] Stefan Sunaert,[8] Paul M. Parizel,[9] Valentin Sinitsyn,[10] Jan Sijbers,[4] Jitka Annen,[11,12] Steven Laureys,[11,12] Angelique Van Ombergen,[13] Floris L. Wuyts[3]*, and Seyed-Ahmad Ahmadi[2]

[1]Institute for Neuroradiology, Faculty of Medicine, Ludwig-Maximilians-University Munich, Munich, Germany, [2]German Center for Vertigo and Balance Disorders, Ludwig-Maximilians-University Munich, Munich, Germany, [3]Lab for Equilibrium Investigations and Aerospace, University of Antwerp, Antwerp, Belgium, [4]Imec/Vision Lab, University of Antwerp, Antwerp, Belgium, [5]SSC RF – Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia, [6]Radiology Department, Federal Center of Treatment and Rehabilitation, Moscow, Russia, [7]Laboratory for Cognitive Research, National Research University, Higher School of Economics, Moscow, Russia, [8]Translational MRI, Department of Imaging & Pathology, KU Leuven, Leuven, Belgium, [9]Radiology Department, Antwerp University Hospital & University of Antwerp, Antwerp, Belgium, [10]Faculty of Fundamental Medicine, Lomonosov Moscow State University, Moscow, Russia, [11]Coma Science Group, GIGA-Consciousness, University of Liège, Liège, Belgium, [12]Centre du Cerveau², University Hospital of Liège, Liège, Belgium, and [13]Department of Translational Neurosciences – ENT, University of Antwerp, Antwerp, Belgium

* indicates equal contribution

Introduction

In long-duration spaceflight (LDSF), spacefarers are known to develop diminished near-distance visual acuity and a series of orbital and retroorbital changes (Mader, Gibson et al. 2011). This syndrome has been termed spaceflight-associated neuro-ocular syndrome (SANS). At the same time, the human brain in LDSF seems to respond with an enlargement of the cerebrospinal fluid compartments at the cost of grey matter volume. The neurophysiological link between these brain-structural alterations and changes in the ocular and retroorbital spaces has never been substantiated. We, therefore, aimed to develop an automatic ocular morphometrics pipeline and establish this connection for human physiology in general and LDSF cosmonauts in particular.

Methods

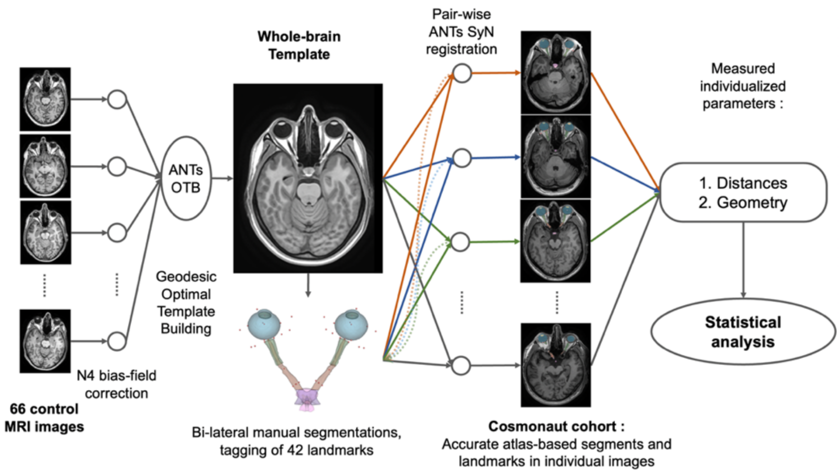


Fig. 1. Graphical abstract of the image processing pipeline. The spacefarers and their matched controls were scanned with T1-weighted MRI at 3T. We computed an unbiased morphological average MRI template (0.2mm isotropic) after denoising and bias-field correction from all unexposed control subjects (one template for each scanning site), using a state-of-the-art template computation algorithm. Key landmarks were tagged in each template. The landmarks together with the respective volume segmentations were transferred into the subject space using high-precision pair-wise atlas registration. Anatomical parameters were measured by computing distances between landmarks. We then investigated statistical hypotheses tests regarding long-term microgravity-induced changes of eye morphology (pairwise pre- vs. post-flight) and structural differences between the left and right eye, retroorbital space and optic nerve.

Structural neuroimaging mission data from thirteen male Russian cosmonauts were used in this study. All but three cosmonauts had previous spaceflight experience. Fifteen age-, gender- and education-matched subjects were analysed as a control cohort. In a complementary analysis, we then added five European astronauts' MRI data and their respective controls. Considering the region of interest in our study, we first built two cohort-specific whole-brain templates for the Russian cosmonauts and European astronauts separately with Advanced Normalization Tools (ANTs). Based on this template, we used 3D Slicer in a supra-resolution approach to segment the ocular and retroorbital structures, from the eye to the optic chiasm. Afterwards, we again applied ANTs for the deformable registration of templates and atlas to the individual subject space. Parallel to this process, we also quantified intracranial tissue spaces through the computational anatomy toolbox (CAT12). We were then able to extract the respective parameters and investigate our hypotheses.

Results

Our findings reveal a profound association of intracranial volume and ocular as well as retroorbital parameters for control subjects and LDSF spacefarers alike. There was a significant correlation observed between the extent of expansion for the third ventricle and the degree of reduction in the volume of the eyeball, as well as the shortening of the eyeball axial length after spaceflight. The optic nerve sheath volume increased by ~0.01cm3 postflight in the right eye and ~0.03 cm3 in the left eye. The right optic nerve lengthened by ~0.38mm postflight, and the left optic nerve by ~0.47mm. Along with these findings, we also observed a significant forward ocular displacement. Surprisingly, we discovered several significant structural differences between the left and right eye in humans, which might play a role in the manifestation of raised intracranial pressure in humans.

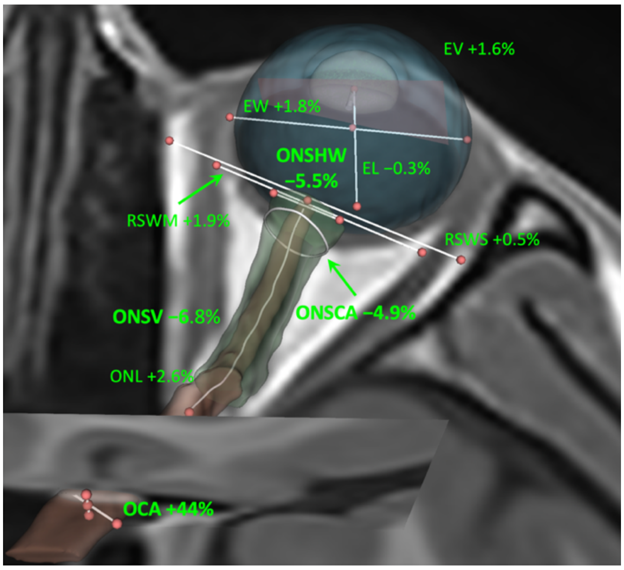


Fig. 2. The overview of significant relative right eye versus left eye differences (%) based on the average left eye ocular and retroorbital parameters. The largest differences were found for the size of the optic canal and along the optic nerve. All the findings were first discovered in the control cohort and were then replicated in the cosmonaut group. EV: eyeball volume; EW: eyeball width; EL: eyeball axial length; ONSHW: optic nerve sheath width; RSWM: retroorbital space width (muscle); RSWS: retroorbital space width (soft tissue); ONSCA: optic nerve sheath cross-sectional area; ONSV: optic nerve sheath volume; ONL: optic nerve length; OCA: optic canal area.

Conclusion

Our data support the hypothesis that the changes observed in the brain and eye after LDSF is likely driven by the same mechanism. The observed significant structural differences between the left and right eye and the corresponding retroorbital spaces might make the right optic nerve tip more susceptible to raised intracranial pressure.

REFERENCES

Mader, T. H., C. R. Gibson, A. F. Pass, L. A. Kramer, A. G. Lee, J. Fogarty, W. J. Tarver, J. P. Dervay, D. R. Hamilton, A. Sargsyan, J. L. Phillips, D. Tran, W. Lipsky, J. Choi, C. Stern, R. Kuyumjian and J. D. Polk (2011). "Optic Disc Edema, Globe Flattening, Choroidal Folds, and Hyperopic Shifts Observed in Astronauts after Long-duration Space Flight." Ophthalmology **118**(10): 2058-2069.

ACKNOWLEDGMENTS

No potential conflict of interest relevant to this study was reported. This work was supported by the German Space Agency (DLR) on behalf of the Federal Ministry of Economics and Technology/Energy (50WB2027).

14:15 Kimia Seyedmadani – "Superior Baseline Oculomotor Performance Viewing with the Dominant Eye"

Superior Baseline Oculomotor Performance Viewing with the Dominant Eye

K. Seyedmadani[1,2], Y. Akay[2], M. Akay[2] and L.Stone[3]
[1]National Aeronautics and Space Administration, Johnson Space Center,2101 E NASA Pkwy, Houston, Texas,77058 United States of America, (Kimia.Seyedmadani@nasa.gov) [2]Department of Biomedical Engineering, University of Houston, Houston, 3517 Cullen Blvd, RM 2018 Houston, Texas 77204 United States of America, (kseyedmaadani@uh.edu, ymakay@uh.edu, makay@uh.edu) [3]National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California, United States of America, (leland.s.stone@nasa.gov)

ABSTRACT

BACKGROUND

Human neurophysiological deconditioning, due to exposure to the many hazards of spaceflight, has been a central challenge for the space program and is now more acute as extended missions are becoming more routine and a multi-year crewed trip to Mars is being planned. Determining the extent to which deconditioning could adversely impact crew performance in operational tasks, assessing how long any impairment might persist post-landing, and identifying any long-term health consequences will be necessary to enable any autonomous, long-duration, deep-space missions (e.g., Mars Clement 2020). In that regard, humans depend particularly on their visual perception and associated visuomotor control to guide nearly all motor action so novel sensitive methods for monitoring the effects of spaceflight on visual/visuomotor function will need to be tailored for use in long term missions. Using oculomotor measures to detect mild visual/visuomotor impairment is a promising tool (Krukowski & Stone, 2005), however, spaceflight applications will require establishing clear baselines.

PROBLEM

NASA has long been observing, and tracking, ophthalmological changes associated with spaceflight driven by fluid shifts associated with microgravity, and other factors. Recent comparison of Optical Coherence Tomography (OCT) measures of retinal thickening pre- vs. postflight indicate that more than two-thirds of US crew members of International Space Station (ISS) show significant changes in retinal structure after missions lasting 6-months or more (Stringer, 2017). This phenomenon, Space Associated Neuro-ocular Syndrome (SANS), consists of visual acuity decrements as well as ocular structural changes (Kramer et al., 2012; Mader et al., 2011; Lee et al 2020; Clement, 2020) that could potentially become severe enough to adversely impact in-flight performance as missions become longer and more autonomous. While some symptoms recover during the first year postflight, others persist and may threaten the long-term visual health of returning crew (Mader et al., 2011). The impact of spaceflight on the visual system, however, is not limited to the retina. Recent comparisons of pre- and post-flight brain images have revealed structural changes throughout the brain (Zahid et al 2020, Wuyts et al 2020) that implicate involvement of central visual, visuomotor and visuo-cognitive pathways. Studies investigating spaceflight effects across the entire brain are needed to create a comprehensive, integrated framework of understanding and, ultimately, in finding effective countermeasures. Structural alterations in visual/visuomotor/visuocognitive brain areas, due to weightlessness, are consistent with observed functional impacts: decreased speed and accuracy of fine goal-oriented movements, somatosensory difficulties, and movement-timing impairment (De la Torre, 2014). Current limitations on inflight testing of the crew make it very difficult to determine when, and under what conditions, SANS and other disruptions of human visual processing and visuomotor control arise. Current studies have thus far focused largely on structural changes with few measures of any functional impacts. The Comprehensive Oculomotor Behavioral Response Assessment (COBRA) tool, which consists of an efficient 5-minute ocular tracking task and generates a set of largely independent metrics related to a range of neural areas and functions (Krukowski & Stone, 2005; Stone et al., 2019), has been shown to provide reliable and sensitive measures of mild neural impairment due to of mild TBI (Liston et al., 2017), sleep deprivation (Stone et al., 2019), and alcohol consumption (Tyson et al., 2021). Our goal here is to investigate human visual and visuomotor performance under monocular viewing conditions and to establish a normal healthy baseline that could be used to interpret monocular performance and inter-ocular differences in pathological states as a basis for future assessments of postflight performance. Any inter-ocular differences due to eye dominance would need to be taken into consideration.

HYPOTHESIS

As in the case of handedness, many aspects of visual and visuomotor performance, as captured by COBRA oculometrics will be better when driven by the dominant eye as compared to when driven by the non-dominant eye.

METHODS

Participants (healthy astronauts-like subjects from NASA Johnson Space Center pool) will be asked to perform a dominance "Mile's" test to determine eye-dominance . We will then the mean and variance in 19 oculomotor measures of performance (oculometrics), as indicators of ocular and neural health, captured by performing a 5-minute radial ocular tracking task under monocular viewing conditions. Each run will consist of 90 trials, with random directional sampling in 4° increments around the circle. The task encompasses a high degree of spatiotemporal uncertainty across many task parameters to ensure the responses are driven visually and not by prediction or anticipation. The computation and interpretation of the various metrics are explained in detail elsewhere (Stone et, 2019).

RESULTS

Our preliminary findings (17 subjects, age: 24—55, 7 females) showed significantly (one-tailed paired t-test) higher initial pursuit acceleration (118.4 vs., 105. 1deg/s2, $p < 0.001$), steady-state pursuit gain (0.86 vs., 0.81, $p < 0.017$), saccade amplitude (1.47 vs., 01.7, $p < 0.015$) and speed responsiveness (14.6 vs. 16.9, $p < 0.04$) when viewing through the dominant eye as opposed to the non-dominant eye.

DISCUSSION AND CONCLUSION

To assess the potential pathology of a given retina postflight using our visuomotor task, it is crucial to characterize normal baseline performance when a single retina is driving ocular tracking and to account for any performance impacts due to eye dominance. This study aims to establish a clear healthy across-subject baseline and distribution of monocular performance and of inter-ocular differences in a well-matched population to allow us to tailor and optimize COBRA to reliably detect postflight retinal impairment and other visual and visuomotor deficits, potentially even without a pre-flight within-subject baseline. The reliable, sensitive, rapid, non-invasive testing of a range of visual and visuomotor functions using oculometrics represents a promising method for the detection and characterization of postflight visual and visuomotor perturbations (retinal or cortical) and could facilitate the assessment of any countermeasure effectiveness as well.

Reference

Beutter B.R., Eckstein M.P., & Stone L.S. Saccadic and perceptual performance in visual search tasks. I. Contrast detection and discrimination. Journal of the Optical Society of America A 20, 1341-55 (2003).

Carvalho, Nicolas, et al. “Saccadic Eye Movements in Depressed Elderly Patients.” *PLOS ONE*, Public Library of Science, 2014, journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0105355.\

Clement, G., Boyle, R., George, K., Nelson, G., Reschke, M., Williams, T., Paloski, W.,2020. Challenges to the central nervous system during human spaceflight missions to Mars. Journal of NeuroPhysiology.

De la Torre, G., 2014. Cognitive Neuroscience in Space. Life 4, 281–294. https://doi.org/10.3390/life4030281

Flynn-Evans, E.E., Tyson, T.L. Cravalho, P.F., Feick, N.H. & Stone, L.S. Low-Dose Caffeine Administration During Acute Sleep Deprivation Eliminates Visual Motion Processing Impairment, but Does Not Improve Saccadic Rate. SLEEP (2019).

Kramer, L. A., Hasan, K. M., Sargsyan, A. E., Wolinsky, J. S., Hamilton, D. R., Riascos, R. F., Carson, W. K., Heimbigner, J., Patel, V. S., Romo, S., & Otto, C. (2015). MR-derived cerebral spinal fluid hydrodynamics as a marker and a risk factor for intracranial hypertension in astronauts exposed to microgravity. Journal of Magnetic Resonance Imaging: JMRI, 42(6), 1560–1571. https://doi.org/10.1002/jmri.24923

Kramer, L.A., Sargsyan, A.E., Hasan, K.M., Polk, J.D., Hamilton, D.R., 2012. Orbital and intracranial effects of microgravity: findings at 3-T MR imaging. Radiology 263, 819–827. https://doi.org/10.1148/radiol.12111986

Kumra S;Sporn A;Hommer DW;Nicolson R;Thaker G;Israel E;Lenane M;Bedwell J;Jacobsen LK;Gochman P;Rapoport JL;, Sanjiv. "Smooth Pursuit Eye-Tracking Impairment in Childhood-Onset Psychotic Disorders." *The American Journal of Psychiatry*, U.S. National Library of Medicine, 2001, pubmed.ncbi.nlm.nih.gov/11481165/

Kuznetsova, G.D., Gabova, A.V., Lazarev, I.E., Obukhov, I.V., Obukhov, K.I., Morozov, A.A., Kulikov, M.A., Shchatskova, A.B., Vasil'eva, O.N., Tomilovskaia, E.S., 2015. [FREQUENCY-TEMPORAL STRUCTURE OF HUMAN ELECTROENCEPHALOGRAM IN THE CONDITION OF ARTIFICIAL HYPOGRAVITY: DRY IMMERSION MODEL]. Aviakosmicheskaia Ekol. Meditsina Aerosp. Environ. Med. 49, 25–32.

Lee NY, Lee DK, Song HS. Effect of virtual reality dance exercise on the balance, activities of daily living, and depressive disorder status of Parkinson's disease patients. *J Phys Ther Sci*. 2015;27(1):145-147. doi:10.1589/jpts.27.145

Lee, A., Mader, T., Gibson , R., Traver, W., Rabiei, P., Riascos, R., … Brunstetter, T. (2020). Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. Nature Microgravity , 6(7).

Li, J., Wang, J., Wei, X.-E., Zhao, Y.-W., Wang, F., & Li, Y.-H. (2020). Examination of Structural Variations of the Circle of Willis by 3D Time-of-Flight Magnetic Resonance Angiography. Frontiers in Neuroscience, 14, 71. https://doi.org/10.3389/fnins.2020.00071

Liston, D.B. & Stone L.S. Effects of prior information and reward on oculomotor and perceptual choices. Journal of Neuroscience, 28, 13866 –13875 (2008).

Liston, D.B. & Stone, L.S. Oculometric assessment of dynamic visual processing. Journal of Vision, 14(14):12, 1-17 (2014).

Liston, D.B., Krukowski, A.E., & Stone, LS. Saccade detection during smooth tracking. Displays, 34, 171–176 (2013).

Liston, D.B., Simpson S., Wong, L.R., Rich, M., & Stone, L.S., Design and validation of a simple eye-tracking system. In: Proceedings of the Ninth Biennial ACM Symposium on Eye Tracking Research & Applications, pp. 221-224. ACM, (2016).

Liston, D.B., Wong, L.R., & Stone, L.S. Oculometric assessment of sensorimotor impairment associated with TBI. Optometry and Vision Science, Optometry and Vision Science, 94:1, 51-59 (2017).

Mader, T.H., Gibson, C.R., Pass, A.F., Kramer, L.A., Lee, A.G., Fogarty, J., Tarver, W.J., Dervay, J.P., Hamilton, D.R., Sargsyan, A., Phillips, J.L., Tran, D., Lipsky, W., Choi, J., Stern, C., Kuyumjian, R., Polk, J.D., 2011. Optic Disc Edema, Globe Flattening, Choroidal Folds, and Hyperopic Shifts Observed in Astronauts after Long-duration Space Flight. Ophthalmology 118, 2058–2069. https://doi.org/10.1016/j.ophtha.2011.06.021

Paloski, W.H., Oman, C.M., Bloomberg, J.J., Reschke, M.F., Wood S.J. Harm, D.L., Peters, B.T., Mulavara, A.P. Locke, J.P., & Stone, L.S. Risk of Sensory-Motor Performance Failures Affecting Vehicle Control During Space Missions: A Review of the Evidence. Journal of Gravitational Biology, 15, 1-29 (2008).

Stenger, M. B., Tarver W. J., et al. "Risk of Spaceflight Associated Neuro-ocular Syndrome (SANS)" *NASA*, 30 Nov. 2017

Stone L.S., Beutter B.R., Eckstein M., & Liston D. "Oculomotor Control: Perception and eye movements" In: The New Encyclopedia of Neuroscience, edited by Larry Squire et al, Elsevier (2009).

Stone, L.S., Beutter, B.R., & Lorenceau, J. Visual motion integration for perception and pursuit. Percept. 29, 771-787 (2000).

Stone, L.S., Tyson, T.L. Cravalho, P.F., Feick, N.H. & Flynn-Evans E.E. Distinct pattern of oculomotor impairment associated with acute sleep loss and circadian misalignment. Journal of Physiology (London), 597(17):4643-4660 (2019).

Stone, Leland S., and Liston, Dorion B. *Comprehensive Oculomotor Behavioral Response Assessment (COBRA).* 15 Aug. 2017, US Patent 9,730,582

Stone, Leland S., Liston, Dorion B. *Comprehensive Oculomotor Behavioral Response Assessment System*. 5 Nov. 2019, US Patent 10,463,249

Stone, Leland S., Liston, Dorion B. *Oculometric Assessment of Sensorimotor Impairment.* 29 Sept. 2019, US Patent 10,420,465

Strenger, M Risk of Spaceflight Associated Neuro-ocular Syndrome. (2017) NASA Johnson Space Center

Tyson, T.L., Feick, N.H., Cravalho, P.F., Tran, T., Flynn-Evans, E.E. & Stone, L.S. Impairment of Human Ocular Tracking with Low-Dose Alcohol, 28th Neural Control of Movement Annual Meeting (2018).

Tyson, T.L., Feick, N.H., Cravalho, P.F., Tran, T., Flynn-Evans, E.E. & Stone, L.S. Increased Dependence on Saccades for Ocular Tracking with Low-Dose Alcohol, Society for Neuroscience annual meeting (2018).

Van Ombergen, A., Demertzi, A., Tomilovskaya, E., Jeurissen, B., Sijbers, J., Kozlovskaya, I.B., Parizel, P.M., Van de Heyning, P.H., Sunaert, S., Laureys, S., Wuyts, F.L., 2017. The effect of spaceflight and microgravity on the human brain. J. Neurol. 264, 18–22. https://doi.org/10.1007/s00415-017-8427-x

Wuyts, F. L., Jillings, S., van Ombergen, A., Jeurissen, B., Tomilovskaya, E., Rumshiskaya, A., ... zu Eulenburg, P. (2019). Novel insight on effect and recovery of long-duration spaceflight on the ventricles of the space traveller's brain. Proceedings of the International Astronautical Congress, IAC, 2019-October, [IAC-19A124x51230].

Zahid, A., Martin, B., Collins, S., Oshinski, J., Ethier, C., 2020. *Quantification of Arterial, Venous, and Cerebrospinal Fluid Flow Dynamics by Magnetic Resonance Imaging Under Simulated Micro-Gravity Conditions: A Prospective Cohort Study. Research Square.* DOI: 10.21203/rs.3.rs-16448/v1

14:30 Jean Pauly – "Psychological and cognitive adaptation during a 3-weeks confinement in a space-like environment"

Psychological and cognitive adaptation during a 3-weeks confinement in a space-like environment

J. Pauly[1], C. Langlet[1], J-P. Hainaut[1], and B. Bolmont[1]
[1]Lorraine Laboratory of Psychology and Neuroscience (jean.pauly@univ-lorraine.fr)

INTRODUCTION

Space missions are a source of multiple stressors that can disturb the psychological states of astronauts. However, studies in space or in space analogous environments (such as confinements) struggles to find a consensus on the psychological adaptation to these stressors (positive, negative, or neutral; Alfano et al., 2018). Therefore, more research is required to better understand the human psychological mechanisms. In addition, not enough studies consider multiple aspects of psychological adaptation at the same time (affective, social, cognitive, behavioral…). Therefore, we do not have an overall perspective on the psychological impact of space conditions.

Multiple confinements in analogous environments are created on earth to simulate some psychological stressors of space missions (isolation, communication delays, workload...) and study their impacts on humans. We used one of these missions in Utah's desert to assess the time-course variations of multiple parameters that may account for psychological adaptation in a 3-weeks confinement simulating a Martian mission. These parameters concern affective states, perceived stress, social functioning, sleep quality and cognitive control. We hypothesize that the isolation could influence these parameters.

METHODS

Two student crews (13 participants: 5 males/8 females, age: 22.38 +/- 1.12) were isolated in a dedicated station in the desert of Utah for three weeks (Mars Desert Research Station - MDRS).

The participants responded to various psychometric tests measuring affective states (ICE-Q emotional adaptation, STAI-YA, POMS, SAM, VAS - valence of thoughts), perceived stress (SOS, ICE-Q occupational adaptation), social functioning (ICE-Q social adaptation) and sleep quality (PSQI, ICE-Q physical adaptation). They also performed cognitive control tasks measuring attentional and executive performance (TAP - Test of Attentional Performance). Each subject performed the protocol five times during the confinement (Day 3, 7, 11, 15, 19).

We used a non-parametric, one-way repeated measures analysis (Friedman test) to observe the time-course variations of every parameters.

RESULTS

The POMS – Tension/Anxiety and the POMS – Anger/Hostility ($p=0.01$ and $p=0.004$ respectively) factors both significantly decreased during the isolation.

We observed a significant increase for the ICE-Q - occupational factor during the mission ($p = 0.02^*$). We also observed a significant decrease for the three items of SOS throughout the isolation (Personal Vulnerability – $p<0.001$, Event Load – $p=0.006$, Total score – $p<0.001$).

Median reaction times in the Alertness task significantly decreased throughout the mission ($p=0.003^{**}$).

No significant difference was observed for the other parameters.

DISCUSSION

Our study proposed to monitor multiple psychological parameters in order to have a global perspective on psychological adaptation during a 3-weeks isolation in space-like conditions. The decrease of Tension/Anxiety and Anger/Hostility indicates a partial improvement of affective states during the mission. In addition, perceived stress appeared to decrease throughout the confinement, indicating a better adaptation to workload and environmental pressure. Sleep quality and social functioning appeared to be preserved during the experiment. Such positive outcomes have been observed previously (Ihle et al., 2006).

Attentional and executive abilities remained stable during the isolation. However, reaction times improved throughout time in an alertness task. We attribute this result to competition among the participants, which could have increased their performance.

We conclude that the 3-weeks confinement was associated with positive effects and a good psychological adaptation from the participants. However, our study only lasted three weeks. This duration is not enough to observe the effects of space stressors such as isolation, workload, or social dynamic during long periods. Therefore, it would be relevant to study whether such results on the multiple psychological adaptation factors are found during longer confinements.

REFERENCES

Alfano, C. A., Bower, J. L., Cowie, J., Lau, S., and Simpson, R. J., 2018, Acta Astronautica, 142, 289-299.

Ihle, E. C., Ritsher, J. B., & Kanas, N., 2006, Aviation, Space, and Environmental Medicine, 77(2), 93-101.

14:45 Gabriel De La Torre – "Neurocognitive assessment in microgravity: Review under a clinical perspective"

Neurocognitive assessment in microgravity: Review under a clinical perspective

G. De la Torre, Gabriel[1], Gonzalez-Torre, Sara[1], and Ramallo, Miguel Angel[1]
[1]Neurotek Lab. University of Cadiz. Spain gabriel.delatorre@uca.es

ABSTRACT

We present a review analysis of existing studies and research performed in neuropsychological assessment in microgravity to this date. Current neuropsychological evaluation of astronauts is mainly based on performance because clinical level complications are not expected. However, for future long-duration missions to the Moon and Mars, a new perspective may be needed—a more clinical approach. Different risks, both environmental, such as radiation, accidents and biopsychological, including neurological problems, represent potential sources of subsequent neurocognitive deficits. We introduce the concept of analog diseases. Analog diseases would be those neurological conditions commonly observed in clinical practice which can be targeted as potential comparison of impairment that astronauts may suffer from exposition to the risks present in long duration missions. Testing current neurocognitive assessment tools in these analog diseases may provide a reference data for future early detection of neurocognitive symptoms. Finally, we present the different neuropsychological functions studied in microgravity during these years as well as the different risks with potential compromise of neurocognitive impairment or brain injury.

It is difficult to know what type of medical problem an astronaut may have in space, but it is more probable that they would have some type of issue in long-duration missions due to a series of risk factors including radiation exposure; accidents and concussion; microgravity related aspects such as vascular and metabolic, respiratory abnormalities and also psychiatric and psychological risks (Table 1). Each factor represents a potential risk for injury to the brain and subsequently to neuropsychological functioning.

Since the number of diseases and different syndromes that may present some form of cognitive or behavioral problem is large, some specific disorders can be selected as analog diseases references due to their similarities in etiology and symptomatology to those that astronauts could develop in long-term missions due to stressors, environmental risks, and other factors (Nasrini, Hermosillo, Dinges, Moore, Gur, & Basner, 2020), studying samples of patients with neurological conditions, especially in early and subclinical stages, represents a very interesting option. This can be an important source of data for early symptom detection and reference, using these neurological diseases as analog diseases models. These type of clinical sample assessments have been used with previous existing cognitive batteries for human spaceflight such as WinSCAT (Kane, Short, Sipes, & Flynn, 2005).

We need to pay more attention to clinical aspects and see how these tools can detect clinical symptoms, especially in preparation for future long-duration missions. Analog disease models in combination with machine learning models may help to predict and to identify possible neurocognitive deficit profiles and associated neuropathology. For this reason, testing batteries such as Cognition in these clinical conditions on Earth, and correlating these tests with neuropsychological tests widely used for neurological disorders and brain injury represents a helpful application. Only if this is sufficiently attended, will we be able to discern if a neuropsychological symptom an astronaut may present is due to performance decrement, fatigue, motivation, etc. related or CNS injury based.

There are many potential risks that can affect neurological and neuropsychological aspects. For all these reasons, having appropriate tools to detect and monitor neurocognitive status and countermeasure for possible injuries and deficits are of paramount relevance. This review covers all studies performed to this date in microgravity using neurocognitive testing (Figure 1), especially those using cognitive batteries specifically designed to be used in spaceflight conditions and the different cognitive domains studied in every case. This review serves as a detailed compendium of existing research in microgravity and human cognitive function and also discuss the performance versus clinical approach for the benefit of future human spaceflight, especially long duration missions.

Risk Factor	Brain effects	Potential Neurological Disease Analogs

Radiation	Localized damage in medial prefrontal cortex (mPFC) and hippocampus Synapses, dendrites and neurons damage Microglia increments and inflammation Cancer	*Alzheimer Disease, Epilepsy, Hypertension, Cushing's Disease* *Remitting-Relapsing Multiple Sclerosis* *Neurodegenerative Diseases* *Brain Tumors*
Traumatic Brain Injury (TBI)/ concussion	Primary (hemorrhage, hematoma) Secondary Injury (diffuse axonal injury, hydrocephalus, edema)	*TBI Potential Deficits:* ***Frontal** (motor, behavioral, attention, language (motor), memory* ***Temporal** (memory, language, behavioral problems, prosopagnosia)* ***Parietal** (neglect, naming, dyscalculia)* ***Occipital** (visual agnosia, diplopia, visuo-spatial deficits)*
Psychological/ Psychiatric/ Circadian rhythms alteration	Chemical and neurotransmitters alterations	*Depression* *Psychosis, Schizophrenia* *Anxiety, Stress, Sleep problems*
Vascular/CSF	Hydrocephalus Hemorrhage Ischemia Hypertension, High Intracranial Pressure	*Cerebrovascular Disease (ictus, embolic, hemorrhagic)* *Neurodegenerative Diseases (vascular)* *Idiopathic intracranial hypertension (IIH)* *Normal-pressure hydrocephalus (NPH)* *Perivascular Spaces Alterations (PVS)*
Metabolic	Imbalance of: Glutamate GABA **Choline-containing phospholipids (ChPh)**	Disturbances in neurological disorders: *Aging (Glutamate↓ GABA↓ ChPh↑)** *Alzheimer Disease (Glutamate ↓ GABA↓)* *Parkinson Disease (Glutamate↑ ChPh↓)* *Huntington Disease(Glutamate↑ ChPh↑)*
Respiratory	Hypoxia Low Blood Oxygen Levels	*Silicosis* *Brain Hypoxia*

Table 1. Risks for neurological and neuropsychological impairment, associated brain effects and potential disease consequences and disease analogs. (*) ↑↓ *represents decrement/ increment in that specific neurotransmitter/ metabolite.*

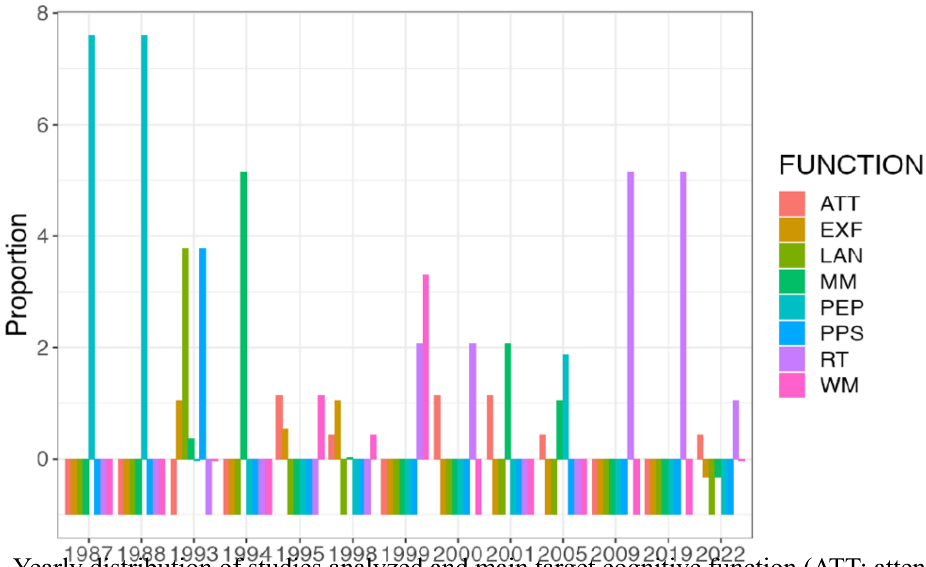


Figure 1. Yearly distribution of studies analyzed and main target cognitive function (ATT: attention; EXF: Executive Functions; LAN: Language; MM: Memory; PEP: Perception; PPS: Processing speed; RT: Reaction Time; WM: Working memory.

REFERENCES

Kane, R. L., Short, P., Sipes, W., & Flynn, C. F. (2005). Development and validation of the spaceflight cognitive assessment tool for windows (WinSCAT). Aviation, Space, and Environmental Medicine, 76(6), B183-B191.

Nasrini, J., Hermosillo, E., Dinges, D. F., Moore, T. M., Gur, R. C., & Basner, M. (2020). Cognitive Performance During Confinement and Sleep Restriction in NASA's Human Exploration Research Analog (HERA). Frontiers in physiology, 11, 394.

15:00 Elisa Raffaella Ferrè – "Cognition in Zero-G: How Altered Gravity Influences Human Brain and Behaviour"

Cognition in Zero-G: How Altered Gravity Influences Human Brain and Behaviour

Elisa Raffaella Ferre
Department of Psychological Sciences, Birkbeck University of London, London, UK (elisa.ferre@bbk.ac.uk)

INTRODUCTION

Human missions to Mars and the Moon along with commercial ventures for space travel are fast becoming a reality. As humanity prepare for a new space exploration age, understanding the impact of spaceflight on the human body and brain has never been timelier. While the effects of non-terrestrial gravities on human bodily physiology, such as the musculoskeletal and cardiovascular systems are well-documented, relatively little is known on the impact of altered gravity on the human brain and behaviour. Here, we critically review key findings across the last 10 years of space research to provide a framework of cognition in zero gravity exploring findings in healthy individuals.

DIFFUSE FRAMEWORK VS. CASCADE FRAMEWORK?

No comprehensive theoretical model exists to outline the impact of non-terrestrial gravity on behaviour. Based on the anatomical and functional features of the brain areas affected by non-terrestrial gravity, we hypothesised that altered vestibular-gravitational signals might affect three main domains of neurocognitive function (Figure 1). First, a Sensorimotor Domain which includes pathways for the integration of sensory signals for orientation, perception and motor control. Second a Cognitive Domain which includes pathways for regulation of attention, executive functions, decision making and other higher cognitive functions. Finally, a Socio-Affective Domain which includes pathways for regulation of social behaviour and emotions. We hypothesised two neurocognitive frameworks to potentially explain the effects of altered gravity on behaviour (Figure 1). First, a Diffuse Framework outlines an independent effect of zero gravity on the three domains. That is, deficits in one domain are independent to others. Independent interactions between altered vestibular-gravitational input would occur with each domain. Alternatively, a Cascade Framework takes a stepped approach whereby vestibular-gravitational alterations firstly impact sensorimotor functioning and then cascade onto cognitive and socio-affective processing. According to this model, deficits in sensorimotor functioning for instance in arm reaching may lead to deficits in measures of cognitive processing such as increased reaction time and slower speed. This framework suggests a reliance of cognition and socio-affective processing on sensorimotor functioning, and potential bidirectional influences between cognition and socio-affective processing.

A NOVEL EFFECT SIZE APPROACH

But, would it be possible to differentiate between the Diffuse Framework and the Cascade Framework? We have attempted to tackle this challenge by estimating the effect size on a selection of studies in the sensorimotor, cognitive and socio-affective domains to capture a representative, though clearly not systematic, sample. We focussed on papers that have been peer-reviewed in the last ten years, that are widely cited and that used established methods to simulate non-terrestrial gravity environments. Focus was given to quantitative reports. Studies exploring social factors, culture, group conflict or team dynamics were not included. Our preliminary search identified approximatively 146 articles relevant for sensorimotor domain, 91 articles for the cognitive domain and 63 for the socio-affective domain. However, for most of these studies it was not possible to compute the effect size estimates based on the reported statistical details. Data support the Cascade Framework; the effect sizes are much higher in the sensorimotor domain compared to the cognitive and socio-affective domains highlighting an interesting neurofunctional architecture for the contribution of gravity on behaviour. Importantly, this is aligned with evidence suggesting that there is a strong interaction between vestibular and sensorimotor cues for controlling orientation, posture and motor control.

CONCLUSION

Having a coherent and accurate perception of the external environment is critical especially during space missions. We have reviewed the effects of non-terrestrial gravity on the human brain and behaviour across the sensorimotor, cognitive and socio-affective domains and have proposed a neurocognitive model based on the effect size of gravity effects on these key functions. The effect sizes are much higher in the sensorimotor domain compared to the cognitive and socio-affective domain, supporting a Cascade Framework. Fundamentally, our exercise highlighted the limitations of current human space research. Future studies should take a more systematic approach with a priori hypotheses driven by neurocognitive and neuroanatomical evidence and models. While the methodological challenges of creating physical zero gravity on Earth are inherently insurmountable, generating theoretically driven approaches, recruiting diverse large samples, using a range of tasks across domains and testing across multiple timepoints can help develop a coherent understanding of the effect of non-terrestrial gravity on the human body and brain. This quantified and systematic approach will not only allow us to identify how gravity constitutes foundational and fundamental signals for cognition, but also enable the development of effective training and interventions for future exciting space exploration, ultimately mitigating against risk.

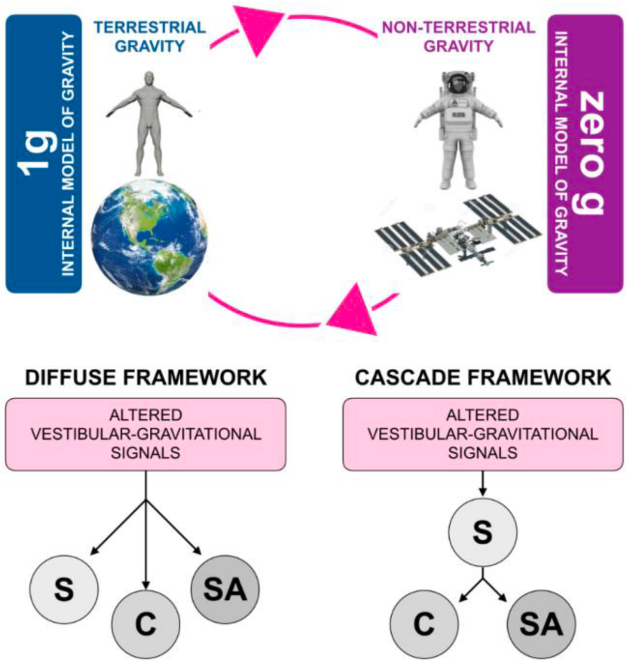


Figure 1. Effects of non-terrestrial gravity on sensorimotor functioning, cognition, and socio-affective processing.

15:15 Steven Jillings – "Brain structural and functional changes after long duration spaceflight"

Brain structural and functional changes after long-duration spaceflight

Steven Jillings [1], Ekaterina Pechenkova[2], Elena Tomilovskaya [3], Ilya Rukavishnikov[3], Ben Jeurissen[1,4], Angelique Van Ombergen[1,5], Inna Nosikova[3], Alena Rumshiskaya[6], Liudmila Litvinova[6], Jitka Annen [7], Chloë De Laet[1], Catho Schoenmaekers[1], Jan Sijbers [4], Victor Petrovichev[6], Stefan Sunaert [8], Paul M. Parizel [9], Valentin Sinitsyn[10], Peter zu Eulenburg[11], Steven Laureys [7,12,13], Athena Demertzi[14,15,*] & Floris L. Wuyts [1,*]

1 Lab for Equilibrium Investigations and Aerospace, University of Antwerp, Antwerp, Belgium. 2 Laboratory for Cognitive Research, HSE University, Moscow, Russia. 3 SSC RF—Institute for Biomedical Problems, Russian Academy of Sciences, Moscow, Russia. 4 imec-Vision Lab, University of Antwerp, Antwerp, Belgium. 5 Department of Translational Neuroscience—ENT, University of Antwerp, Antwerp, Belgium. 6 Radiology Department, National Medical Research Treatment and Rehabilitation Center of the Ministry of Health of Russia, Moscow, Russia. 7 Coma Science Group, GIGA Consciousness, GIGA Institute, University and University Hospital of Liège, Liège, Belgium. 8 Department of Imaging & Pathology, Translational MRI, KU Leuven—University of Leuven, Leuven, Belgium. 9 Department of Radiology, Royal Perth Hospital and University of Western Australia Medical School, Perth, WA, Australia. 10 Faculty of Fundamental Medicine, Lomonosov Moscow State University, Moscow, Russia. 11 Institute for Neuroradiology, Ludwig-Maximilians-University Munich, Munich, Germany. 12 Joint International Research Unit on Consciousness, CERVO Brain Research Centre, Laval University, Quebec, QC, Canada. 13 International Consciousness Science Institute, Hangzhou Normal University, Hangzhou, China. 14 Physiology of Cognition, GIGA-CRC In Vivo Imaging, University of Liège, Liège, Belgium. 15 Department of Psychology, Psychology and Neuroscience of Cognition Research Unit, University of Liège, Liège, Belgium. *These authors contributed equally: Athena Demertzi, Floris L. Wuyts.

Abstract

The prospect of continued manned space missions warrants an in-depth understanding of how prolonged microgravity affects the human brain. Previous studies have revealed both structural and functional changes [1]. These findings provide first insights into the brain changes that occur following spaceflight, though many other studies with different complementary approaches are required to be conducted. In this work, resting-state functional magnetic resonance imaging (fMRI) was used to gain a measure for brain functional architecture and interregional communication that can be analyzed longitudinally to study brain adaptation following spaceflight.

A total of 15 male cosmonauts (median age = 45 years) were considered in the subject pool for this analysis, as well as 14 control subjects (median age = 41 years). All cosmonauts were scanned before (median interval scan and launch = 96 days) and shortly after (median interval return and scan = 9 days) a long-duration space mission (median days in space = 173). From these cosmonauts, 11 were also scanned at a 8-month follow-up time point after spaceflight. The control subjects were scanned at two time points with an interval matching the interval of the pre- and postflight measurements of the cosmonauts. Data were acquired using a 3T MRI machine (Discovery 750, GE Healthcare) and using a 16 channel head and neck array coil located at the Federal Center for Treatment and Rehabilitation in Moscow, Russia. The MRI protocol included an anatomical T1-weighted scan, as well as a resting-state fMRI scan. The fMRI scan was acquired with following parameters: echo time = 30ms, repetition time = 2000ms, flip angle = 77°, voxel size = 3x3x3 mm^3, and a field of view = 192x192x126. A total of 300 dynamic volumes were acquired after discarding the first 8 dummy scans for the purpose of T1 saturation effects. Total acquisition time was 10min per scan. Resting-state fMRI data were preprocessed in SPM12 (Matlab R2018b) by performing movement correction, slice timing correction, warping to standard MNI space, and spatial smoothing using a 3D Gaussian kernel of 6mm full-width at half-maximum. T1-weighted images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) and were all warped to standard MNI space. Next, the resting-state fMRI data were denoised by removing outlier volumes using the artefact removal toolbox (ART), by regressing out the outlier volumes as well as the movement correction parameters in all 6 degrees of freedom, by linear detrending, by regressing out the signal arising from WM and CSF, and lastly by applying a bandpass filter of 0.008Hz to 0.09Hz. Next, the intrinsic connectivity contrast (ICC) was calculated in each voxel of the preprocessed and denoised fMRI data. The ICC reflects the degree of connectivity between one voxel and all other voxels in the brain, where connectivity is defined as the correlation of the signal fluctuations in time between two voxels [2]. We investigated whether the ICC would change as a result of spaceflight by performing three statistical tests. The first is a paired t-test comparing pre- and postflight data (n=15). The second and third include a t-contrast test that models changes between pre- and postflight that either sustain or either reverse back to preflight levels at the 8-month follow-up measurement (n=11). A statistical threshold of $p<0.005$

uncorrected at the voxel-level was applied, followed by a threshold of $p<0.05$ at the cluster-level, corrected for multiple comparisons using the family-wise error rate.

Our results show that ICC decreased in a cluster of voxels covering the posterior cingulate cortex when comparing pre- to postflight. The sustain model (n=11) revealed sustained decreased ICC in the PCC and the mediodorsal thalamus. Sustained increased ICC was found in the right angular gyrus. ICC decreases that were reversed back to preflight levels upon our follow-up measurement were found in the bilateral middle to anterior insular cortex.

Our results demonstrate that resting-state functional connectivity changes as a result of spaceflight, even without having the cosmonauts engage in specific tasks. The regions where we show changes are predominantly multimodal brain regions that play a role in integrating different modalities of information. The posterior cingulate cortex is part of the default mode network and involved in maintaining a balance between internally and externally directed attention and in environmental change detection [3]. The mediodorsal thalamus is mainly involved in various adaptive cognitive functions [4]. The angular gyrus plays a role in spatial cognition [5] and in detecting mismatches between actual and expected outcomes from one's actions [6]. The insular cortex is known to be a key part of the salience network which is triggered by new and unfamiliar input. It also plays a role in interoception, which is based on sensory signals from the internal organs and the vestibular system [6].

In conclusion, our results demonstrate various connectivity changes that reflect adaptation in the brain at the functional level after long-duration spaceflight. These findings can be used to perform more directed studies in the future that may expand our knowledge on the true adaptations occurring during space travel.

References

[1] M. Roy-O'Reilly et al., *NPJ Microgravity*, 2021. DOI: 10.1038/s41526-021-00133-z

[2] Martuzzi et al., *Neuroimage,* 2011. DOI: 10.1016/j.neuroimage.2011.06.075

[3] Pearson et al., *Trends Cogn. Sci.*, 2011. DOI: 10.1016/j.tics.2011.02.002

[4] Parnaudeau et al. *Biol. Psychology*, 2017. DOI: 10.1016/j.biopsych.2017.11.008

[5] Fiori et al., *J Neurophysiol.,* 2015. DOI: 10.1152/jn.00289.2015

[6] Farrer et al. *Cerebral Cortex*, 2008. DOI: 10.1093/cercor/bhm050

[7] Craig, *Nat Rev Neurosci,* 2002. DOI: 10.1038/nrn894

15:30 Jennifer-Vernice Pauly – "Brains on ICE - Hippocampal Changes in Response to Prolonged Isolation and Confinement"

Brains on ICE – Hippocampal Changes in Response to Prolonged Isolation and Confinement

J.V. Pauly[1], A. Friedl-Werner[1], K. Brauns[1], P.C. Barsby[1], S. Kühn[2], and A. C. Stahn[3]
[1]Charité University Medicine Berlin, Germany (jennifer-vernice.pauly@charite.de, anika.werner@charite.de, katharina.brauns@charite.de, poppy.barsby@charite.de), [2]Max-Planck-Institute for Human Development Berlin, Germany (kuehn@mpib-berlin.mpg.de), and [3]University of Pennsylvania, Philadelphia, PA (astahn@pennmedicine.upenn.edu).

Long-duration space missions (LDSM) can pose a considerable risk for developing adverse behavioral conditions and psychiatric disorders. The neural signatures of these risks are currently not well understood. Previous data have shown that prolonged isolation and confinement can lead to significant structural brain changes that are associated with key neurotrophins and translate to impaired cognitive performance (Stahn et al., 2019). Importantly, the response to isolation and confinement is characterized by significant inter-individual differences, and it remains to be determined how different crews and crew dynamics respond to prolonged isolation and confinement and how these relate to phenotypic vulnerabilities.

One brain area impacted by stress is the hippocampus as shown by scientific studies. The hippocampus is involved in memory formation, spatial navigation as well as mood regulation, which makes it of particular interest if negatively affected by LDSM. As a subregion of the hippocampus, the dentate gyrus is one of the two known regions in the adult human brain where active neurogenesis can occur. Whereas physical activity and environmental enrichment can stimulate the dentate gyrus, it has been shown that various conditions and stressors can have adverse effects on hippocampal function and structure.

With this study we will investigate the impact of long-duration Antarctic missions on hippocampal structure and shape. We hypothesize that the isolation and confinement experienced during such missions results in changes specific to different hippocampal subfields such as the dentate gyrus. A total of 4 crews (each comprising N=9 crew members) overwintering at Neumayer-Station III in Antarctica for 12 to 14 months will be investigated. To asses neurostructural and neurofunctional changes crew members underwent neuroimaging once before and once after the mission. The magnetic resonance imaging (MRI) data (Siemens Tim Trio 3T scanner) obtained includes high-resolution T1- and T2-weighted sequences. Changes in the volumes of hippocampal subfields will be analyzed in the post-processing of those sequences using the automated segmentation of hippocampal subfield (ASHS) tool (Yushkevich et al., 2010). The algorithm allows for analysis of various hippocampal segments, including the head, tail, CA1-3, dentate gyrus, subiculum, entorhinal cortex, and parahippocampal gyrus. Data analyses are currently ongoing and preliminary findings will be presented at the conference.

With this study we will relevantly contribute to ESA's and NASA's goal to provide knowledge, technologies, and tools to enable safe, reliable, and productive human space exploration by providing data on critical neurobehavioral functions. Our findings relate to the relevance for facilitating effective countermeasure tools critical in support of exploration class missions to monitor and mitigate crew health and performance risks.

SUPPORT

Supported by DLR through grant 50WB1525 and 50WB1330.

REFERENCES

Stahn A.C., Gunga H.C., Kohlberg E., Gallinat J., Dinges D.F. and Kühn S., 2019, *New England Journal of Medicine*, *381*(23), 2273–2275.

Yushkevich P.A., Wang H., Pluta J., Das S.R., Craige C., Avants B.B., Weiner M.W. and Mueller S., 2010, *NeuroImage*, *53*(4), 1208–1224.

16:15 Adrien Robin – "Venous filling and emptying properties during 5-day strict dry immersion microgravity simulation"

Venous filling and emptying properties during 5-day strict dry immersion microgravity simulation

Adrien Robin[1*], Nastassia Navasiolava[1], Carla Bariot[1,2], Baptiste Chupin[1], Marc-Antoine Custaud[1]
1. Univ Angers, CHU Angers, CRC, Inserm, CNRS, MITOVASC, SFR ICAT, 49000 Angers, France
2. ESEO engineer school, Angers, France
* adrien.robin@univ-angers.fr

CONTEXT

Impaired orthostatic tolerance has been reported after spaceflight when returning to Earth gravity. Possible mechanisms include hypovolemia (Blomqvist C.G. et al, 1994; Coupé M. et al, 2011; Gharib C. & Hughson R.L., 1992; Jordan J. et al, 2022), changes in cardiac and baroreflex autonomic control (Fritsch J.M. et al, 1992; Hughson R.L. et al, 1994), and increased overall venous compliance of lower limbs (Fortrat J.O. et al, 2017). Returning to the Moon and having Mars in sight is the goal of the present decade, thus investigating the astronauts' venous function and its contribution to orthostatic intolerance remains crucial. Altered venous filling has already been shown during bedrest (Louisy F. et al, 1997) and real short-term (Louisy F. et al, 2001) and long-term spaceflight (Fortrat J.O. et al, 2017) using lower limb plethysmography in men.

METHOD

We assessed the venous function on 18 women and 19 men before, during, and after 5 days of strict dry immersion (DI). Venous plethysmography was performed using an Air Plethysmograph APG® 1000 (ACI Corporation, San Marcos, CA, USA). Venous occlusion was performed using a manual pneumatic thigh cuff with changes in calf volume determined at 30, 40 and 50 mmHg. Venous occlusion was applied long enough to reach the plateau of the pressure curve as visually estimated by the operator (AR), each occlusion step lasted a maximum of 5 min.

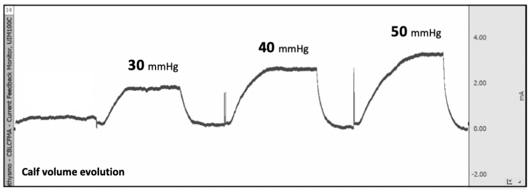



Figure 1: Venous occlusion cuff on the thigh, and air plethysmograph around the calf to record volume changes.

RESULTS

We assessed the integrative venous function before, during, and after DI in women and men. We studied the arterial filling speed, maximal filling volume, venous filling index and emptying rate of 50% and 90% of pooled venous volume. Preliminary data indicates a decrease in venous compliance in women during DI compared to baseline values.

DISCUSSION

In our study, we observed a decrease in leg venous distensibility during DI. It could be due to the chronic hydrostatic compression effect of the water mass around the body. Long-term compression therapy is known to be an efficient conservative treatment of venous incompetence.

REFERENCE

Blomqvist, C. G., Buckey, J. C., Gaffney, F. A., Lane, L. D., Levine, B. D., & Watenpaugh, D. E., 1994, *Journal of Gravitational Physiology: A Journal of the International Society for Gravitational Physiology*, 1, P122-124.

Coupé, M., Yuan, M., Demiot, C., Bai, Y. Q., Jiang, S. Z., Li, Y. Z., Arbeille, P., Gauquelin-Koch, G., Levrard, T., Custaud, M.-A., & Li, Y. H., 2011, *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 301, R1748-R1754.

Fortrat, J.-O., de Holanda, A., Zuj, K., Gauquelin-Koch, G., & Gharib, C., 2017, *Frontiers in Physiology*, 8, 694.

Fritsch, J. M., Charles, J. B., Bennett, B. S., Jones, M. M., & Eckberg, D. L, 1992, *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 73, 664-671.

Gharib, C., & Hughson, R. L., 1992, *Advances in Space Biology and Medicine*, 2, 113-130.

Hughson, R. L., Maillet, A., Gharib, C., Fortrat, J. O., Yamamoto, Y., Pavy-Letraon, A., Rivière, D., & Güell, A, 1994, *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 77, 69-77.

Jordan, J., Limper, U., & Tank, J, 2022, *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, 43, 3039-3051.

Louisy, F., Cauquil, D., Andre-Deshays, C., Schroiff, P., Lazerges, M., Lafaye, C., Camus, A. L., & Fomina, G, 2001, *European Journal of Applied Physiology*, 85, 383-391.

Louisy, F., Schroiff, P., & Güell, A, 1997, *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 82, 1726-17

16:30 Ana Diaz Artiles – "Quantification Of The Internal Jugular Vein Characteristics During Fluid Shift Induced By Lower Body Negative Pressure"

Quantification Of The Internal Jugular Vein Characteristics During Fluid Shift Induced By Lower Body Negative Pressure

A. Diaz-Artiles[1], R. S. Whittle[2]
[1]Aerospace Engineering, Texas A&M University (adartiles@tamu.edu), [2]Aerospace Engineering, Texas A&M University (rswhittle@tamu.edu).

ABSTRACT

With upcoming exploration class missions, such as NASA's Artemis lunar exploration program that aims to send the first female astronaut to the lunar surface by 2030 as well as a long-term goal of Martian exploration, maintaining astronaut health (both males and females) during extended-duration spaceflight is critical for achieving mission objectives. Exposure to weightlessness results in the removal of hydrostatic pressure gradients and a permanent headward fluid shift, causing a redistribution of blood. Additionally, in space, postural changes do not occur and thus, astronauts are not exposed to daily fluid shifts (between upright and supine) as we are on Earth. This has currently unknown consequences, but it might be related to a series of neuro-ocular and functional changes developed in some astronauts during both short and long-duration spaceflight, collectively known as Spaceflight Associated Neuro-Ocular Syndrome (SANS) (Lee et al., 2020). SANS has been diagnosed in both males and females, contrary to initial reports suggesting a male-only condition (Laurie et al., 2022). While the exact etiology of SANS is currently unknown, chronic fluid redistribution affecting intravascular, interstitial, and cerebrospinal fluids and pressures is widely hypothesized to be a contributing factor. Additionally, recently demonstrated stagnant and retrograde blood flow and venous thrombosis in the left internal jugular vein (IJV) during spaceflight could also be associated with sustained headward shift (Marshall-Goebel et al., 2019).

Based on the current knowledge and hypotheses, countermeasures focused on producing hydrostatic gradients to reduce the microgravity-induced fluid shifts, such as lower body negative pressure (LBNP) and centrifugation, become particularly relevant. It has been shown that LBNP is a promising countermeasure in reducing headward fluid shift, intra-ocular pressure (IOP), and intracranial pressure (ICP) (Petersen et al., 2019). Although previous studies in altered gravity have provided a small window into understanding the effect of fluid shift on certain physiological responses, the overall relationship between fluid shift and the resulting responses is largely unknown. In this research effort, we focus on generating quantitative dose-response curves of the IJV characteristics on both males and females when exposed to LBNP.

Twenty-four subjects (12M: 28.8 ± 5.4 years, 12F: 28.1 ± 4.2 years) were exposed to different LBNP levels (from 0 to –50 mmHg, in intervals of 10 mmHg) in both supine and 15° HDT. At each pressure level, we collected the characteristics of the internal jugular vein, including the internal jugular vein cross-sectional area (AIJV), internal jugular vein pressure (IJVP), and internal jugular vein flow. All variables presented a strong dependency on the level of LBNP. In the supine position, AIJV decreased from (mean ± SEM) 50.8 ± 4.6 mm2 at 0 mmHg to 21.7 ± 2.7 mm2 at –50 mmHg. At 15° HDT, AIJV decreased from 106.5 ± 7.4 mm2 at 0 mmHg to 43.9 ± 4.4 mm2 at –50 mmHg. Similarly, in the supine position, IJVP decreased from 20.3 ± 1.3 mmHg at 0 mmHg to 7.8 ± 0.9 mmHg at –50 mmHg. At 15° HDT, IJVP decreased from 35.6 ± 2.6 mmHg to 13.2 ± 1.0 mmHg at –50 mmHg. Internal jugular vein flow was measured on a qualitative scale from grade 1 (fully forward flow) to grade 4 (retrograde flow) (Marshall-Goebel et al., 2019). In the supine position, the probability of having grade 1 flow increased from 0.566 ± 0.122 at 0 mmHg to 0.997 ± 0.002 at –50 mmHg. At 15° HDT, the probability of having grade 1 flow also increased from 0.248 ± 0.096 at 0 mmHg to 0.989 ± 0.008 in HDT.

There was no significant difference in IJVP or AIJV between males and females (IJVP effect size: 1.14 ± 1.71 mmHg, 89% Credible Interval (CrI) –1.53 to 3.88 mmHg, p.d. 77.1%; AIJV effect size: 10.6 ± 8.1 mm2, 89% CrI –2.2 to 23.5 mm2, p.d. 90.2%). Similarly, there was no effect of sex on IJV flow (log odds: 0.11 ± 0.68, 89% CrI –0.98 to 1.18, p.d. 58.0%)1.

---

[1] Probability of direction, *p.d.*, is a Bayesian measure representing evidence for existence of an effect. It is roughly analogous to p-value via: $p_{two-sided} = 2(1 - p.d.)$.

These results constitute terrestrial models that can be used as a reference for microgravity or future partial gravity spaceflight missions, as well as the development of countermeasures. These efforts complement our previous investigation on gravitational dose-response curves during tilt (Whittle et al., 2022; Whittle & Diaz-Artiles, 2023). Future efforts include a similar experiment using the Aerospace Human Centrifuge at Texas A&M University. Our combined results from these investigations inform future countermeasure development and in-flight countermeasure prescriptions and constitute a more inclusive conceptual framework for fluid-shift response prediction, contributing to the assessment of the pathogenesis of SANS and spaceflight venous thromboembolism events.

This research effort is supported by the NASA Human Research Program, Grant number 80NSSC20K1521.

REFERENCES

Laurie, S. S., Macias, B. R., Pardon, L. P., Brunstetter, T., Gibson, R., Greenwald, S. H., Marshall-Goebel, K., Jasien, J. v., Mason, S., & Tsung, A. (2022). Evidence Report : Risk of Spaceflight Associated Neuro-ocular Syndrome (SANS).

Lee, A. G., Mader, T. H., Gibson, C. R., Tarver, W., Rabiei, P., Riascos, R. F., Galdamez, L. A., & Brunstetter, T. (2020). Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. Npj Microgravity, 7, Art. 6. https://doi.org/10.1038/s41526-020-0097-9

Marshall-Goebel, K., Laurie, S. S., Alferova, I. v., Arbeille, P., Auñón-Chancellor, S. M., Ebert, D. J., Lee, S. M. C., Macias, B. R., Martin, D. S., Pattarini, J. M., Ploutz-Snyder, R., Ribeiro, L. C., Tarver, W. J., Dulchavsky, S. A., Hargens, A. R., & Stenger, M. B. (2019). Assessment of Jugular Venous Blood Flow Stasis and Thrombosis During Spaceflight. JAMA Network Open, 2(11), e1915011. https://doi.org/10.1001/jamanetworkopen.2019.15011

Petersen, L. G., Lawley, J. S., Lilja-Cyron, A., Petersen, J. C. G., Howden, E. J., Sarma, S., Cornwell, W. K., Zhang, R., Whitworth, L. A., Williams, M. A., Juhler, M., & Levine, B. D. (2019). Lower body negative pressure to safely reduce intracranial pressure. Journal of Physiology, 597(1), 237–248. https://doi.org/10.1113/JP276557

Whittle, R. S., & Diaz-Artiles, A. (2023). Gravitational effects on carotid and jugular characteristics in graded head-up and head-down tilt. Journal of Applied Physiology, 134(2), 217–229. https://doi.org/10.1152/japplphysiol.00248.2022

Whittle, R. S., Keller, N., Hall, E. A., Vellore, H. S., Stapleton, L. M., Findlay, K. H., Dunbar, B. J., & Diaz-Artiles, A. (2022). Gravitational dose-response curves for acute cardiovascular hemodynamics and autonomic responses in a tilt paradigm. Journal of the American Heart Association, 11(14). https://doi.org/10.1161/JAHA.121.024175

16:45 Richard Hughson – "Is fainting after 14-day head-down bed rest in 55-65 year-old men and women a chicken and egg question?"

Is fainting after 14-day head-down bed rest in 55–65-year-old men and women a chicken and egg question?

R. L. Hughson[1], C. J. Mastrandrea[2], E. T. Hedge[3], A. D. Robertson[4]
[1]Schlegel-UW Research Institute for Aging (hughson@uwaterloo.ca), [2](c2mastrandrea@uwaterloo.ca), [3](ethedge@uwaterloo.ca) and [4](andrew.d.robertson@uwaterloo.ca).

A progressive or sudden fall in blood pressure at the level of the middle cerebral artery (BPMCA) is taken as the cause of reduced cerebral blood flow, measured as velocity through the middle cerebral artery (MCAv), that results in syncope during a head-up tilt test. However, we previously observed a progressive reduction in blood velocity through the MCAv that was linked to a decrease in end-tidal PCO2 (PETCO2) and preceded the fall in BPMCA (Zuj et al. 2013). The underlying mechanism responsible for the reduction in PETCO2 is not known. In a 14-day head-down bed rest (HDBR) study with 22 participants (11 female) ages 55-65 years with one-half randomly assigned to daily exercise (total 1-h/day including aerobic and high intensity interval cycling) we investigated cardio- and cerebrovascular responses to a maximum of 15-min head-up tilt (HUT). We hypothesized that HDBR would cause a more rapid decline in MCAv that was associated with reductions in BPMCA, PETCO2, and cardiac output (C.O.).

Examples of the time series data for two participants, one unable to complete 15-min tilt post-HDBR (R1), are shown in Figure 1. Pre-HDBR, all but 3 participants (female in Exercise group) completed the 15-min HUT. Post-HDBR, only 4 participants (2 male Control, 1 female Exercise and 1 male Exercise) completed 15-min HUT. There were no significant differences in tilt end times post-HDBR between men (8.2±5.3 min) and women (5.6±5.0 min), and between Control (7.0±4.8 min) and Exercise (6.8±5.8 min). The reduction in cerebral blood flow during HUT was correlated with each of PETCO2, BPMCA and C.O. (Table and Figure 2).

R^2	$P_{ET}CO_2$ - MCAv	BP_{MCA} - MCAv	C.O. - MCAv
Pre-HDBR	0.64±0.22	0.61±0.23	0.53±0.23
Post-HDBR (R1)	0.75±0.22	0.72±0.17	0.53±0.29

These data show that both BPMCA and PETCO2 are directly correlated with the decline in MCAv both before and after HDBR. The complex interactions between these variables raise questions about which comes first? Is the reduction in MCAv a consequence of the decline in BPMCA or of the progressive reduction in PETCO2? An alternative is that the reduction in PETCO2 is a necessary consequence of the decline in MCAv and that the effects of BPMCA and PETCO2 are additive. The data also show that in contrast to younger men and women there were no sex differences and that this specific exercise countermeasure was not beneficial for orthostatic tolerance after 14-days HDBR.

Supported by Canadian Institutes of Health Research and Canadian Space Agency.

REFERENCE

Zuj KA, Arbeille P, Shoemaker JK, Hughson RL. Cerebral critical closing pressure and CO2 responses during the progression towards syncope. J Appl Physiol. 114: 801-807, 2013

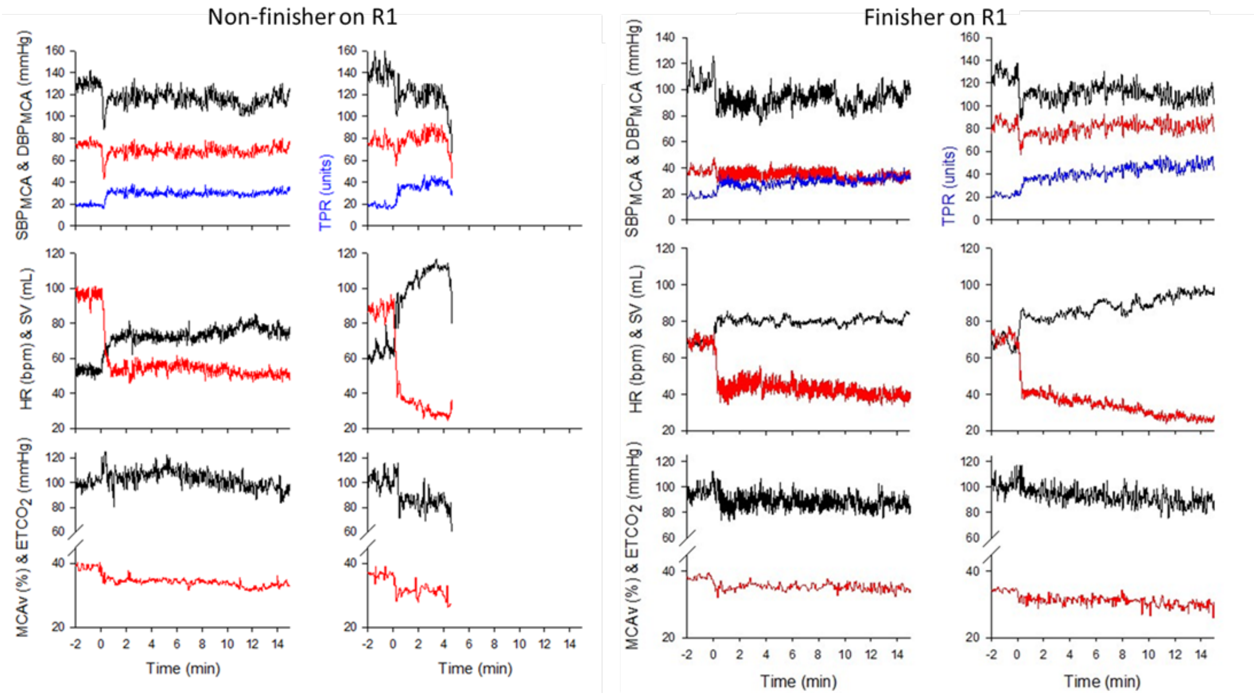


Figure 1: Time series data from two participants showing the head-up tilt baseline data collection period (left columns) and head-up tilt as the first event after 14-days head-down bed rest (R1). Participant on the left had vasovagal syncope after about 4-min tilt on R1 while participant on the right was able to complete 15-min tilt.

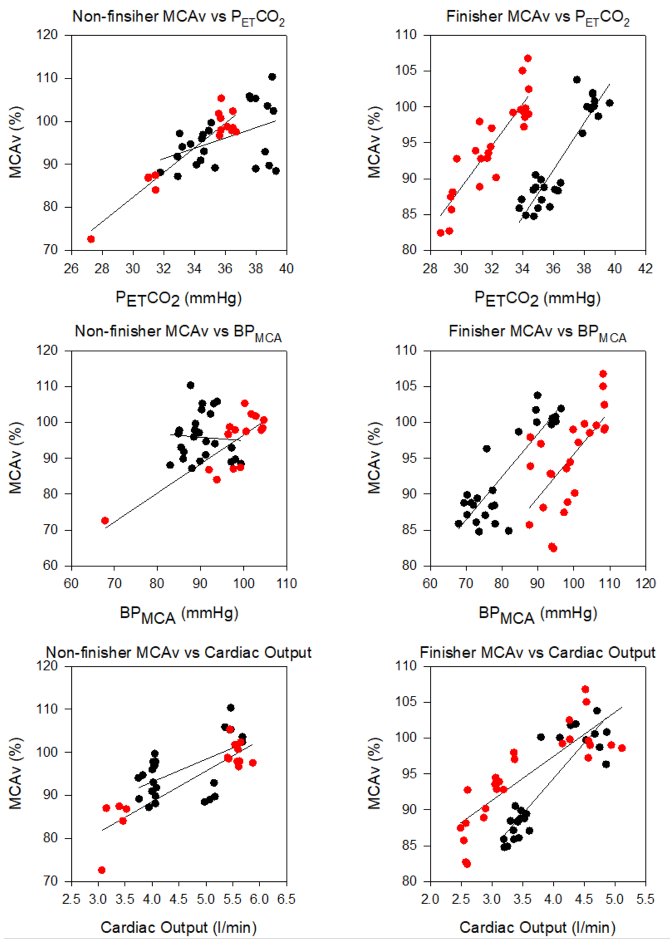


Figure 2: Linear regressions showing relationships between blood velocity through the MCA and each of PETCO2, BPMCA and cardiac output. Black symbols are pre-bed rest, red symbols are immediate post bed rest (R1).

17:00 Jérémy Rabineau – "Correlation of changes in aortic stiffness with other parameters of cardiovascular health after 60-day head-down bed rest"

Correlation of changes in aortic stiffness with other parameters of cardiovascular health after 60-day head-down bed rest

J. Rabineau[1], P. Balali[2], C. Tordeur[3], M. Issertine[4], E. G. Caiani[5], F. Hoffmann[6], D. Gerlarch[7], V. Faoro[8], P.-F. Migeotte[9], J. Tank[10], and P. van de Borne[11]

[1]LPHYS, Département de Cardiologie, Université libre de Bruxelles, jeremy.rabineau@ulb.be, [2]LPHYS, Département de Cardiologie, Université libre de Bruxelles, paniz.balali@ulb.be, [3]LPHYS, Département de Cardiologie, Université libre de Bruxelles, cyril.tordeur@ulb.be, [4]LPHYS, Département de Cardiologie, Université libre de Bruxelles, margotissertine@gmail.com, [5]Electronic, Information and Biomedical Engineering Department, Politecnico di Milano, enrico.caiani@polimi.it, [6]Institute of Aerospace Medicine, German Aerospace Center (DLR), Fabian.Hoffmann@dlr.de, [7]Institute of Aerospace Medicine, German Aerospace Center (DLR), Darius.Gerlach@dlr.de, [8]LPHYS, Département de Cardiologie, Université libre de Bruxelles, vitalie.faoro@ulb.be, [9]LPHYS, Département de Cardiologie, Université libre de Bruxelles, pierre-francois.migeotte@ulb.be, [10]Institute of Aerospace Medicine, German Aerospace Center (DLR), Jens.Tank@dlr.de, and [11]LPHYS, Département de Cardiologie, Université libre de Bruxelles, Philippe.Van.de.Borne@erasme.ulb.ac.be.

INTRODUCTION

There is more and more evidence supporting an increased aortic stiffness following long duration exposure to weightlessness. However, the underlying mechanisms for these adaptations remain poorly understood and so is the inter-subject variability. The objective of this research was to study how microgravity-induced changes in aortic pulse wave velocity (PWV) and its equivalent corrected for blood pressure (CAVI) were correlated to changes in other parameters, following 60-day exposure to strict -6° head-down-tilt bed rest (HDBR).

METHODS

As part of the AGBRESA study, 24 subjects (8 females) were investigated during 60-day HDBR. Subjects were equally distributed into three groups, with different countermeasures, but previous analyses have shown that none of the studied parameters were impacted by these countermeasures. These parameters include: PWV and CAVI assessed by 4D-flow cardiac MRI, orthostatic tolerance time (OTT) measured with a standardized lower body negative pressure protocol, the heart rate variability parameter LF/HF, as well as the height of the subject. They were all measured before and at the end of the HDBR period: -9/56 days for PWV, CAVI, and LF/HF; or 0/60 days for OTT and height. OTT was not normally distributed, so it was log-transformed. Spearman correlation analyses were performed, with results expressed as correlation coefficient R and 95% confidence interval. Other results are expressed as median [first quartile; third quartile]. Statistical significance was considered for p-values below 0.05.

RESULTS

OTT decreased during HDBR (-467 [-131; -763] s, $p<0.001$), while PWV and CAVI increased (+0.86 [+0.52; +1.46] m/s, $p<0.001$ and +1.14 [+0.54; +2.95], $p<0.001$). After removing one outlier, Δlog(OTT) was negatively correlated with ΔPWV (R=-0.50 [-0.76; -0.10], p=0.008) and ΔCAVI (R=-0.57 [-0.80; -0.20], p=0.002). After being exposed to HDBR, the height and LF/HF also increase (+2.8 [+1.8; +4.0] cm, $p<0.001$ and +0.42 [+0.08; +1.28], p=0.005, respectively). There was a trend for negative correlation between Δheight and ΔPWV (R=-0.34 [-0.67; 0.10], p=0.056), while a trend for positive correlation was observed between ΔLF/HF and ΔCAVI (R=0.29 [-0.17; 0.65], p=0.098).

DISCUSSION

The increased sympathovagal balance may be one of the factors leading to an increased aortic stiffness, following exposure to microgravity. Previous studies with astronauts hypothesized that this increased stiffness may decrease the resistance to venous return and thus play a protective role regarding postflight orthostatic intolerance. Here, the results show the opposite, meaning either that this hypothesis must potentially be re-evaluated, or that different changes occur in the aorta and in the other vessels. The trend for negative correlation between microgravity-induced changes in height and aortic stiffness is counterintuitive and may result from changes in baroreceptor sensitivity, which requires further studies.

17:15 Jacques-Olivier Fortrat – "Increased Cardiovascular SelfOrganized Critically After Prolonged Head-Down Bed Rest"

Increased Cardiovascular Self-Organized Criticality After Prolonged Head-Down Bed Rest

N. Navasiolava[1,2], A. Robin[1,2], G. Gauquelin[3], C.Gharib[4], M.A Custaud[1,2] and J. O. Fortrat[2]
[1]Centre de Recherche Clinique, Centre Hospitalier Universitaire d'Angers, France (nastia.navasiolava@gmail.com), [2]Mitovasc UMR INSERM 1083-CNRS 6015, Université d'Angers, France, (jofortrat@chu-angers.fr), [3]CNES, Paris, France (guillemette.gauquelinkoch@cnes.fr), and [4] Pathologie et Génétique du Neurone et du Muscle, Faculté de Médecine Lyon-Est, Lyon, France (gharib.claude@gmail.com).

INTRODUCTION

Self-Organized Criticality (SOC) is a physics principle that refers to the ability of natural dynamic systems to maintain a critical equilibrium without external control (Muñoz, 2018). The criticality results in large spontaneous events, such as avalanches or catastrophes, which follow power-law distributions. We have previously found evidence of cardiovascular SOC through power-law distributions in heart rate variability and vasovagal events, with a clearer power law in the standing position (Fortrat & Gharib, 2016; Fortrat, 2020, Fortrat & Ravé, 2020). We aimed to test the hypothesis that orthostatic intolerance induced by simulated spaceflight will impact cardiovascular SOC by evaluating heart rate variability power laws before and after a prolonged head-down bed rest (HDBR).

MATERIAL AND METHODS

Twelve healthy male subjects were in the head-down position for 21 days and underwent a presyncopal orthostatic test: 80° head-up tilt test (HUTT) for 15 min, followed by 3-min Lower Body Negative Pressure steps from -10mmHg to -80mmHg before and after HDBR (first rising, Guinet et al., 2020). RR-intervals were recorded in both supine and standing positions to determine heart rate variability self-organized criticality as previously described (Fortrat, 2020). Shortly, bradycardia sequences were counted and classified based on their length to obtain their distribution. Linear regression of distributions was performed to obtain the regression coefficients of the short and long bradycardia (Fortrat, 2020).

RESULTS

Heart rate increased and orthostatic test duration decreased after head-down bed rest (Guinet et al., 2020). The maximum length of bradycardia sequences increased during the head-up position before HDBR as expected (Fortrat, 2020). This pattern of change was altered after HDBR with values in the supine position equivalent to this of standing before HDBR (Fig 1).

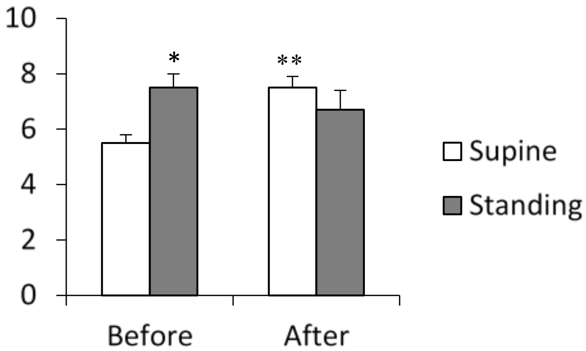


Fig 1. Maximum length of bradycardia sequence used a witness of cardiovascular criticality during a head-up tilt test (Supine and Standing) Before and After head-down best rest. *: $p<0.05$, **: $p<0.01$ vs Supine Before, Friedman/Wilcoxon.

Slope of bradycardia distributions did not differ between phases because of missing values. An unpaired analysis showed differences in the slope of short bradycardias, which increased in head-up position before HDBR but decreased after.

DISCUSSION

The concept of self-organized criticality in the cardiovascular system has been established by the distribution of vasovagal events (Fortrat & Gharib, 2016). These events are more common in the standing position, indicating increased criticality (Fortrat & Ravé, 2020). Head-down bed rest leads to cardiovascular deconditioning and increased risk of orthostatic intolerance and syncope, suggesting increased criticality. Our study showed that after head-down bed rest, cardiovascular criticality in the supine position is similar to the known increase in cardiovascular criticality in the standing position before head-down bed rest, indicating heightened criticality in the supine position after head-down bed rest.

The study of criticality during HDBR has challenges, one being that this dynamic is better observed in the standing position, which is suppressed during HDBR. Orthostatic intolerance after HDBR also reduces available data length in the standing position, resulting in missing values and restrictions on statistical analysis.

Future studies are needed to validate these results and address the challenge of characterizing cardiovascular criticality in an upright position after head-down bed rest. There is a need to investigate if criticality could help identify individuals who are at risk of fainting after spaceflight.

REFERENCES

Muñoz M.A., 2018, Review of Modern Physics, 90, 031001.

Fortrat J.O. and Gharib C, 2016, Frontiers in Physiology, 30;7:113.

Fortrat J.O., 2020, Entropy, 6;22:413.

Fortrat, J.O. and Ravé G, 2020. In Proceedings of the European Study Group on Cardiovascular Oscillations, Pisa, Italy, 27–29 April 2020.

Guinet P., MacNamara J.P., Berry M., Larcher F., Bareille M.P., Custaud M.A., Pavy-Le Traon A., Levine B.D. and Navasiolava N., 2020, Frontiers in Physiology, 11:812.

17:30 Stefan Möstl – "Autonomic cardiovascular control following 30 days strict head down tilt bedrest - Results from the SANS CM studies"

Autonomic cardiovascular control following 30 days strict head down tilt bedrest – Results from the SANS CM studies

S. Moestl[1], J. N. Hoenemann[1,2], A. Diedrich[3], J. Jordan[1,4] and J. Tank[1]
[1]German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne, Germany Stefan.Moestl@dlr.de [2]University Hospital Cologne, Department of Cardiology, Cologne, Germany, JanNiklas.Hoenemann@dlr.de [3]Department of Medicine, Division of Clinical Pharmacology, Autonomic Dysfunction Service, Vanderbilt University, Nashville, USA, Andre.Diedrich@vumc.org [4]University of Cologne, Head of Aerospace Medicine, University of Cologne, Germany, Jens.Jordan@dlr.de and [1]German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne, Germany Jens.Tank@dlr.de

BACKGROUND

Autonomic cardiovascular regulation is impaired, both, after bedrest and after space flight. Impaired autonomic control likely limits cardiovascular adaptation to physiological stresses such as standing. We hypothesized that 6 hours daily lower body negative pressure (LBNP) helps maintaining heart rate (HR), heart rate variability (HRV) and baroreflex sensitivity (BRS) following strict head-down tilt bedrest, an established terrestrial space-analogue.

METHODS

We included 23 healthy persons (11 women, 34.5±9 years, 23.9±2.8 kg/m2) in the 30 days SANS CM strict head down tilt bedrest study. Subjects were randomly assigned to 6 hours daily LBNP training with moderate intensity (LBNP, -25 mmHg) or 6 hours daily sitting (SEAT). We measured HR and finger blood pressure before bedrest and at the end of bedrest during head-up tilt testing. We assessed HRV in the time and frequency domains, systolic blood pressure variability in the low frequency range (LF-SBP), and baroreflex sensitivity using cross spectral analysis (BRS-LF). We analyzed stationary time series during the last 5 minutes supine and during minutes 2-6 at 80 degrees head-up tilt.

RESULTS

Supine HR increased during bedrest (LBNP group: 67±9 vs. 74±11; SEAT: 66±10 vs. 71±12bpm; $p<0.001$) and the orthostatic HR response was enhanced (HR upright: 90±10 vs. 112±13bpm; 86±12 vs. 109±13bpm; $p<0.0001$). HRV in the time domain was reduced after bedrest while supine (rmssd: 37±24 vs. 27±14ms; 50±44 vs. 30±20ms; $p<0.017$) and upright (15±5 vs. 8±4ms and 28±21 vs. 14±10ms; $p<0.001$). HRV in the frequency domain (total power, LF- and HF power) tended to decrease while supine and standing but the bedrest effect did not reach significance. Supine and upright LF/HF ratios did not differ after bedrest. The tilt induced increase of LF-SBP tended to be enhanced after bedrest ($p=0.0547$). Baroreflex mediated vagal HR control was reduced after bedrest at rest ($p=0.011$) and during tilt ($p<0.0001$). No interactions between groups and conditions were detected.

CONCLUSIONS

Six hours of daily moderate intensity LBNP training or sitting upright do not completely preserve autonomic cardiovascular autonomic control during 30 days strict head-down tilt bedrest. Additional countermeasures, such as physical exercise may be required.

PERSPECTIVE

Physical exercise followed by 6 hours veno-occlusive thigh cuffs is currently being tested as a countermeasure in the third and fourth campaign of the SANS CM study. Within these two campaigns, we also have a control group that receives no countermeasure. For comparison with LBNP and SEAT, data will be recorded and analyzed as described above. The results will be presented on the ISGP meeting.

16:15 Britt Schoenrock – "Muscle stiffness in astronauts during long duration missions onboard the International Space Station (MYOTONES)"

Muscle stiffness in astronauts during long duration missions onboard the International Space Station (MYOTONES)

B. Schoenrock [1], *P.E. Muckelt* [2], *M. Hastermann* [3], *M. Salanova* [1], *M.B. Warner* [2], *K. Albracht* [4], *R. MacGregor* [5], *M. Stokes* [2], *D. Blottner* [1]

[1] Charité Universitätsmedizin Berlin, Institute for integrative Neuroanatomy, Berlin, Germany (britt.schoenrock@charite.de)
[2] School of Health Sciences, University of Southampton, Southampton, UK
[3] Experimental and Clinical Research Center (ECRC) and NeuroCure Clinical Research Center (NCRC), AG Klinische Neuroimmunologie
[4] Institute of Biomechanics and Orthopaedics, German Sport University, Cologne, Germany.
[5] Airbus DS Space Systems, Inc., Houston, Texas, USA.

INTRODUCTION

Portable, easy-to-use technology is needed to monitor the outcome/effectiveness of exercise countermeasures in order to mitigate the negative effects of muscle unloading in microgravity. Biomechanical properties (e.g. stiffness) can be used to monitor changes in the health status of astronauts' muscles inflight.
The present MYOTONES study aims to detect changes in biomechanical properties using non-invasive Myoton technology (MyotonPRO device) to monitor muscle health across an entire International Space Station (ISS) mission cycle.

METHODS

Ten astronauts (male n=7, female n=3) participated in the experiment. Data collection consists of 2 pre-, 4 inflight and 4 postflight sessions, which include recording biomechanical parameters (stiffness [N/m]) of resting tissues (muscle, tendon) at various skin measurement points using the MyotonPRO (space qualified) device.
Myoton technology uses mechanical impulses to the skin and records damped natural oscillations from the underlying muscle system. A mean of 5 short impulses (duration 15 ms, force 0.4 N) with a pre-compression force of 0.18 N was recorded for each measurement point.
Routine inflight countermeasure (CM) involved performing multimodal aerobic resistance exercise (6 times per week, T2, ARED, CEVIS) for up to 2-2.5h/day according to ISS flight rules.
Existing reliability data demonstrated that the protocol was robust (Muckelt et al 2022).
Statistical analysis includes Shapiro-Wilk test for normality of distribution and one-way ANOVA with Tukey's multiple comparison with adjusted p-values to compare results between time points (pre- to in- to postflight).

RESULTS

The calf muscles (Gastrocnemius and Soleus) showed inverse changes relative to one another in stiffness (N/m) during microgravity. Soleus showed decreased stiffness preflight to inflight ($p=0.01$) and Gastrocnemius showed increase in stiffness inflight ($p<0.001$). In both muscles, stiffness recovered postflight to preflight values. Achilles Tendon showed decreased stiffness pre- to inflight ($p<0.0001$) with recovery after return.
Knee extensor Rectus Femoris showed only a negative trend (NS) in stiffness pre- to inflight with increase upon return ($p=0.001$). The corresponding Tendon (Patellar Tendon) showed an inverse change with increased stiffness inflight (pre-to inflight $p=0.01$; inflight- to postflight $p=0.003$).
The foot lever muscle (Tibialis Anterior) showed decreased stiffness inflight compared to pre- ($p<0.0001$) and postflight ($p<0.0001$). Postflight values were significantly higher than preflight values ($p=0.002$).

CONCLUSIONS

The MYOTONES study documents muscle health of astronauts during their long duration mission.
Calf muscles showed inverse changes relative to one another in stiffness inflight, which may reflect selective effectiveness of regular CM exercise. Knee extensor Rectus femoris appears to be less affected by unloading or targeted thoroughly by exercise. However, the reduced stiffness may be due to greater hip flexion (shorter muscle length) during testing in microgravity than during testing on the ground. The foot lever muscle (Tibialis Anterior) appears to be negatively affected by unloading in space and may need targeting in CM exercise programmes. Both Tendons showed significant changes inflight, which may be influenced by different joint positions in space and muscle shortening (Achilles Tendon)/lengthening (Patellar Tendon).
Future onboard CM exercise protocols may need to be targeted for certain muscle groups to be better prepared for extra vehicular activities and to enhance astronauts´ reconditioning on returning to Earth.

Sponsors: Federal Ministry for Economic Affairs and Climate Action (BMWi) through

German Aerospace Agency (DLR) Bonn-Oberkassel (grant 50WB2029 to DB); UK Space Agency (# ST/R005680/1 to MS, MW and PM); intramural funding from authors' academic institutions.

REFERENCES

Muckelt P. et al, 2022, Protocol and reference values for minimal detectable change of MyotonPRO and ultrasound imaging measurements of muscle and subcutaneous tissue. Sci. Rep. 2022;12:13654. doi: 10.1038/s41598-022-17507-2

16:30 Elena Fomina – "The weight load simulation mode every other day is quite effective for preserving muscular strength on long space missions"

THE WEIGHT LOAD SIMULATION MODE EVERY OTHER DAY IS QUITE EFFECTIVE FOR PRESERVING MUSCULAR STRENGTH ON LONG SPACE MISSIONS

Fomina E.V.[1], Kukoba T.B.[2], Romanov P.V.[1], Babich D.R.[1]

[1] State Research Center of Russian Federation - Institute of biomedical problems of the RAS, Moscow (fomin-fomin@yandex.ru)

[2] Federal State Organization "Gagarin Research &Test Cosmonaut Training Center", Zvezdnyi Gorodok (tatyana-kukobra@yandex.ru)

Factors of long-term spaceflight are reducing performance of the tasks of manned expeditions into deep space (Ade C. et al, 2015; Sutterfield S. L. et al, 2019). It is necessary to determine the magnitude and frequency of simulated weight loading in flight, sufficient to trigger physiological mechanisms that counteract to the negative effects of weightlessness. The effectiveness of the simulated magnitude and frequency of weight loading in flight can be evaluated by the post-flight change in the "strength index" - the ratio of muscle strength to the body weight. The aim of this study was to evaluate post-flight changes in the "strength index" of the cosmonauts after weight load simulation in flight with magnitude not exceeding 120% of cosmonauts the body weight, performed every other day.

The experiment involved 14 cosmonauts who had performed space flights with duration from 115 to 203 days. The maximum voluntary contraction (MVC) of 23 cases on total were analyzed, including 20 cases in two repeated flights by 10 cosmonauts and 3 cases of one flight.

The results of the study were processed using standard methods of mathematical statistics in the program "STATISTICA 12". The study was approved by the Bioethics Commission of the State Research Center - IMBP RAS (protocol № 14-001-Ren-3and the International Council for Research with Human Participation (Human Research Multilateral Review Board – HRMRB) (protocol NASA MPA № NASA7116301606HR, protocol FWA № 00019876). All subjects signed an informed agreement to participate in the experiment in accordance with the Declaration of Helsinki

In post-flight studies on the 4th and 15th days after the flight a statistically significant decrease of hip and lower leg muscle "strength index" was observed. Hip muscle "strength index" at knee extension on the 4th day after the flight decreased by 12% ($p<0.01$); decrease on the 15th day after the - 9.5% ($p<0.001$). No statistically significant differences were found between the hip flexion muscle strength index on the 4th and 15th days. Hip muscle strength at knee flexion on the 4th day after the flight decreased 13.3% ($p<0.01$); on the 15th day - 6.8% ($p<0.01$). A statistically significant increase in hip muscle «strength index» at knee flexion on the 15th day after the flight was found compared to the 4th day after the flight ($p<0.01$ Ankle extension "strength index" decreased by16.4% on the 4th day after the flight ($p<0.01$); on the 15th day - by 9.4% compared to pre-flight level ($p<0.05$). A statistically significant increase in hip muscle "strength index" at knee flexion on the 15th day after the flight in comparison with the 4th day averaged at 9.7% ($p<0.01$). Ankle flexion "strength index" decreased by 9.8% on the 4th day after the flight vs. pre-flight level ($p<0.01$); on the 15th day - 7.2% vs. pre-flight level ($p<0.01$). No statistically significant differences were found between shin muscle «strength index» at flexion on the 4th and 15th days.

The results of the «strength index» studies are consistent with earlier studies (English K. L. et al, 2015; Gopalakrishnan R. et al, 2010). On average, the values of absolute and weight normalized leg muscle strength are lower in the post-flight period than in the pre-flight period. The "strength index" was incorporated into NASA [3] health standards, based on a study by Ryder et al. who evaluated performing of functional tasks. The correlation of threshold values for functional tasks suggested by NASA (Ryder J. W. et al, 2013) with our results showed that on the 4th day after the fligh hip muscle «strength index» exceeded the value of 1.9 N*m/kg in 14 of 23 cases, was between 1.7 and 1.9 N*m/kg in 3 cases, and between 1.0 and 1.7 N*m/kg - in 6 cases. A hip flexion knee "strength index" above 1.9 N*m/kg, is the lower threshold for successful and rapid performance of all types of test tasks. A hip «strength index» between 1.7 and 1.9 N*m/kg, according to Ryder et al, indicates an increased risk of failing or taking too long to complete the task of getting out of the chair and passing the obstacle course. Such comparisons, expressed numerically when expanding the list of functional tasks, can help in making risk-oriented decisions when performing on-planet activities, as well as in changing the training profile appropriate to the current values and tasks, which is one of the goals of creating a system of health standards. Thus, the "strength index" indicates the sufficiency of performing a weight-loading simulation in long-duration spaceflight every other day. Only in six cases a "strength index", were below the required to perform simulated operations.

Supported by RAS base funding 63.1 and RSF project 23-65-10045.

References

1. Ade C., Broxterman R., Barstow T. V˙ O2max and Microgravity Exposure: Convective versus Diffusive O2 Transport //Medicine & Science in Sports & Exercise. – 2015. – T. 47. – №. 7. – C. 1351-1361.
2. Sutterfield S. L. et al. Prediction of Planetary Mission Task Performance for Long-Duration Spaceflight //Medicine and science in sports and exercise. – 2019. – T. 51. – №. 8. – C. 1662-1670.
3. Ryder J. W. et al. Influence of muscle strength to weight ratio on functional task performance //European journal of applied physiology. – 2013. – T. 113. – №. 4. – C. 911-921.
4. English K. L. et al. Isokinetic strength changes following long-duration spaceflight on the ISS //Aerospace medicine and human performance. – 2015. – T. 86. – №. 12. – C. A68-A77.
5. Gopalakrishnan R. et al. Muscle volume, strength, endurance, and exercise loads during 6-month missions in space //Aviation, space, and environmental medicine. – 2010. – T. 81. – №. 2. – C. 91-104.

16:45 Philip Carvil – "Effects of a proposed microgravity countermeasure, the MK VI SkinSuit, upon markers of lumbar geometry and kinematics following unloading"

Effects of a proposed microgravity countermeasure, the MK VI SkinSuit, upon markers of lumbar geometry and kinematics following unloading

Alexander Breen[1], Philip Carvil[2,3], David Andrew Green[3,4,5], Thais Russomano[6], Alan Breen[1]

[1]Faculty of Science and Technology, Bournemouth University, Poole BH12 5BB, UK [Alex Breen - alexbreen@bournemouth.ac.uk | Alan Breen imrci.ABreen@aecc.ac.uk]

[2]Science and Technology Facilities Council, Daresbury Laboratory, Warrington, WA4 4AD, UK philip.carvil@stfc.ac.uk

[3]Centre of Human and Applied Physiological Sciences, King's College London, Strand London WC2R 2LS, UK

[4]Space Medicine Team, European Astronaut Centre, European Space Agency, Cologne, Germany David.green@esa.int

[5]KBRwyle, Cologne, Germany

[6]CEMA, Faculty of Medicine University of Lisbon, Portugal, Avenida Professor Egas Moniz (Edifício comum ao Hospital de Santa Maria) 1649-028 Lisboa Portugal trussomano@hotmail.com

BACKGROUND

Prolonged spinal unloading in microgravity has been associated with increased risk of intervertebral disc (IVD) herniation, particularly in the lumbar spine. This is accompanied by biomechanical and morphological changes associated with spinal elongation. Novel countermeasures to reintroduce axial loading in space are therefore required. This study evaluated the impact of 8h of unloading (using a novel microgravity analogue – Hyper Buoyancy Floatation; HBF), followed by 4h reloading with a proposed countermeasure, the Mk VI SkinSuit, on lumbar geometry (magnetic resonance imaging; MRI) and intervertebral kinematics (quantitative fluoroscopy; QF). The specific aims of this study were:

- Investigate the effect of 4-hour axial reloading upon markers of disc swelling in the lumbar spine, induced by the Mk VI SkinSuit.
- Determine if reloading acts to increase intervertebral motion, by comparing parameters of intervertebral restraint between conditions (with and without SkinSuit loading)

METHODS

This pilot study was approved by the South West 3 Research Ethics Committee and conducted at the Anglo European Chiropractic College (Bournemouth, UK). Twenty healthy male volunteers aged 21-36 without back pain participate. Each was measured and fitted for a Mk VI SkinSuit, that provided on average 0.19±0.03Gz axial loading at the foot. For the study, participants acted as their own control, taking part in in two sessions spaced ~6 weeks apart. For each session, the participants lay overnight on the HBF bed (which is filled with saturated saline water) for 12 hours. On the second occasion, participants donned the Mk VI SkinSuit during the last 4 hours of flotation. At the end of each session, participants were transported directly to the Imaging Centre, lying supine on a trolly. Imaging consisted of

- Supine Magnetic Resonance Imaging (MRI)
- Lateral Recumbent Quantitative Fluoroscopy (QF)
- Upright (seated) Magnetic Resonance Imaging (MRI)
- Upright (standing) Quantitative Fluoroscopy (QF)

Supine Magnetic Resonance Imaging (MRI)

Participants were transferred supine onto the MRI platform and positioned by the radiographer for scanning (Paramed ASG MROpen, 0.5T, Genoa, Italy). Sagittal and axial scans were taken with eleven T2 weighted sagittal slices (5mm thickness, 2597/1117ms repetition/echo time, 30cm field of view), parallel to the spine on coronal localisers and 20 (four blocks of five slices) axial slices (4mm thickness, 5368/132ms repetition/echo time, 25cm field of view) aligned through each IVD L1-S1 to facilitate IVD height and cross-sectional area measurement. At the end they were transferred supine back onto the trolley.

Lateral Recumbent Quantitative Fluoroscopy (QF)

Participants were transferred horizontally onto a computer-controlled motor operated table, which allowed passive motion of the spine (through flexion and extension). Imaging was performed with a Siemens Arcadis Avantic VC10A digital fluoroscope, (Henkestrasse, Germany) with image acquisition synchronised with the controller of the horizontal motion frame. The central ray was positioned at L3-4 disc with all vertebrae from L2-S1 in the field of view during continuous imaging. Fluoroscopic images acquired at 15 Hz were obtained in this passive recumbent configuration, first in 40˚of flexion, followed by 40˚of extension.

Upright (seated) Magnetic Resonance Imaging (MRI)

After the first QF, participants were allowed to stand, then positioned on the MRI table in a seated configuration for the scan, using a similar protocol to the first MRI session.

Upright (standing) Quantitative Fluoroscopy (QF)

Following the second MRI, participants received standing fluoroscopic imaging while stabilised against an upright controller. This guided them through 60˚of flexion and 20˚of extension in separate sequences synchronised to the fluoroscopic imaging.

Image analysis

Images were checked by a consultant radiologist for underlying pathologies, anonymised and compared between conditions. MRI scans were analysed using commercial software (OsiriX Lite, Pixmeo Sarl, Switzerland), examining the lumbar length, curvature, IVD disk height, and cross-sectional area. QF image processing/analysis were performed using custom code written in MATLAB (V7.12, The Mathworks, Cambridge, UK). The inter-vertebral motion parameters measured were; maximum IV-RoM, dynamic anterior disc height, maximum translation, laxity, motion sharing inequality (MSI) and motion sharing variability (MSV).

RESULTS

Following 4-hour SkinSuit reloading there were no significant difference in IVD cross-sectional area and volume. However, participants did evidence more flexion RoM at L3-4 (p=0.01) and L4-5 (p=0.003), more translation at L3-4 (p=0.02), lower dynamic disc height at L5-S1 (p=0.002), lower lumbar spine length (p=0.01) and greater lordosis (p=0.0001).

CONCLUSION

Short term (4 hour) wear of the Mk VI SkinSuit appears to restore lumbar mobility and lordosis following a microgravity analogue exposure in a healthy control population and may be an effective countermeasure for post space flight lumbar disc herniation.

AGKNOWLEDGEMENTS

This study was funded by the European Space Agency and supported by King’s College London and the Anglo European Chiropractic College. Our thanks to the participants and technical staff involved in this study and the wider SkinSuit project team.

17:00 Margot Issertine – "The NEBULA Project: effect of preflight endurance and resistance training as a countermeasure against microgravity-induced musculoskeletal deconditioning"

The NEBULA Project: effect of preflight endurance and resistance training as a countermeasure against microgravity-induced musculoskeletal deconditioning

Margot Issertine[1], Théo Fovet[2], Pierre Delobel[3], Clémence Bronstein[4], Guillaume Py[5], Thomas Brioche[6] and Angèle Chopard[7]

[1]DMeM, Montpellier University, INRAE, Montpellier, France – margot.issertine@umontpellier.fr

[2] U1059 INSERM – SAINBIOSE, Université Jean Monnet, Saint-Priest-en-Jarez, France, theo.fovet@gmail.com

[3] DMeM, Montpellier University, INRAE, Montpellier, France - pierre.delobel@inrae.fr

[4] DMeM, Montpellier University, INRAE, Montpellier, France - clemence.bronstein@etu.umontpellier.fr

[5] DMeM, Montpellier University, INRAE, Montpellier, France -guilhaume.py@umontpellier.fr

[6] DMeM, Montpellier University, INRAE, Montpellier, France – thomas.brioche@umontpellier.fr

[7] DMeM, Montpellier University, INRAE, Montpellier, France – angele.chopard@umontpellier.fr

Musculoskeletal system deconditioning is a major consequence of spaceflight for astronauts, leading to a rapid decrease in muscle strength, mass, oxidative qualities and enhance intra-muscular fat infiltrations. These deconditioning impacts astronauts during their mission and after their return to Earth, especially in postural muscles, which are at the roots of healthy mobility and physical capacity. Therefore, muscle recovery becomes a critical point after a mission, and requires astronauts to go through intense readaptation programs to recover muscle capacity and protect their health after returning to Earth. In order to mitigate it, countermeasures are being studied by space agencies, and physical exercise appears to be the most efficient method for now (S. M. C. Lee et al., 2014; Chopard et al., 2005). However, even if astronauts are already in great physical form before flying, and exercising up to 2h per day on the ISS (Petersen et al., 2016), their current training programs are still not fully efficient in the prevention of muscle loss. Recent human dry immersion studies have demonstrated that the first days of inactivity are critical for muscle deconditioning (Demangel et al., 2017; Fovet et al.,2021). Indeed, the management of these few first days plays a decisive role in the efficiency of the countermeasures implemented during the spaceflight. In that way, studies have investigated the effect of moderate intensity endurance training sessions carried out before the induction of microgravity, and have shown significant protective effects of these training, delaying the onset of muscle waste (Brocca et al., 2021).

Our study aimed to evaluate the impact of a specific physical training program during the pre-flight period (preconditioning) in order to delay muscle wasting. The pre-conditioning method consists of specific high intensity endurance and resistance training exercises during the weeks preceding the microgravity event. Indeed, endurance and resistance exercise are both performed by astronauts on the ISS (ARED, CEVIS and TVIS) and are together allowing the activation of several pathways involved in muscle plasticity.

Sixty 14 weeks old males C57B6J mice were dispatched into 5 groups (n=10) performing or not a pre-conditioning training (endurance and resistance), followed by a 1 or 3 weeks hindlimb suspension (HLS) period before being euthanized (Figure 1). Throughout the experimentation, we longitudinally followed the evolution of the mice's rear paw grip force (RPGF), as well as their aerobic qualities (Maximum Aerobic Velocity (MAV) on a treadmill) and their global muscular and body fat composition via EchoMRI. Terminal data consisted of hindlimb muscle mass and myofibers typologies and cross-sectional area, as well as biomolecular insights in muscle protein (protein balance, REDOX balance, apoptosis, satellite cells, inflammation, mitochondrial dynamics), and mRNA content.

We expect to observe delays in muscular atrophy during the first week of HLS in group performing preconditioning, as well as a less severe deconditioning in the following HLS weeks. We expect trained mice to show a smaller strength and endurance loss after HLS, as well as a different protein and RNA content between timepoints, due to the different molecular pathways activated in the short and long term by the physical training. Trained mice should show a more stable body muscle and fat composition throughout the protocol.

As space agencies are planning travels to the Moon and Mars involving periods of microgravity from a few days to several months or years, finding countermeasures to preserve the muscular integrity of the astronauts is a major concern. Finally, space research is also a great opportunity to develop new therapeutic protocols in the clinical field for patients (elders, athletes…) suffering from earth-bound pathologies.

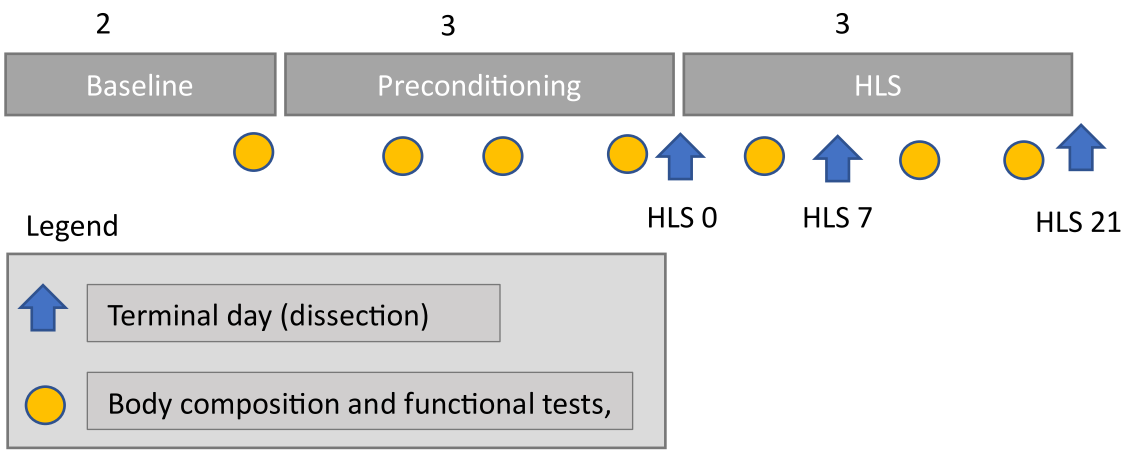


Figure 1: Design of the experimentation

REFERENCES

S. M. C. Lee et al., "WISE-2005: Countermeasures to prevent muscle deconditioning during bed rest in women," J. Appl. Physiol. Bethesda Md 1985, vol. 116, no. 6, pp. 654–667, Mar. 2014, doi: 10.1152/japplphysiol.00590.2013.

A. Chopard, N. Arrighi, A. Carnino, and J. F. Marini, "Changes in dysferlin, proteins from dystrophin glycoprotein complex, costameres, and cytoskeleton in human soleus and vastus lateralis muscles after a long-term bedrest with or without exercise," FASEB J., vol. 19, no. 12, pp. 1722–1724, 2005, doi: 10.1096/fj.04-3336fje.

R. Demangel et al., "Early structural and functional signature of 3-day human skeletal muscle disuse using the dry immersion model," J. Physiol., vol. 595, no. 13, pp. 4301–4315, 2017, doi: 10.1113/JP273895.

T. Fovet et al., "Early Deconditioning of Human Skeletal Muscles and the Effects of a Thigh Cuff Countermeasure," Int. J. Mol. Sci., vol. 22, Nov. 2021, doi: 10.3390/ijms222112064.

Petersen et al., "Exercise in space: the European Space Agency approach to in-flight exercise countermeasures for long-duration missions on ISS," Extreme Physiol. Med., vol. 5, p. 9, Aug. 2016, doi: 10.1186/s13728

L. Brocca, M. Rossi, M. Canepari, R. Bottinelli, and M. A. Pellegrino, "Exercise Preconditioning Blunts Early Atrogenes Expression and Atrophy in Gastrocnemius Muscle of Hindlimb Unloaded Mice," Int. J. Mol. Sci., vol. 23, no. 1, p. 148, Dec. 2021, doi: 10.3390/ijms23010148.

17:15 Thomas Angeli – "Implementation of Blood Flow Restricted Exercising for Strength Increase in Quadriceps Muscle on the Multifunctional Dynamometer for Application in Space"

Implementation of Blood Flow Restricted Exercising for Strength Increase in Quadriceps Muscle on the Multifunctional Dynamometer for Application in Space

T. Angeli[1], and C. Kumpf[1]
[1] Institute of Engineering Design and Product Development, TU Wien, Austria (thomas.angeli@tuwien.ac.at).

INTRODUCTION

This study focuses on an interdisciplinary investigation of different exercise intensities and methods, used for the maintenance of the musculoskeletal system in the microgravity of space. Especially for weight bearing structures, such as muscles and bones, the ruling weightlessness in the cosmos causes enormous changes that threaten or even prevent long-term missions. Various exercise devices try to counteract this problem and allow astronauts an earth-like stimulus for selective resistance training to reduce muscle and bone atrophy. Previous developments have repeatedly pointed out various weaknesses in microgravity, and none of them does meet the requirements so far, therefore the demand for new machines or methods for this purpose is large. An interesting and promising development is represented by the "Multifunctional Dynamometer for Application in Space" (MDS), which was developed at TU Wien and was already successfully tested in the 520-day isolation project "Mars 500" (June 2010 - November 2011). To underline a future application of the MDS, a complete training study on several subjects was conducted to examine the issue of microgravity completely contrary to the previous study. While not examining the principle of optimum load from a biomechanical point of view, but rather at the biochemical level and specifically related to the problem of fluid shift in weightlessness. After new findings suggest that muscle and bone atrophy greatly depend on the changed blood pressure gradient in these structures, due to the relocation of the blood from the lower to the upper body, a long-term research study including eleven subjects who exercised up to six weeks under vascular blood flow reduction on the MDS was carried out.

METHODS

The study was executed on eleven healthy subjects (3 women, 8 men). Analyzed was the development of strength and hypertrophy in the quadriceps muscle, over a training period of 4-6 weeks, where participants had to finish twelve training sessions at least, divided up to 2-3 sessions per week. Exercise was limited to a single-legged leg press exercise on the Multifunctional Dynamometer, for four sets of continuous vascular occlusion. Each session consisted of a training. On certain appointments isometric maximum (IM) tests were performed to document strength development.

Before blood flow restriction (BFR) pressure was calculated for every subject individually, the process started with a measurement of the systolic blood pressure on hearth level, to exclude possible hypertonia and give a comparison for the later calculated BFR-pressure. BFR-pressure was finally defined as 50% of measured systolic blood pressure at hip level, as suggested in literature (Scott et al., 2015).

Additional body measurements were taken at the beginning, in which weight, body fat and water content were measured with a common body fat balance. Thigh circumference was measured manually with a measuring tape taken at positions 1/4, 1/3 and 1/2 of femur length. The measurements started from the top of the patella in a standing position. Isometric Maximum Force: To define an individual exercising load, every participant had to execute an isometric maximum (IM) test.

The training sequence was the same for everyone. Participants had to execute a seated single-legged leg press exercise at a load based on their individual isometric maximum force. With each leg a full cycle volume of 75 repetitions had to be performed under continuous vascular occlusion. Repetitions were divided up to four sets of 30/15/15/15 repetitions with a 60 seconds rest period between the sets. In the first session the intensity was defined at 20% of isometric maximum, to allow the participant to get familiar with the training principle. All other sessions were performed at 25%, with the exception of session five, where intensity was increased up to 30% to check individual responses to higher intensity. After session seven rest time was halved from 60 to 30 seconds for further response understandings. For five sessions (1, 3, 5, 8, 12) an isometric maximum test was performed before the training and intensity-load was adapted to the results.

The common exercise cycle started with a warm up, which was half body weight for most people. Furthermore, this was already above exercise intensity for all participants. After this, an inflatable cuff from Boso (cuff width=14cm) was applied by the participants as proximal as possible on the thigh (figure 1a). This position is commonly recommended BFR protocols. Additionally it also showed that a more distal position was not effective in occluding the venous return and partially led to discomfort. Inflation was done by the participants, too. A pressure about 50% of the measured arterial pressure on hip level was adjusted by an inflation bulb and controlled with a pressure gauge (figure 2b).

(a) (b)

Figure 1: Application of the inflatable cuff (b) proximal on the thigh (a).

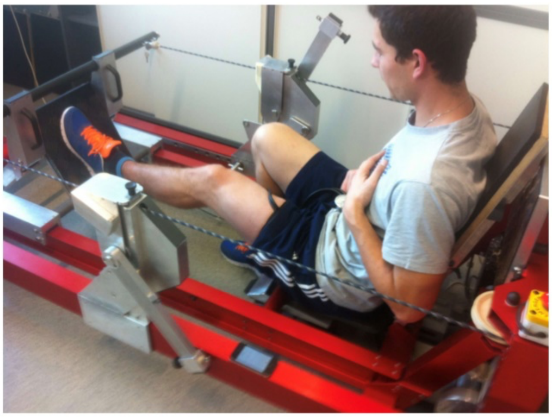


Figure 2: Single-legged leg press exercise with an inflatable cuff applied proximal on the left thigh.

RESULTS

The results show significant strength increases. Between individual isometric maximum strength tests, within two weeks, partly an increase of 5-8 % of isometric maximum force was measured.

DISCUSSION AND CONCLUSION

The number of subjects that participated in this training study was too small for high statistical accuracy. The problem was that strength was increasing so fast that tendons could not adapt at this speed, therefore the subjects often had to train about two weeks with light weight to avoid any injuries. The good results are probably mostly caused by the participation of subjects that were completely inexperienced in strength training. In conclusion, more research needs to be done in this interesting area.

REFERENCES

Scott, B., Loenneke, J., Slattery, K., and Dascombe, B., 2015, *Exercise with blood flow restriction: an updated evidence-based approach for enhanced muscle development.* Sports Med 45(3), 313-32

17:30 Mona Nasser – "Jaw movement in microgravity"

Jaw movement in microgravity

M. Nasser, C. Y. Gonzalez[2]
[1] Dental School, Faculty of Health, University of Plymouth (mona.nasser@plymouth.ac.uk), [2] Native Sky Inc

INTRODUCTION

Movement of the jaws are key aspect of our masticatory function and speech. Psychological stress can affect those movement as people start clenching and bruxism that had long term impact on the maxillofacial structure. Due to the repetitive nature of these jaw movements, even small changes in the movement can result in long term impact on the function and structure. Moreover, several studies have shown that the orientation of the jaws in space and their movement can affect body posture and coordinations.

METHODS

This study is the initial steps to build upon a larger work around measuring how jaw movements change and adapt in microgravity. We conducted two scoping reviews – one review focusing on how microgravity environment can potentially affect short-term or long-term jaw movements. This was complicated by a mapping of currently available devices to monitor jaw movements. We mapped the latter across requirements for future studies on microgravity to see which instruments can be used to study jaw movement further in microgravity.

RESULTS

Microgravity environment can have a range of effect on different parts of the maxillofacial structure. There are a series of physiological factors that can be affected e.g. blood flow increases in the skull and unlike the lower limbs, the bone density seem to increase. Masticatory muscles are more resilient than leg muscles towards microgravity. In addition to physiological factors, psychological factors like stress and loneliness are present in space environment. However, the complex interaction of these factors is not currently adequately explored.

We evaluated the current instruments to measure these movements to evaluate whether we can design future studies to better understand jaw movement in future space flight. Our initial mapping showed that the measurement tools range from smaller wearable instruments that are limited to record a small range of indicators to large optical devices that require to immobilize the patients.

CONCLUSION

Although the initial data demonstrates that microgravity environment can affect jaw movement and potentially have long term effect due to the importance and repetitive nature of these movements. However, there is not enough data to have a good overview – this can be partially due to limitations of current equipment to be used in microgravity. Our next step is to work further on running simulations on impact of microgravity on jaw movements.

17:45 Elie-Tino Godonou – "Changes in urinary concentration of the cartilage degradation marker Coll2-1NO2 in response to bed rest immobilization and countermeasures"

Changes in urinary concentrations of the cartilage degradation marker Coll2-1NO2 in response to bed rest immobilization and countermeasures

A.-M. Liphardt[1,2,] E.-T. Godonou[1,2], N. Djalal[1,2], M. Dreiner[3], A. Mündermann[4], Y. Henrotin[5,6,7,] F. Zaucke[8], M. Heer[9,10], G. Schett[1,2], A. Niehoff[3,10].

[1]Dep. of Internal Medicine 3 – Rheumatology & Immunology, Universitätsklinikum Erlangen & Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Erlangen, Germany [2]Deutsches Zentrum für Immuntherapie (DZI), Universitätsklinikum Erlangen & FAU Erlangen-Nürnberg, Erlangen, Germany [3]Inst. of Biomechanics & Orthopaedics, German Sport University Cologne, Köln, Germany [4]Dep. of Orthopaedics and Traumatology, University Hospital Basel, Basel Switzerland [5]Artialis S.A., Liège, Belgium [6]musculoSKeletal Innovative research Lab (mSKIL), University of Liège, Center for Interdisciplinary Research on Medicines, Liège, Belgium [6]Department of Physical Therapy and Rehabilitation, Princess Paola Hospital, Vivalia, Marche-en-Famenne, Belgium [7]Research Unit for Osteoarthritis, Dep. of Orthopaedics (Friedrichsheim), University Hospital Frankfurt, Goethe University, Frankfurt/Main, Germany [8]Dep. of Nutrition and Food Science, Nutritional Physiology, University of Bonn, Bonn, Germany, [10]Cologne Center for Musculoskeletal Biomechanics (CCMB), Medical Faculty, University of Cologne, Köln, Germany.

BACKGROUND

The lack of mechanical stress or joint fluid circulation during immobilization can alter cartilage morphology, function, and composition resulting in cartilage destruction (Hudelmaier M. et al, 2006; Hagiwara Y. et al, 2009). Specific immunoassays for a peptide of the triple helix of type II collagen (Coll2-1) and its nitrated form (Coll2-1NO2) have been developed for measuring cartilage degradation (Henrotin Y. et al, 2004) and radiological progression of knee OA (Deberg M. A. et al, 2005). This study aims to investigate the effects of 21 days of 6° head-down tilt bed rest (HDT) combined with nutrition and exercise countermeasures on the urinary levels of Coll 2-1 NO2 in healthy male individuals.

METHODS

The ESA-funded "Medium duration nutrition and vibration exercise" (MNX)-study was conducted at the Institute de Médecine et de Physiologie Spatiales (MEDES) in Toulouse, France in a randomized cross-over design with three campaigns (C1 – 3): 7 days of baseline data collection (BDC), 21 days of HDT, and 7 days of recovery (R+). A 4-month washout period separated study campaigns. HDT was applied alone (control, CON) or in combination with resistive vibration exercise (RVE) or RVE + whey & bicarbonate supplementation, NeX) in twelve healthy male subjects (age 34.2 ± 8.3 years; BMI 22.4 ± 1.7 kg/m²). Urinary (u) Coll2-1NO2 concentrations were measured by immunoassay (Artialis, Liège, Belgium) in 24hrs urines at BDC -3 to -1, HDT2, HDT3, HDT5, HDT7, HDT14, HDT21, R+1, and R+6. For the regression model, BDC was calculated as the mean of BDC -3 to -1. Linear Mixed-Effects Models were used to detect significant changes in uColl 2-1 NO2 (nM), considering time points and countermeasures as predictors.

RESULTS

At baseline (BDC-3) of campaign 1, uColl2-1NO2 (mean (sd)) was 0.108 (0.04) nM for all study participants. Time points ($p=0.011$) and countermeasures ($p<0.001$) had a significant effect on uColl2-1NO2. More specifically, overall (main effect) uColl2-1 NO2 was lower (Figure 1.A) on R+1 compared to BDC by 47.3% ($\beta=-0.04$, 95%CI: -0.08 – -0.01, $p=0.008$); when the countermeasure is NEX (Figure 1.B), uColl 2-1 NO2 concentrations were increased by 53.7% ($\beta=0.04$, 95%CI=0.03 – 0.06, $p<0.001$).

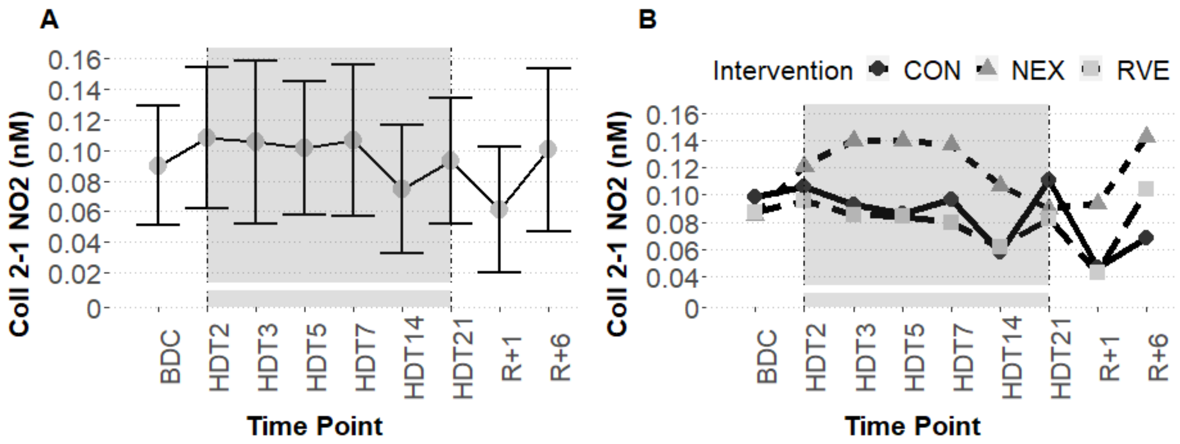


Figure 1: Mean urine concentration of Coll 2-1 NO2 in all groups over time (A), and by intervention (B).

CONCLUSION

Three weeks of bed rest immobilization resulted in lower levels of uColl 2-1NO2 at R+1 day (vs. BDC), potentially reflecting the oxidative-stress related cartilage remodeling. The nutrition countermeasure (whey & bicarbonate supplementation) in combination with resistive vibration exercise (NEX) lead to higher Coll 2-1NO2 urinary levels compared to the control intervention. While the seen difference between NEX and other interventions (CON, RVE) is notable during bed rest, the overall observed bed rest effect on Coll2-1NO2 is not as prominent as previously reported in other cartilage biomarkers (Liphardt et al. 2009, 2018, 2020). Coll2-1NO2 may not be as sensitive to mechanical loading as other markers which is in line with the previously observed response of uColl2-1NO2 in response to marathon running.

REFERENCES

Hudelmaier M. et al, 2006, J Musculoskelet Neuronal Interact 6, 284–290. Hagiwara Y. et al, 2009, J Orthop Res. 27, 236–242.

Henrotin, Y. et al, 2004, Biorheology, 41, 543–547. Deberg, M. A. et al, 2005, Osteoarthritis Cartilage, 13, 1059–1065.

Liphardt et al. 2009, Osteoarthritis Cartilage. Dec;17(12):1598-603. Liphardt et al. 2018, J Orthop Res. May;36(5):1465-1471.

Liphardt et al. 2020, J Orthop Res. Nov;38(11):2373-2382. Henrotin et al. 2013, Springerplus Dec;2(1):92.