Human Physiology Workshop

5th of December 2020

Online event

DLR

Human Physiology Workshop

We are pleased to welcome you to the 5th German Human Physiology Workshop 2020. The workshop shall provide a forum for researchers at all stages (student to professor) to meet and discuss their latest findings in human physiological research and space research and give room for mutual exchange and benefit between space and non-space scientists.

Organizers

Jörn Rittweger, Carina Arndt, Friederike Wütscher (German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne)
Katrin Stang, Michaela Girgenrath, Christian Rogon (German Aerospace Center (DLR), Space Administration, Microgravity Research and Life Sciences, Bonn)

Administrative secretariat

humanphysiologie-ws@dlr.de

Scientific Committee

Markus Braun	Deutsches Zentrum für Luft- und Raumfahrt (DLR), Raumfahrtmanagement, Forschung unter Weltraumbedingungen/Lebenswissenschaften, Bonn
Peter zu Eulenburg	LMU München
Joachim Fandrey	Universität Duisburg-Essen, Institut für Physiologie
Nandu Goswami	Medizinische Universität Graz, Institut für Physiologie, Gravitationsphysiologie und -medizin, Graz, Österreich
Markus Gruber	Universität Konstanz, Sensorimotor Performance Lab
Pete Hodkinson	King's College London, Centre for Human & Applied Physiological Sciences, Großbritannien
Ylva Hellsten	Universität Kopenhagen, Department of Nutrition Exercise and Sports, Dänemark
Jens Jordan	Deutsches Zentrum für Luft- und Raumfahrt (DLR), Institut für Luft- und Raumfahrtmedizin, Köln
Justin Lawley	Universität Innsbruck, Institut für Sportwissenschaft, Österreich
Anja Niehoff	Sporthochschule Köln, Institut für Biomechanik und Orthopädie, Köln
Jörn Rittweger	Deutsches Zentrum für Luft- und Raumfahrt (DLR), Institut für Luft- und Raumfahrtmedizin, Muskel- und Knochenstoffwechsel, Köln
Michele Salanova	Charité Universitätsmedizin Berlin, Institut für Physiologie, Center for Space Medicine and Extreme Environments, Berlin
Simon Schäfer	Klinikum der Universität München
Frank Weber	Zentrum für Luft- und Raumfahrtmedizin der Luftwaffe, Fürstenfeldbruck
Tobias Weber	ESA, Köln

Program

Saturday, December 5, 2020

Session 1:
Chair: Peter Hodkinson, Julie Hides

09:00 **De Gioannis, Riccardo:** Increased inspiratory carbon dioxide improves blood oxygenation during prolonged hypoxia exposure.

09:15 **Lindsay, Kirsty:** Thirteen sessions of FRED exercise reduced back pain symptoms after 60-day strict head-down tilt bed rest

09:30 **Köhlmoos, Anika:** Effect of synbiotic intake on liver lipid metabolism and their potential impact on non-alcoholic fatty liver disease

09:45 **Schreiber, Kristina:** Genetic variants in the prolinhydroxylase 2 gene and hypoxia tolerance: end-stage-lung-disease as a model for chronic, moderate hypoxia in long-term-space-flights

10:00 **Zuccarelli, Lucrezia:** Peripheral impairments of oxidative metabolism after a 10--day bed rest are upstream of mitochondrial respiration

10:15–10:30 break

Session 2:
Chair: Gabriele Pfitzer, Dominik Pesta

10:30 **Aebi, Mathias:** Cerebral and physiological responses to submaximal exercise in pilot trainees in various normobaric/hypobaric and normoxic/hypoxic conditions

10:45 **Marume, Kyohei:** Daily Generation of Footward Fluid Shift Attenuates cerebral hypoperfusion Associated with 3 days Head-Down Tilt Bedrest

11:00 **Schubert, Martin:** Myasthenia gravis, an autoimmune neurodegenerative disease, as an experimental model to investigate the effects of microgravity in skeletal muscle.

11:15 **Huber, Judita:** Inaugural quantitative analysis of perivascular spaces in long-duration space flyers

11:30 **Monti, Elena:** Early neuromuscular and contractile maladaptations to short-term bed rest

11:45 **Baldassarre, Giovanni:** Work rate decrease during exercise at a fixed submaximal heart rate: a new method to evaluate exercise (in)tolerance microgravity?

12:00–12:45 Break

Session 3:

Chair: Stefan Sammito, Jessica Koschate

12:45 **Hinterwaldner, Luis:** Ankle joint immobilization using an unloading orthosis induces a muscle atrophy pattern that is similar to head down-tilt bedrest

13:00 **Klein, Timo:** Importance of exercise during short-term isolation

13:15 **Tran, Vienna:** Artificial gravity does not mitigate deconditioning of the gluteal muscles after prolonged head-down tilt bed rest

13:30 **De Martino, Enrico:** Intermittent short-arm centrifugation is a partially effective countermeasure against upright balance deterioration following 60-day strict head-down tilt bed rest

13:45 **Henkel, Sara:** Resting energy expenditure (REE) of master athletes: Accuracy of predictive equations and primary determinants

14:00–14:30 break

Session 4:

Chair: Mauro Marzorati, Kirsten Albracht

14:30 **Thier, Nikolas:** Evaluation of ultrasound data from the MARES Sinusoidal Perturbation Protocol for the analysis of vibration-induced changes in fascicle length and pennation angle as a function of vibration frequency and muscular preload

14:45 **Eggelbusch, Moritz:** Skeletal muscle ultrastructural changes in myofibrillar morphology and nutrient storage after short- and long-term bed rest

15:00 **Marcos, David:** Cervical intervertebral disc expansion, vertebral compliance modulation and neck pain is induced by 4h of Hyper-Buoyancy Flotation and is only partially reversed by 15 mins re-exposure to gravity

15:15 **V. Wooten, Savannah:** Cardiovascular Sex-Differences in Master Athletes Determined by Echocardiography

15:30 **Invited Talk: Rolf Erdmann: How Space Exploration will be Boosted by Private Industry**

16:00 ***Awards***

16:15 **Adjourn**

Human Physiology Workshop

Abstracts

1

Increased inspiratory carbon dioxide improves blood oxygenation during prolonged hypoxia exposure.

Riccardo De Gioannis[1], *Daniel Rooney*[1], *Martin Wittkowski*[1], *Titiaan Post*[2], *Jörn Rittweger*[1], *Daniel Aeschbach*[1]

[1] Division of Sleep and Human Factors Research, Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany
[2] Centre for Human Drug Research (CHDR), Leiden, The Netherlands

Introduction: The physiological response to sustained hypoxia exposure is characterized by an initial increase in minute ventilation. This mechanism prevents a sudden decrease of oxygen delivery to the body. Although this is effective in the short term, minute ventilation decreases after some minutes due to the onset of hypocapnia. In this study we hypothesize that increased inspiratory carbon dioxide (CO_2) sustains the hypoxic ventilatory response determining an improvement in blood oxygenation in healthy subjects.

Methods: We examined seventeen healthy participants (eight females, 27.8 ± 4.2 years) in a randomized double blind cross-over trial. Subjects spent on two study days six hours in a hypobaric chamber. On both days the pressure in the chamber was decreased to simulate an altitude of 8000 feet (2438 meters). Partial pressure of CO_2 was maintained at 1 hPa (low CO_2) on one study day and 10 hPa (high CO_2) on the other. A breath by breath spirometry, blood oxygen saturation measurement (SpO_2) and head and muscle near-infrared spectroscopy measurements (NIRS) were performed hourly. We performed a capillary blood gas analysis (cBGA) after 15 minutes of hypoxia exposure and before returning to normobaric conditions. All measurements were additionally conducted before hypoxia exposure.

Results: In the high CO_2 condition SpO_2 decline from baseline was significantly smaller (F=171, p=0.0001) when compared to the hypoxia exposure with low ambient CO_2. This observation was consistent across all six time points; the relative decline of mean SpO_2 ranged from -3.6 to -4.6% with high and from -5.2 to -6.8% with low CO_2. The average relative decline from baseline of capillary pO_2 was 21.1% vs. 32.0% after 10 min (t(16)=-2.89, p=0.01) and 24.1% vs. 30.1% after 6 hr (t(16)=-2.41, p=0.03) at altitude on test days with high and low CO_2 respectively. With high ambient CO_2 the increase in minute ventilation was higher (0.76 ± 2.92 L/min) compared to the low CO_2 condition (0.09 ± 2.57 L/min), t(94)=2.13, p=0.04. However the increase of oxygen saturation and minute ventilation were not correlated. Muscle and brain oxygen saturation, heart rate and arterial pressure showed no difference in the two conditions.

Conclusions: The altitude simulated in this study reproduces the atmospheric conditions inside the cabin of a commercial aircraft. Although atmospheric oxygen partial pressure at 8000 feet is considered safe for most passengers, in some of them, oxygen saturation can drop below 90%. Our results show the potential of CO_2 enrichment of the aircraft cabin as a countermeasure to in-flight hypoxia in healthy subjects during a long haul flight.

Thirteen sessions of FRED exercise reduced back pain symptoms after 60-day strict head-down tilt bed rest

Kirsty Lindsay[1], Enrico De Martino[1], Paul Hodges[2], Julie Hides[3], Dorothée Debuse[1], Andrew Winnard[1], David Beard[3], Jonathan A. Cook[3,4], Robert Ekman[6], Luis Hinterwaldner[6], Jonathan Scott[6,7], Tobias Weber[6,7], Nick Caplan[1]

[1] Aerospace Medicine and Rehabilitation Laboratory, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK
[2] The University of Queensland, NHMRC Centre for Clinical Research Excellence in Spinal Pain, Injury and Health, School of Health and Rehabilitation Sciences, Brisbane, Australia
[3] School of Allied Health Sciences, Griffith University, Nathan Campus, Brisbane, Australia
[4] NIHR Oxford Biomedical Research Centre, Nuffield Department of Orthopaedics, Rheumatology and 5 Musculoskeletal Sciences, University of Oxford, Oxford, UK
[5] Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[6] European Astronaut Centre, Space Medicine Team (HRE-OM), European Space Agency (ESA), Cologne, Germany
[7] KBR GmbH, Cologne, Germany

Introduction: Both post-flight astronauts and terrestrial chronic non-specific low back pain patients experience muscle atrophy in the deep lumbopelvic muscles which may increase their risk of injury and disability. Low back pain (LBP) is also reported in both groups and can reduce the patients' health-realted quality of life. Long-duration, head down-tilt bedrest can be used to model lumbopelvic deconitioning. During the reconditioning phase, physical exercise is crucial to reduce LBP and the risk of disc injury. The Functional Re-Adaptive Exercise Device (FRED) was designed to recruit the lumbopelvic muscles during cyclical lower limb movement. Importantly, preliminary evidence showed that FRED exercise reduced pain intensity and functional disability in chronic LBP patients by recruiting the deep lumbopelvic muscles. This study investigated whether daily FRED exercise reduced pain symptoms and characteristics following 60 days of strict head-down bedrest in the ESA/NASA Artifical Gravity Bedrest (AGBRESA) study compared with the standard rehabilitation.

Methods: 24 participants were randomly assigned to receive either 13 sessions of FRED exercise (FRED, n= 12) or no-FRED intervention control (CON, n=12) following 60-days of head-down tilt bed rest. Both groups received standard DLR reconditioning and physiotherapy. Pain data were collected via written questionnaires on recovery days 1, 6 and 12 (R+1, R+6, and R+12). Questionnaires consisted of a body chart showing pain location and area in mm^2, visual analogue scale (VAS) showing pain intensity between 0-10cm, maximum pain duration in hours and back pain report count. The analysis consisted of descriptive statistics due to the small sample. The FRED group exercised daily from R+1 to R+13 for up to 30 minutes under supervision in addition to standard DLR reconditioning. The control group undertook DLR deconditioning only.

Results: At R+1 maximum pain duration (FRED= 6.4±3.1hr, CON=8.1±4.8hr) was similar between groups and the CON group reported fewer incidences of LBP(FRED=13, CON=9). Pain intensity was less in FRED group following one training session (FRED=28.2±12.2mm, CON =44.6±22.1), while pain area was larger in the FRED group (FRED=117.7±96.7mm^2, CON= 70.4± 28.3mm^2). By R+6, the FRED group reported fewer back pain reports (FRED= 3, CON= 12) and less maximum pain duration (FRED=2.7±0.5hr, CON=9.5±5hr). Pain intensity was comparable between groups (FRED= 15.4±5.5mm and CON= 14.8±7.2mm), and pain area was larger in the FRED group (FRED=84.1±48.1mm^{2,} CON=70.7±33mm^2). At R+12, both groups reported low levels of back pain (three reports each) and maximum pain duration (FRED=2.5±1.9hr, CON= 3.3±1.9hr). The FRED group reported lower pain intensity than the CON group (FRED=14±6mm and CON=39.7±6.1) and larger pain area (FRED= 69.6±41mm^2, CON= 37±41.1 mm^2).

Conclusions: These preliminary results indicated that daily FRED exercise in addition to standard reconditioning reduced the number of back pain reports, pain intensity, and maximum pain duration compared to standard reconditioning alone during the early phase of the rehabilitation following 60- days of head-down tilt bed rest. However, pain area was larger in the FRED group. The reduced pain intensity and duration seen in the FRED group may be due to improved lumbopelvic control, pelvic position and posture, however future studies are needed to explore this association.

3

Effect of synbiotic intake on liver lipid metabolism and their potential impact on non-alcoholic fatty liver disease

Anika Köhlmoos[1], Daniela Schelski[2,3], Leonie Koban [4,5], Hilke Plassmann[4,5], Marie-Christine Simon[1]

[1] Institute of Nutrition and Food Science (IEL), Nutrition and Microbiota, University Bonn, Germany
[2] Center for Economics and Neuroscience, University of Bonn
[3] Institute of Experimental Epileptology and Cognition Research, University of Bonn
[4] INSEAD, Fontainebleau, France
[5] Control-Interoception-Attention Team, Paris Brain Institute (ICM), Paris, France

Introduction: There is an anatomical connection between the gut and the liver. Thus, modulation of the gut microbiome may have an impact on liver metabolism and may serve as therapy for nonalcoholic fatty liver disease (NAFLD), which is the most common liver disease in western population. Therefore we hypothesize, that modulation of the microbiome with a synbiotic supplement alters human liver metabolism.

Methods: A randomized, placebo-controlled, double-blinded seven-week intervention study was conducted with 117 metabolic healthy male participants. They were allocated to two groups, either receiving a synbiotic supplement or placebo, without changing their dietary habits or physical activity. The synbiotic contained 2x109 probiotic bacteria from five strains (*Bifidobacterium lactis*, *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Lactococcus lactis*) and inulin from the agave as prebiotic. The placebo consisted of microcrystalline cellulose. During two visits (T1 and T2), anthropometry and parameters of the liver metabolism were measured.

Results: After seven weeks of intervention a significant reduction of alanine aminotransferase (ALAT) (T1: 31.30 ± 24.08 U/L vs. T2: 27.57 ± 19.67 U/L, p=0.02) and gamma-glutamyltransferase (T1: 22.55 ± 12.40 U/L vs.T2: 21.98 ± 12.22 U/L, p=0.04) was observed in the synbiotic group while the placebo group remained stable. Furthermore, there was a significant difference in the relative change of ALAT from T1 to T2 between the synbiotic and the placebo group (synbiotic: -5.80 ± 26.12 % vs. placebo: 7.91 ± 27.05 %, p=0.02). But there was no change in markers of lipid metabolism. A stratified analysis by body fat percentage revealed a significant decrease in ALAT in overfat participants of the synbiotic group (T1: 39.22 ± 30.27 U/L vs. T2: 34.83 ± 29.05 U/L, p=0.03) and a significant difference in the relative change of ALAT between the groups (synbiotic: -8.52 ± 23.33 % vs. placebo: 12.60 ± 28.22 %, p=0.03).

Conclusions: The supplementation of a specific synbiotic might be able to delay the progression of NAFLD, which could be useful in addition to the recommended lifestyle modification therapy.

4

Genetic variants in the prolinhydroxylase 2 gene and hypoxia tolerance: end-stage-lung-disease as a model for chronic, moderate hypoxia in long-term- space-flights

Schreiber K[1], Lenhart B[1], Hatz R[2,3], Kneidinger N[3,4,] Koch I[2,3], Rump K[5], Kauke T[2], Schäfer ST[1]

[1] Department of Anaesthesiology, University Hospital, LMU Munich, Munich, Germany
[2] Center for Thoracic Surgery Munich, University hospital, LMU Munich, Munich/Asklepios Lung Clinic Gauting, Gauting, Germany/ Asklepios Biobank for Lung Disease
[3] Member of the German Centre for Lung Research (DZL), Germany
[4] Department of Internal Medicine V, University Hospital, LMU Munich, Comprehensive Pneumology Center (CPC-M), Member of the German Centre for Lung Research (DZL), Germany
[5] Department of Anaesthesiology, Intensive Care Medicine and Analgesic Therapy, University Hospital Knappschaftskrankenhaus Bochum, Bochum, Germany

Introduction: Humans' tolerance to hypoxia is regulated through a group of transcription factors, the so-called hypoxia-inducible-factors, and the activity of its degrading prolyl-hydroxylases (PHD). It is known that genetic variants in these genes are common in high altitude residents and allow to tolerate chronic, moderate hypoxia, e.g. by blunted hypoxic pulmonary vasoconstriction (HPV). In this regard genetic variants in the HIF-pathway, e.g. in the *PHD2* gene (C/T; SNP rs516651 and T/C; SNP rs480902) are associated with improved adaptation to hypoxia, reduced 30-day mortality in adult respiratory distress syndrome (ARDS) or disease severity in septic patients and most important the development of pulmonary hypertension (PH) in Caucasians. Thus, there is an interindividual difference in the genotype- dependent hypoxia-tolerance. Genetic variants in the HIF-pathway depict a strong phenotype e.g. regarding HPV and inflammatory responses. Whether these genotypes should be determined in long-term-space-flight applicants, who might experience chronic, moderate hypoxia during the mission, is unclear. Now, we have chosen patients with end-stage lung disease awaiting sequential bilateral lung transplantation (BLTx), as a model for chronic moderate hypoxia. These patients are an ideal sample population as they suffer chronic, moderate hypoxia and are followed up extensively. Therefore, we tested the hypotheses that 1) SNPs in the HIF-1α or PHD2 genes are common in patients with end-stage lung disease being listed for BLTx in Europe and 2) the genetic variants are associated with altered tolerance towards chronic hypoxia.

Methods: After ethics approval, 262 patients receiving BLTx were analysed using retrospectively acquired biosamples. Patients' characteristics, nature of the end-stage lung disease and genotypes (Taqman Genotyping Assay) were determined. Preoperative partial pressures of oxygen (paO2), intraoperative Horowitz values and hemodynamic measurements (e.g. mPAP values) were defined as indicators for tolerance towards chronic hypoxia and analysed using the Mann-Whitney-U-test. Kaplan-Meier survival probabilities after lung transplantation were separately analysed according to the patients' genetic variants. Differences in survival between groups were compared using the log-rank test. Patients with CC genotype were compared to T-allel carriers (combined CT and TT genotype for PHD2 SNP rs516651 and rs480902) due to low TT frequencies.

Results: *Hypoxia inducible factor-1α (C/T rs11549465) polymorphism*
The Hif-1α -SNP rs11549465 is a common genetic variant in end-stage-lung-disease patients listed for BLTx in Europe, with 23.3% T-allel carriers (TT 3.1%, CT 20.2%, CC 76.7%). Patients' clinical characteristics (e.g. gender, age, BMI, days spent in hospital, days in ICU, pretransplant diagnosis) did not differ between underlying groups. Of note, our null hypotheses had to be rejected and there was no impact of this genetic variant on hypoxia-tolerance (mPAP, p=0.841; paO_2, p=0.124; Horowitz, p=0.905), and 90-day mortality after BLTx (p=0.859;Kaplan-Meier-analysis) in contrast to septic patients.
Prolylhydroxlase 2 (C/T; rs516651) polymorphism
The PHD-2-SNP rs516651 is a common genetic variant in end-stage-lung-disease patients listed for BLTx in Europe, with 23.3% T-allel carriers (TT 0.4%, CT 22.9%, CC 76.7%). Patients' clinical characteristics (e.g. gender, age, BMI, days spent in hospital, days in ICU, pretransplant diagnosis) did not differ between underlying groups. Of note, our null hypothesis had to be rejected and in contrast to ARDS patients, there was no impact of this genetic variant on hypoxia-tolerance (mPAP, p=0.212; paO_2, p=0.536; Horowitz, p=0.923), and 90-day mortality after BLTx (p=0.669; Kaplan-Meier-analysis). Similar results were obtained for the Prolylhydroxylase 2 (T/C; rs480902) polymorphism.

Conclusions: This is the first study to show that these genetic variants in the HIF-pathway are common in European patients suffering from end-stage lung disease. Of note, in end-stage-lung-disease patients undergoing BLTx, as a model group for moderate hypoxia, these genetic variants did not have an impact on pulmonary hypertension, oxygenation and 90-day mortality. One possible explanation is that BLTx represents a complex surgical procedure and thus perioperative outcome parameters such as organ quality or perioperative complications overshadow the impact of genetic variants. Further studies are required to analyse whether aforementioned genetic variants are relevant for hypoxia tolerance in otherwise healthy volunteers applying for long-term space flights. Understanding the role of HIFs and PHDs in chronic hypoxia can be essential for decision making in long-term space missions, e.g. regarding optimal inspired oxygen content and ambient pressure.

5

Peripheral impairments of oxidative metabolism after a 10-day bed rest are upstream of mitochondrial respiration

Lucrezia Zuccarelli[1], *Giovanni Baldassarre*[1], *Benedetta Magnesa*[1], *Cristina Degano*[1], *Marina Comelli*[1], *Mladen Gasparini*[2], *Giorgio Manferdelli*[3], *Mauro Marzorati*[3], *Irene Mavelli*[1], *Andrea Pilotto*[1,3], *Simone Porcelli*[3,4], *Letizia Rasica*[3], *Boštjan Šimunič*[5], *Rado Pišot*[5], *Marco Narici*[6], *Bruno Grassi*[1]

[1] University of Udine, Department of Medicine, Udine, Italy
[2] Splošna Bolnišnica Izola, Izola, Slovenia
[3] National Research Council, Institute of Biomedical Technologies, Milan, Italy
[4] University of Pavia, Department of Molecular Medicine, Pavia, Italy
[5] Science and Research Centre, Institute of Kinesiology Research, Koper, Slovenia
[6] University of Padova, Department of Biomedical Sciences, Padova, Italy

Introduction: Exposure to simulated microgravity by bed rest (BR), leads to an impairment of oxidative metabolism. The sites of this impairment are still debated. Aim of this study was to identify markers of impaired oxidative metabolism along the O_2 pathway, from ambient air to skeletal muscle mitochondria, following 10 days of BR.

Methods: Before (PRE) and after (POST) 10 days of horizontal BR, ten recreationally active young males (age 23 ± 5 years [mean±SD]) performed on a cycle ergometer an incremental exercise (INCR) up to exhaustion (to determine peak pulmonary $\dot{V}O_2$ [$\dot{V}O_2p$] and the gas exchange threshold [GET]) and two repetitions of constant work-rate (CWR) exercises at 80% of GET (MOD). $\dot{V}O_2p$ and *vastus lateralis* muscle fractional O_2 extraction by near-infrared spectroscopy (NIRS) (Δ[deoxy(Hb±Mb)]) were recorded continuously. Peripheral vascular and endothelial functions were evaluated by the blood flow response (Doppler ultrasound) in the femoral artery during 1-min passive leg movements (PLM). Mitochondrial respiration was evaluated: (i) *ex vivo* by high-resolution respirometry on permeabilized *vastus lateralis* fibers obtained by biopsy and (ii) non-invasively *in vivo* by NIRS by calculating skeletal muscle $\dot{V}O_2$ ($\dot{V}O_2m$) recovery kinetics (repeated transient occlusions method) following MOD. The time constants (τ) of the monoexponential $\dot{V}O_2m$ kinetics during the first 7 minutes of recovery were determined.

Results: Peak $\dot{V}O_2p$ was lower (P=0.001) in POST (41.5 ± 6.5 $ml \cdot kg^{-1} \cdot min^{-1}$) *vs.* PRE (44.5 ± 7.4). The area under the blood flow *vs.* time curve during PLM was lower (P=0.038) in POST (274 ± 233 mL) *vs.* PRE (427 ± 291). Skeletal muscle citrate synthase activity, an estimate of mitochondrial mass, was not different (P=0.115) in POST (131.2 ± 15.9 $UI \cdot mg^{-1}$ protein) *vs.* PRE (137.9 ± 18.8). Maximal ADP stimulated mitochondrial respiration (66.4 ± 17.5 $pmol \cdot s^{-1} \cdot mg^{-1}$ wet weight [POST] *vs.* 72.3 ± 14.0 [PRE], P=0.127) and oxidative phosphorylation coupling efficiency (respiratory control ratio, 4.10 ± 1.19 [PRE] *vs.* 3.59 ± 1.11 [POST], P=0.443) were not affected by BR. τ of $\dot{V}O_2m$ recovery was not different (P=0.079) in POST (22.2 ± 5.9 s) *vs.* PRE (21.7 ± 5.7).

Conclusions: These preliminary data suggest that the whole-body impairment of oxidative metabolism during exercise, following 10 days of horizontal BR, is associated with an impairment of peripheral vascular and endothelial functions whereas mitochondrial volume and maximal respiratory function are unaffected.
Funding: ASI, MARS-PRE Project, n. DC-VUM-2017-006.

6

Cerebral and physiological responses to submaximal exercise in pilot trainees in various normobaric/hypobaric and normoxic/hypoxic conditions

Mathias R. Aebi[1,2,3], Nicolas Bourdillon[2,4], Denis Bron[2], Grégoire P. Millet[2]

[1] Aeromedical Center (AeMC), Swiss Air Force, Dübendorf, Switzerland
[2] University of Lausanne, Institute of Sport Sciences (ISSUL), Lausanne, Switzerland
[3] Armasuisse, Wissenschaft & Technologie, Thun, Switzerland.
[4] be.care SA, Renens, Switzerland

Introduction: In hypoxic environment, cerebral blood flow regulation is vital to maintain adequate oxygen supply to the brain. The present study aimed to evaluate change in cerebral blood flow velocity (MCAv), cerebral oxygen delivery (cDO_2) and physiological responses at rest vs. moderate-intensity exercise, in acute normobaric vs. hypobaric normoxic/hypoxic conditions.

Methods: Eighteen healthy pilot trainees (26±3 years old, 177±10 cm, and 70±11 kg) performed a 6-min moderate-intensity exercise (1 W/kg, at 80 rpm) on a cycle ergometer (eBike II basic, GE medical systems, Germany) in four randomized conditions (normobaric normoxia, NN; hypobaric hypoxia, HH and normobaric hypoxia, NH at 5000m; and hypobaric normoxia, HN). Inspired oxygen pressure (P_IO_2) was matched between normoxic (NN vs. HN, 141.2±0.8 vs. 141.5±1.5 mm Hg) and hypoxic (NH vs. HH, 75.7±0.4 vs. 74.3±1.0 mm Hg). Pulse oxygen saturation (SpO_2), heart rate (HR), MCAv (Transcranial Doppler, ST3, Spencer Technology, Seattle, WA) and gas exchanges (K5, Cosmed, Italy) were measured at rest and during exercise as well as rating of perceived exertion (RPE). Repeated measures ANOVAs were performed to assess statistical significance.

Results: At rest, MCAv was higher in HH (48±7 cm/s) than in NN (43±6 cm/s, p=0.022) and HN (42±5 cm/s, p=0.003). HR was higher and SpO_2 lower in NH and HH than in HN and NN (i.e., hypoxic effect, p<0.001). During exercise, MCAv was higher in HH (57±6 cm/s, p=0.01) than in NN and HN (49±6 and 48±6 cm/s, respectively). Moreover, HH induced greater HR (131±17 bpm, p=0.002) and lower SpO_2 (69.2±5.7 %, p<0.001) than NH (119±15 bpm and 81.4±4.8 %). Minute ventilation during exercise was higher only in HH (p<0.01) while breathing frequency and tidal volume were higher in hypoxic than in normoxic conditions (p<0.001). RPE was higher (p<0.001) in NH (11.3±2.2) and HH (11.8±2.3) compared to NN (8.1±1.3) and HN (9.1±1.3). There was no significant difference in cDO_2 between conditions either at rest or during exercise. No significant difference was reported between NN and HN.

Conclusions: Hypoxemia in NH and HH induced an increase in MCAv to maintain cDO_2 (Brugniaux et al., 2007). The present results (i.e., lower SpO_2 and greater HR) confirm the more severe condition in HH than in NH (DiPasquale et al., 2015; Savourey et al., 2003), inducing a higher ventilatory response during exercise in HH than in NH. Contradictory, the effect of hypobaria seems negligible in normoxic conditions since there was no difference in MCAv, HR and SpO_2 between NN and HN. These findings are of clinical importance for pilots training in flight simulator (i.e. NH) to prepare for flights hypoxic events at real altitude in HH.

7

Daily Generation of Footward Fluid Shift Attenuates cerebral hypoperfusion Associated with 3 days Head-Down Tilt Bedrest

Kyohei Marume[1], Carmen Possnig[1], Gautam Bab[2,3], Sylvan Janssen[4], Christopher Hearon Jr[2,3], Katrin Dias[2,3], Satyam Sarma[2,3], Justin Lawley[1,2,3], Benjamin Levine[2,3]

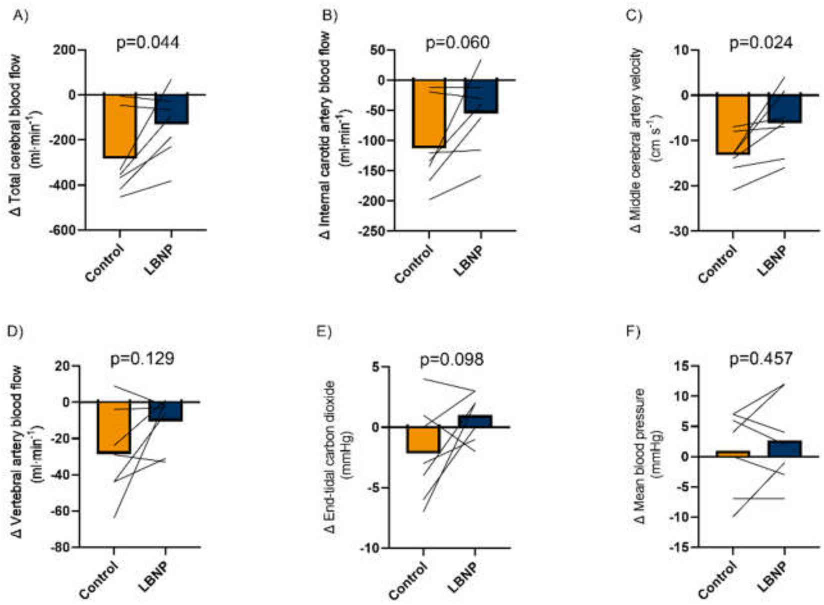
[1] Department of Sports Science, Division of Physiology, University of Innsbruck, Innsbruck, Austria
[2] Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Dallas, Dallas, TX, USA
[3] University of Texas Southwestern Medical Center, Dallas, TX, USA
[4] University Medical center, Radboud University, Nijmegen, Netherlands

Introduction: Decreased cerebral blood flow (CBF) may be associated with cognitive impairment during spaceflight. Previous studies showed that CBF decreases from 2-4 weeks during a 6-month spaceflight. The decrease in CBF may be associated with increased cerebral vessel resistance and increased intracranial pressure, both of which can be countered by a gravitational stimulation during space flight. Lower body negative pressure (LBNP) can load gravitational stimulation and can be used on the international space station. The purpose of this study is to investigate whether LBNP can attenuate the decrease in CBF induced during bed rest.

Methods: Five men and two women were subjected to −6 deg head-down tilt (HDT) bed rest for 3 days in a randomized, counter-balanced, cross-over design, with and without daily −20mmHg LBNP for 8 hours.

Results: After 3-days of −6 deg HDT, blood flow in the internal carotid artery (ICA) (477±176 to 364±140 ml·min-1; p=0.006), vertebral arteries (VA) (105±36 to 77±24 ml·min-1; p=0.024), and total CBF (1164±345 to 881±260 ml·min-1; p=0.006) was decreased. Transcranial Doppler ultrasound showed a decreased middle cerebral artery velocity (MCAv) (56±7 to 44±7 cm·s-1; p<0.001). When participants spent 8 hours per day under −20mmHg LBNP, the decrease in TCBF was attenuated by 55% (−131±148 vs. −288±181 ml·min-1; p=0.044, Figure A). This protective effect was predominantly seen in the anterior cerebral circulation as ICA blood flow (−55±64 vs. −113±71 ml·min-1; p=0.068, Figure B) and MCAv (−11±15 vs. −28±25 ml·min-1; p=0.129, Figure C) were attenuated by daily LBNP whereas VA blood flow was decreased similarly (−11±15 vs. −28±25 ml·min-1; p=0.129, Figure D).

Conclusions: LBNP can attenuate the decrease in CBF during the 3-days of −6 deg HDT bed rest.



8

Myasthenia gravis, an autoimmune neurodegenerative disease, as an experimental model to investigate the effects of microgravity in skeletal muscle - *"NEMUCO" 1g ground based analyses*

Martin Schubert[1], Gabor Trautmann[1], Katharina Block[2], Andreas Pelz[3], Siegfried Kohler[3], Andreas Meisel[3], Dieter Blottner[1,2], Michele Salanova[1,2]

[1] Charité University Medicine Berlin, Institute of Integrative Neuroanatomy, Neuromuscular Signaling, Berlin, Germany
[2] Center of Space Medicine Berlin; Berlin, Germany
[3] Charité University Medicine Berlin, Department of Neurology with Experimental Neurology, Berlin, Germany

Introduction: Disturbance of neuromuscular transmission, denervation and synaptic remodeling is an important issue during aging and long-term spaceflights. Recently, emerging evidence suggest that exposure to microgravity results in an altered performance of the immune system, which is responsible for a progression of immunological conditions such as allergy and autoimmunity. To further investigate skeletal muscle postsynaptic molecular adaptations of autoimmune neurodegenerative diseases, here we explored Homer protein expression and NMJ subcellular localization in hind limb muscles of a mouse experimental model of myasthenia gravis (EAMG). This autoimmune neurodegenerative disease is characterised by the presence of circulating autoantibodies against NMJ nicotinic acetylcholine receptors (nAChRs), a key molecular player of the NMJ postsynaptic signal transduction machinery.

Methods: Mice were immunized at the Department of Experimental Neurology of Charité by subcutaneous injection of Torpedo AChR-antigens in presence of adjuvants compound (EAMG) for three times with four-week intervals; an age-matched cohort of mice was used as a control group (CTR). Clinical scoring of disease severity was done by grip-strength examination. Mice were sacrificed at two different time intervals (around 20 w and 68 w) after first immunization in order to study acute and chronic immunization effects. Hind limb soleus (SOL), extensor digitorum longus (EDL) and gastrocnemius (GAS) muscles were dissected and snap frozen in N2-liquid nitrogen for further examination. Muscle samples from right hind limb were dedicated for laser confocal experiments. Muscle cryosections were double stained with anti-Homer antibodies and α-bungarotoxin (α-BTX), a specific marker of nAChRs. nAChR-density analysis was performed at the NMJs to evaluate the pathogenic destruction of endplates caused by the autoantibodies. Muscles from left hind limb were dedicated for biochemical and gene transcription analysis. Myofiber-type cross-sectional area was performed to investigate atrophy.

Results: Homer fluorescence pixel intensity increased in NMJs postsynaptic microdomains of MG mice in both myofiber type-II dominant GAS and EDL muscles and, to a lesser extent, in type-I dominant SOL muscle. Through biochemical analysis, anti-Homer antibodies identified immunoreactive bands with a predicted Homer molecular weight in both type of muscles. Densitometry analysis showed that, there was a 125% increase in Homer protein expression in EDL, and a 45% decrease in SOL of EAMG mice suggesting only a Homer protein translocation from cytosol to sub-synaptic subcellular compartment rather than an increase in relative Homer protein expression in SOL. In contrast, nAChRs fluorescence pixel intensity decreased in endplates of EAMG mice, more distinct in type-I dominant SOL muscle. Morphometric myofiber cross-sectional area of EAMG vs CTR did not show any significant changes despite EAMG mice having clinical symptoms of increased muscle fatigue and general weakness. The RT-PCR analysis of "Homer protein isoform" and "nAChR subunits" expression pattern in both type of muscles of EAMG vs CTR mice will provide more detailed information regard the altered NMJ postsynaptic signalling pathways in our EAMG model.

Conclusions: The expression pattern of postsynaptic scaffold adapter proteins of the Homer family is upregulated in postsynaptic endplates of EAMG mice. Since all models of muscle-disuse atrophy (e.g. bed rest, denervation and hind limb unloading) highlight a significant decrease in Homer gene expression, the increased Homer signalling in postsynaptic endplates of our MG experimental model might be the result of a skeletal muscle adaptation to the impaired acetylcholine receptor signalling. Taken together these results provide evidences for a functional coupling between Homer and nAChR. If the described findings are confirmed in human MG patients, they might highlight the importance of Homer in muscle cell neurophysiology / NMJ transmission and propose it as a potential molecular biomarker for NMJ-postsynaptic dysfunction or even a potential target for countermeasure intervention.

9

Inaugural quantitative analysis of perivascular spaces in long-duration space flyers

Judita Huber[1,2], 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], and Floris L. Wuyts[3]*, Peter zu Eulenburg[1,14,15]*

[1] Ludwig-Maximilians-Universität München, Graduate School of Systemic Neurosciences , Germany [2] Ludwig-Maximilians-Universität München, Research Training Grant 2175, 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 [13] Department of Translational Neurosciences – ENT, University of Antwerp, Antwerp, Belgium [14] Institute for Neuroradiology, Faculty of Medicine, Ludwig-Maximilians-University Munich, Munich, Germany [15] German Center for Vertigo and Balance Disorders, Ludwig-Maximilians-University Munich, Munich, Germany

Introduction: Perivascular spaces (PVS) are the spaces surrounding blood vessels which function as flow pathways for cerebrospinal (CSF) and interstitial fluid (ISF). Enlarged PVS indicate impaired brain fluid clearance [1]. Since spending prolonged time in microgravity causes brain changes related to the perturbations cranial microcirculation due to the cephalic fluid shift [2, 3], we aimed to quantify PVS volume in cosmonauts before and after their respective missions after noticing PVS on the structural data.

Methods: Structural neuroimaging data from 12 male Russian cosmonauts was used in this study. All but three cosmonauts had previous spaceflight experience. Twelve age-, gender- and education- matched subjects were analysed as a control cohort. In a complementary analysis, we then added datapoints of four European astronauts and their respective controls. Identification and labelling of PVS was performed by using a very cautious and conservative T1-weighted approach within the open-source software package Slicer 4.11.0 [4]. We used a semi-automatic quantification procedure after image denoising and white matter masking applying the established geometric characteristics of PVS from the literature (elongation, roundedness, length).

Results: We found that that PVS volume of cosmonauts at all time points (preflight, postflight, follow- up) was greater on average than in controls but did not differ significantly due to a large variability ($t(12.16) = -1.25$, $p=0.24$). However, taken together with the data points from the four European astronauts and four of their respective controls, the trends that were observed in the cosmonauts solidified. PVS volume of the pooled data set pre-flight was significantly higher on average when compared to controls ($t(16.43)=2.50$, $p=0.02$). Furthermore, a small, but significant increase of PVS was found in the pooled data set shortly after return to earth, when compared to the pre-flight data ($t(24.88) =1.97$, $p=0.06$). An average 0.6% increase from the mean baseline PVS volume pre-flight was estimated for the merged data set.

Conclusions: A significant increase in total PVS volume in astronauts/cosmonauts on average, when compared to controls was found preflight with a further transient increase postflight which returned to baseline levels at follow-up after half a year back on earth. Generally, pre-flight PVS volume varied substantially across astronauts and cosmonauts. We hypothesise that individual factors during cosmonaut training or acquired tissue changes from previous missions may contribute to this variance. Another contributing factor aside from the established cerebrospinal fluid accumulation intracranially could be the individual sleep pattern of the spaceflyers. Previously a higher PVS load was found to be related to poor sleep quality [5, 6] and sleep disturbances were already reported in space crews [7].

References: **1**. Wardlaw et al., *Perivascular spaces in the brain: anatomy, physiology and pathology.* Nat Rev Neurol, 2020; **2**. Van Ombergen et al., *Brain ventricular volume changes induced by long-duration spaceflight.* Proc Natl Acad Sci U S A, 2019; **3**. Jillings et al., *Macro- and microstructural changes in cosmonauts' brains after long-duration spaceflight.* Science Advances, 2020; **4**. Fedorov et al., *3D Slicer as an image computing platform for the Quantitative Imaging Network.* Magnetic resonance imaging, 2012; **5**. Song et al., *Moderate-to-severe obstructive sleep apnea is associated with cerebral small vessel disease.* Sleep Med, 2017; **6**. Del Brutto et al., *Enlarged basal ganglia perivascular spaces and sleep parameters. A population-based study.* Clinical neurology and neurosurgery, 2019; **7**. Barger et al., *Prevalence of sleep deficiency and use of hypnotic drugs in astronauts before, during, and after spaceflight: an observational study.* The Lancet Neurology, 2014

10

Early neuromuscular and contractile maladaptations to short-term bed rest

Elena Monti[1], *Martino V. Franchi*[1], *Luana Toniolo*[1], *Fabio Sarto*[1], *Giuseppe Sirago*[1], *Lorenzo Marcucci*[1], *Leonardo Nogara*[1], *Emiliana Giacomello*[2], *Sandra Zampieri*[1,3], *Boštjan Šimunic*[4], *Rado Pišot*[4], *Carlo Reggiani*[1,4], *Marco V. Narici*[1,4]

[1] *Biomedical Sciences, University of Padova*
[2] *Department of Medicine, Surgery and Health Sciences, University of Trieste*
[3] *Department of Surgery, Oncology, and Gastroenterology, University of Padova*
[4] *Institute for Kinesiology Research, Science and Research Centre Koper*

Introduction: Inactivity and unloading induce rapid skeletal muscle atrophy and functional decline, detectable after 2-3 days (1). Importantly, muscle force is lost to a much greater extent than muscle mass (2). We aimed to investigate the early determinants of the disproportionate loss of muscle force with respect to the decrease in muscle size in response to unloading caused by short-term bed rest.

Methods: 10 male volunteers (22.9±4.7 years; 181.2±3.9 cm; 77.5±10.0 kg) underwent 10 days of bed rest (BR). At baseline (BR0) and at the end (BR10), quadriceps femoris (QF) volume and knee- extensors force (MVT) were obtained by MRI and dynamometry, respectively. At BR0, BR5 (mid-BR) and BR10 blood samples and a biopsy from the vastus lateralis (VL) were collected. Damage to the neuromuscular junction (NMJ) was assessed from serum levels of C-terminal Agrin Fragment (CAF) and presence of denervated fibers was determined by positivity for neural cell-adhesion molecule (NCAM) staining. Mechanical properties of single fibers were also assessed. The study was approved by the local Ethics committee.

Results: From BR0 to BR10, QF volume and MVT respectively decreased by 5.2% ($p<.01$) and 14.3% ($p<.01$). A significant increase of NCAM positive fibers was observed with respect to baseline:
5.5 (n.s.) fold at BR5 and 26.1 fold at BR10 ($p<.01$), accompanied by a 10.6% increase in serum CAF levels at BR5 (n.s) and 19.2% at BR10 ($p<.05$), reflecting increased instability of the NMJ. In addition, fast fiber CSA decreased by 15% ($P=.055$) at BR10, while single fibers maximal tension (force/CSA) was unmodified. However, the handling of Ca^{2+} by the sarcoplasmic reticulum was altered as calcium release induced by caffeine exposure decreased at BR10 by 35.1% ($P<.01$) and 30.2% ($P<.001$) in fast and slow fibers, respectively. Myofiber functionality expressed as pCa50 (Ca^{2+} concentration at which 50% of the maximum force is produced) did not change.

Conclusions: These findings show that early onset of NMJ damage, denervation and impairment in calcium dynamics are major determinants of the greater loss of muscle force than of muscle size accompanying disuse. The identification of such mechanisms warrant the implementation of early countermeasures against the maladaptive changes of the neuromuscular system to unloading.

Funding by *ASI, MARS-PRE Project, n. DC-VUM-2017-006*, is acknowledged.

References

1. R. Demangel, *et al.*, Early structural and functional signature of 3-day human skeletal muscle disuse using the dry immersion model. *J. Physiol.* **595**, 4301–4315 (2017).
2. M. D. de Boer, C. N. Maganaris, O. R. Seynnes, M. J. Rennie, M. V. Narici, Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. *J. Physiol.* **583**, 1079–1091 (2007).

11

Work rate decrease during exercise at a fixed submaximal heart rate: a new method to evaluate exercise (in)tolerance in actual and simulated microgravity?

Giovanni Baldassarre[1], Lucrezia Zuccarelli[1], Giorgio Manferdelli[2], Valentina Manfredini[1], Letizia Rasica[3], Andrea Pilotto[1-3], Mauro Marzorati[3], Simone Porcelli[3-4], Boštjan Šimunic[5], Rado Pišot[5], Marco Narici[6], Bruno Grassi[1]

[1] Department of Medicine, University of Udine, Udine, Italy
[2] Faculty of Biology and Medicine, Institute of Sport Sciences, University of Lausanne, Lausanne, Switzerland
[3] Institute of Biomedical Technologies, National Research Council, Segrate, Italy
[4] Department of Molecular Medicine, University of Pavia, Italy
[5] Institute for Kinesiology Research, Science and Research Center, Koper, Slovenia
[6] Department of Biomedical Sciences, University of Padova, Italy

Introduction: Aerobic exercise prescription, in terms of intensity, is usually set at specific heart rate (HR) values. However, it has been recently demonstrated that during exercise carried out at a HR slightly above that corresponding to the gas exchange threshold (GET), the work rate ($\dot{w}$) has to decrease in order to maintain HR constant. It is well documented that simulated microgravity (bed rest, BR) determines a significant impairment of skeletal muscle oxidative metabolism and exercise tolerance; this led us to hypothesize that the $\dot{w}$ decrease at a fixed HR could be significantly aggravated by BR. If confirmed, this hypothesis would significantly affect exercise tolerance evaluation and exercise prescription in microgravity.

Methods: Ten young and healthy men (age, 23±5 yr) participated in this study. Subjects were tested before (PRE) and after (POST) a 10-day horizontal BR and performed on a cycle ergometer: a) an incremental exercise up to voluntary exhaustion (INCR); b) a 15-min ''HRCLAMPED'' exercise, in which $\dot{w}$ was continuously adjusted to maintain a constant HR, slightly higher than that determined at GET in PRE. Pulmonary O_2 uptake ($\dot{V}O_2$) was assessed breath-by-breath by a metabolic cart. HR was recorded by chest band, and stroke volume (SV) and cardiac output (CO) were estimated by impedance cardiography. Fractional O_2 extraction changes (Δ[deoxy(Hb+Mb)]) in muscles of the anterior and posterior compartments of the thigh were evaluated by near-infrared spectroscopy (NIRS).

Results: During INCR, $\dot{w}_{peak}$ (230±41 *vs.* 251±50 W, P=0.02) and $\dot{V}O_{2peak}$ (41.5±6.5 vs. 44.5±7.5 ml·kg^{-1}·min^{-1}, P=0.001) were significantly lower in POST *vs.* PRE, whereas HR_{peak} remained unchanged (189±6 *vs.* 187±8 b·min^{-1}). Δ[deoxy(Hb+Mb)]$_{peak}$ significantly decreased (p<0.05) in POST compared to PRE. $\dot{w}$ and HR at GET were not different in POST (113±33 W and 145±9 b·min^{-1}, respectively) *vs.* PRE (118±33 W and 136±14 b·min^{-1}). During $HR_{CLAMPED}$ (set at 145±11 b·min^{-1}), the decrease in $\dot{w}$ needed to maintain a constant HR was more pronounced in POST *vs.* PRE (-39±10 vs. -29±14%, P<0.001) and it was associated with decreases (both in POST and in PRE) of $\dot{V}O_2$ (-14 and -13%) and Δ[deoxy(Hb+Mb)] (- 41 and -18% in quadriceps femoris muscles; -75 and 70% in hamstring muscles), whereas SV and CO did not change.

Conclusions: During 15-min cycling initially set at a $\dot{w}$ corresponding to a HR slightly above GET (as frequently done for exercise prescription), in order to keep HR constant $\dot{w}$ had to decrease by ~29% and ~39%, respectively, before and after a 10-day bed rest. The $\dot{w}$ decrease, whose cause(s) still need to be determined, is a sign of reduced exercise tolerance, and was more pronounced compared to that needed to prevent "slow components" of $\dot{V}O_2$ and muscle fractional O_2 extraction kinetics. Exercise prescription at fixed submaximal HR is associated with a marked decrease in work rate; the problem is likely to be more pronounced in microgravity.

Ankle joint immobilization using an unloading orthosis induces a muscle atrophy pattern that is similar to head down-tilt bedrest

Luis Hinterwaldner[1,2,3], *Tobias Weber*[1,3,4], *David A. Green*[3,4], *Michel Ducos*[1], *Jochen Zange*[1], *Matthias Siegert*[1], *Rainer Burgkart*[2], *Jörn Rittweger*[1,5]

[1] German Aerospace Center (DLR), Institute of Aerospace Medicine, Köln, Germany
[2] Technical University of Munich, University Hospital rechts der Isar, Department of Orthopedics and Sports Orthopedics, München, Germany
[3] European Astronaut Centre, Space Medicine Team (HRE-OM), European Space Agency (ESA), Cologne, Germany
[4] KBR GmbH, Cologne, Germany
[5] Department of Pediatrics and Adolescent Medicine, University of Cologne, Cologne, Germany

Introduction: Latest data on inactivity-induced atrophy patterns in humans is mainly generated via spaceflight and it's on earth pendant: -6° head down tilt (HDT) bedrest. Originally invented for the discrimination of bone-modelling stimulators, Hephaistos, a newly developed unloading device, prevents the muscle activity of the lower limb muscles. The orthosis enables normal gait but reduces plantar flexor torque and muscle activity while walking to about 35 percent at the same time. This can be ensured by imitating the Achilles tendon function with an elastic front part of the sole which stores and releases kinetic energy during the stance phase of the gait. As a cost-effective and individually less-restrictive alternative to bedrest, Hephaistos might be considered for upcoming research on muscle atrophy. Hence, aim of this study was the investigation of atrophy rates of muscle groups of the lower leg, either following an intervention with the unloading orthosis or after bedrest. Both methods were then compared on the basis of the induced muscle atrophy patterns.

Methods: The present work compares data from two sub-studies within unrelated large projects. Two groups were created; 1) Hephaistos group: participants wearing the unloading orthosis during all locomotive activities for 56 days (N=11); 2) bedrest group: 60 days -6° HDT bedrest without countermeasures as part of the artificial gravity bedrest study AGBRESA (N=8). For both studies MRI of the calf of each study subject was performed before and after intervention. Muscle CSAs of six muscle groups of the lower leg, predefined according to anatomical or functional synergisms, were manually measured for each layer. The muscle groups were gastrocnemius medialis (GMED), gastrocnemius lateralis (GLAT), soleus (SOL), peroneus group (PER), tibialis posterior group (TIBP), anterior tibial group (ANT). After summing up the products of CSA with the slice thickness and normalizing the post intervention data to its baseline volume muscle wasting rates were assessed. To minimize confounding factors, the atrophy rates of the individual muscle groups were always considered in relation to the atrophy rate of tibialis anterior group (ANT). Statistical analysis was performed in a nested data design.

Results: There was a significant loss of total muscle mass of the lower leg in both groups (P=0,002), which was comparable between bed rest and Hephaistos (P=0.46). The predefined muscle groups were affected to varying degrees (P=0,007) which, again, was comparable between interventions (P=0.95). When comparing the differential atrophy across muscle groups, it was found that atrophy rates of SOL (-8.5%, SD 5.9%) and of GM (-6.9%, SD 8.6%) were greater than in ANT (-1.3%, SD 4.9%, both P<0.01), whilst the other muscle groups were comparable to ANT (all P>0.10).

Discussion: These data show differential atrophy for the lower leg. Greater changes of muscle mass for the plantar flexor groups SOL and GM compared to ANT group and similar atrophy rates of the other muscle groups of the lower leg compared to ANT group were detected in both intervention groups. Hence, the atrophy pattern triggered by Hephaistos ankle-joint immobilization can be described as similar to those of the bedrest campaign. The results of relative change of muscle mass in both studies suggests that the Hephaistos orthosis may be considered as a future alternative for the investigation of inactivity induced muscle atrophy in the lower leg.

13

Importance of exercise during short-term isolation

Timo Klein[1,2], Jan Weber[1,3], Tina Foitschik[1], Leonard Braunsmann[1], Stefan Schneider[1,2,4], Vera Abeln[1]

[1] German Sport University Cologne, Institute of Movement and Neurosciences, Germany
[2] University of the Sunshine Coast, VasoActive Research Group, School of Health and Sport Sciences, Australia
[3] University of Tuebingen, Graduate Training Center of Neuroscience, Germany
[4] Memorial University Newfoundland, Offshore Safety and Survival Centre, Marine Institute, Canada

Introduction: Isolation is known to be stressful for the human organism and negatively effects sleep and mood of crewmembers and might also negatively affects the brain in its structure and function. Physical exercise is known for its positive effects on the brain. We assumed that physical exercise during isolation might positively influence sleep quality and affect CNS function and structural markers, brain cortical activation and neurotropic factors. The aim was to investigate the effect of exercise during isolation and the underlying factors to preserve neuro-psychological function.

Methods: 32 participants were included and tested at the Human Exploration Research Analogue (HERA) at NASA Johnson Space Centre in Houston Texas. Participants of campaign 3 were isolated for 30 days and exercised on a daily basis (ISO_{100}). Participants of campaign 4 were isolated for 45 days and exercised every second day (ISO_{50}). Additionally, 26 participants were included and separated into two non-isolated control groups, an exercising (CON_{Ex}) and a non-exercising control group (CON_{NonEx}) and tested at the German Sport University Cologne. Participants of the CON_{NonEx} group exercised at 3 times a week for the past 2 years and were not allowed to perform any type of exercise for 30 days. At the start (T1) and the end (T2) of the isolation/non-isolation periods morning cortisol, melatonin, BDNF and IGF-1 were assessed. Sleep was assessed with actigraphy (Phillips, USA) and participants completed self-assessment questionnaire for sleep and awakening quality (SSA). Positive Affect and Negative Affect Scale (PANAS-X) was assessed. Resting state brain activity was assessed by a five-minutes resting electroencephalography in a relaxed sitting position with eyes-closed (Brain Products, Munich) and cognitive function with the NASA standard cognitive function assessment for astronauts (Joggle). Effects of group (ISO_{100} vs ISO_{50} vs CON_{Ex} vs CON_{NonEx}) and time (T1 and T2) were determined using repeated measures ANOVA. Cardiorespiratory fitness of CON_{NonEx} was tested with an independent t-test.

Results: Cardiorespiratory fitness of the CON_{NonEx} group reduced from T1 to T2 ($p< .05$). Cortisol was highest in ISO_{100} and ISO_{50} ($p< .05$) and remained unchanged over time ($p= .84$). BDNF in ISO_{50} was higher than CON_{NonEx} ($p< .05$) and reduced across all groups over time ($p< .05$). Melatonin was higher in CON_{NonEx} than CON_{Ex} ($p< .05$) and showed a reduction from T1 to T2 in only the CON_{NonEx} group ($p< .05$). IGF-1 was not different between groups ($p= .16$). Self-reported sleep and awake quality were higher in ISO_{100} and CON_{Ex} than ISO_{50} and CON_{NonEx} ($p< .001$) respectively. Sleep efficiency was lower in CON_{NonEx} ($p< .05$) compared to the other groups and reduced from T1 to T2 ($p< .05$), whereas it increased in ISO_{50} from T1 to T2 ($p< .05$). General negative affect scale was lower in CON_{Ex} ($p< .05$) compared to other groups and remained unchanged over time in all groups. General positive affect was lowest in CON_{NonEx} ($p< .05$) and reduced from T1 to T2 ($p< .05$). Psychomotor vigiliance, a sensitive measure of vigilant attention circadian misalignment reduced from T1 to T2 in CON_{NonEx} ($p< .01$) only. No group differences were observed in EEG measures.

Conclusions: Although high levels of cortisol were observed for the isolated groups, this did not negatively affect the brain, cognitive function, sleep and mood. Our data supports the assumption that a physical exercise routine is necessary, which might positively affected sleep, CNS function and structural markers, as brain cortical activation and neurotropic factors were not impaired in both the isolated and non-isolated exercise groups. Impairments of sleep quality, mood, cognitive function and neurotropic factors are already observed after a short period of time with the absence of physical activity. Consequently, regular exercise routines, with the most promising results at every second day, should be promoted for future missions but also on earth e.g. during pandemic lockdowns to prevent sleep, mood and cognitive impairments.

14

Artificial gravity does not mitigate deconditioning of the gluteal muscles after prolonged head-down tilt bed rest

Vienna Tran[1], Enrico De Martino[2], Julie Hides[3], James Elliott[4,5], Jonathan Scott[6,7], Tobias Weber[6,7], Gordon Cable[1], Nick Caplan[2]

[1] Adelaide Medical School, The University of Adelaide, Adelaide, Australia
[2] Aerospace Medicine and Rehabilitation Laboratory, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK
[3] School of Allied Health Sciences, Griffith University, Nathan Campus, Brisbane, Australia
[4] Northwestern University Feinberg School of Medicine Department of Physical Therapy and Human Movement Sciences, Chicago, Illinois, USA
[5] Northern Sydney Local Health District & The University of Sydney, Faculty of Medicine and Health, The Kolling Research Institute Sydney, Australia
[6] European Astronaut Centre, Space Medicine Team (HRE-OM), European Space Agency (ESA), Cologne, Germany
[7] KBR GmbH, Cologne, Germany

Introduction: The gluteal muscles (maximus, medius, minimus) are important antigravity muscles responsible for hip extension, abduction, and stabilization of the hip and pelvis in upright standing. Exposure to microgravity or body unloading in the axial direction induces rapid adaptations in the antigravity gluteal muscles, such as muscle atrophy and increased relative intramuscular lipid concentration (ILC). This process impairs upright function and may increase the risk of falls and injury. Artificial gravity (AG) has been shown to partially mitigate some cardiovascular, musculoskeletal, and neurovestibular adaptations induced by 6° head-down tilt (HDT). However, the effect of AG on the gluteal muscle morphology is still unknown. This study investigated whether AG mitigates deconditioning in the gluteal muscles following 60-day strict HDT bed rest. It was hypothesized that exposure to daily AG would sufficiently stimulate the gluteal muscles by creating a head-to-feet force of 1G along the body axis while in a supine position, resulting in the mitigation of gluteal muscle deconditioning.

Methods: Twenty-four healthy participants participated in the study: Eight received 30 minutes continuous AG; eight received 6x5 minutes AG, with rests in between; and eight belonged to a control group with no AG exposure. Magnetic resonance imaging (MRI) of the hip region was conducted at baseline data collection (BDC) and at the end of HDT on Day 59 (HDT59). T1-weighted images were used to assess the morphology of the gluteus maximus, medius, and minimus muscles. A chemical shift-based 2-point fat/water Dixon sequence was used to measure ILC. The gluteal muscles were manually segmented on a semi-automated Matlab-based program, and the volume and ILCs were automatically calculated. Mixed model ANOVAs were used to compare each outcome measure between Groups (CRTL vs. cAG vs. iAG) and Time (BDC vs. HDT59), and Group*Time interaction.

Results: Results showed that, in all groups, muscle volumes decreased by 9.5% for gluteus maximus, 9% for gluteus medius, and 12.5% for glutes minimus after HDT59 (all $P<0.001$). The ILC increased by 1.3% for gluteus maximus and 0.5% for gluteus medius after HDT59 (both $P<0.05$). The ANOVA revealed no Group*Time interaction effects.

Conclusions: Neither of the AG protocols used in the study mitigated gluteal muscle deconditioning following 60-day HDT bed rest. The increase in ILC in the gluteus maximus and medius may indicate an increased relative ILC or substantial fluid shifts from the intramuscular space. Altered muscle composition in atrophied muscles may impair gluteal muscle function after axial unloading, which may result in an increased risk of falls and injury. However, this relationship needs further investigation. Further studies are needed to investigate whether other AG protocols, including longer exposures or the inclusion of exercise, can mitigate gluteal muscle deconditioning following axial unloading.

15

Intermittent short-arm centrifugation is a partially effective countermeasure against upright balance deterioration following 60-day strict head-down tilt bed rest

Enrico De Martino[1], Sauro Salomoni[2], Paul Hodges[2], Julie Hides[3], Kirsty Lindsay[1], Dorothée Debuse[1], Andrew Winnard[1], James Elliott[4,5], Mark Hoggarth[4,6], David Beard[7], Jonathan A. Cook[7,8], Robert Ekman[9], Luis Hinterwaldner[9], Jonathan Scott[9,10], Tobias Weber[9,10], Nick Caplan[1]

[1] Aerospace Medicine and Rehabilitation Laboratory, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK
[2] The University of Queensland, NHMRC Centre for Clinical Research Excellence in Spinal Pain, Injury and HealthBrisbane, Australia
[3] School of Allied Health Sciences, Griffith University, Nathan Campus, Brisbane, Australia
[4] Northwestern University Feinberg School of Medicine Department of Physical Therapy and Human Movement Sciences, Chicago, Illinois, USA
[5] Northern Sydney Local Health District & The University of Sydney, Faculty of Medicine and Health, The Kolling Research Institute Sydney, Australia
[6] Department of Biomedical Engineering, McCormick School of Engineering, Northwestern University, Evanston, Illinois, USA
[7] NIHR Oxford Biomedical Research Centre, Nuffield Department of Orthopaedics, University of Oxford, Oxford, UK
[8] Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[9] European Astronaut Centre, Space Medicine Team (HRE-OM), European Space Agency (ESA), Cologne, Germany
[10] KBR GmbH, Cologne, Germany

Introduction: Standing balance is intimately related to control of the trunk muscles. Maintenance of standing balance and coordination of lumbopelvic muscles depend on receiving and integrating accurate information from multiple somatosensory receptors. As a result, prolonged sensory input deprivation induced by exposure to microgravity has been implicated in impaired standing balance and altered anticipatory postural adjustments (APAs) of paraspinal muscles after space missions. On Earth, artificial gravity (AG) has been shown to partially mitigate some cardiovascular, musculoskeletal, and neurovestibular adaptations induced by head-down tilt (HDT) bed rest by creating a head-to-feet force along the body axis while in a supine position. This study investigated whether AG mitigated deterioration in standing balance and anticipatory postural adjustments (APAs) of trunk muscles following 60-day strict HDT bed rest.

Methods: Twenty-four participants were allocated to one of three groups; 1) control group (N=8); 2) 30 minutes continuous AG daily (N=8); 3) intermittent 6x5 minutes AG daily (N=8). Before (BDC) and immediately after bed rest (R0), standing balance was assessed in four conditions: eyes open and closed on a stable surface, and eyes open and closed on foam. Measures including sway path, root- mean-square, and peak sway velocity, sway area, sway frequency power, and sway density curve were extracted from the center of pressure displacement. APAs were assessed during rapid arm movements using intramuscular or surface electrodes to record electromyography (EMG) from rectus abdominis, obliquus external and internal abdominis, transversus abdominis, erector spinae at L1, L2, L3, and L4 vertebral levels, and deep lumbar multifidus muscles. The relative latency between the EMG onset of the deltoid and each of the trunk muscles was calculated. Mixed model ANOVAs were used to compare each outcome measure between Groups (CRTL vs. cAG vs. iAG) and Time (BDC vs. R0), and Group*Time interaction.

Results: All groups had poorer balance performance in most of the parameters ($P<0.001$) and delayed APAs of the lumbar and abdominal muscles compared with measures made before bed rest ($P<0.05$). However, deterioration in anteroposterior sway path, sway velocity, and sway frequency power was between 50 and 150% less in those exposed to intermittent AG than the control group at R0 ($P<0.05$), particularly in measurement tasks that involved reduced sensory feedback. Although vertical gravitational unloading also induced delay in relative latency of several trunk muscles in association with rapid arm movement, there was no impact of AG on the preservation of this parameter.

Conclusions: These data show compromised standing balance and trunk muscle APAs after exposure to vertical gravitational unloading and that the effects on balance, but not APAs, can be partially alleviated by daily exposure to iAG. Although these results indicate that iAG can partially mitigate the increased anteroposterior sways during standing balance, these effects do not appear to be related to improved control/coordination of the trunk muscles. AG may be considered as a potential countermeasure for mitigating balance instability in future planetary surface explorations. However, refinement of AG or supplementation with other interventions should be investigated to determine whether additional mitigation can be achieved.

16

Resting energy expenditure (REE) of master athletes: Accuracy of predictive equations and primary determinants

Sara Henkel[1], Petra Frings-Meuthen[1], Michael Boschmann[2], Philip D. Chilibeck[3], José Ramón Alvero Cruz[4], Fabian Hoffmann[1], Stefan Möstl[1], Uwe Mittag[1], Edwin Mulder[1], Natia Rittweger[1], Wolfram Sies[1], Hirofumi Tanaka[5], Jörn Rittweger[1]

[1] German Aerospace Center (DLR), Cologne, Germany
[2] Charité Universitätsmedizin Berlin, Experimental & Clinical Research Center, Germany
[3] College of Kinesiology,University of Saskatchewan, Saskatoon SK, Canada
[4] Universidad de Málaga, Instituto de Investigación Biomédica de Málaga, Facultad de Medicina, Málaga, Spain
[5] Department of Kinesiology and Health Education, The University of Texas at Austin, Austin, Texas, USA

Introduction: Resting energy expenditure (REE) is determined mainly by fat-free mass (FFM). FFM depends also on daily physical/exercise activity (EA). REE normally decreases with increased age due to decreases in FFM and EA. However, an increasing number of people, mostly older than 35 years, still participates in competitive sports - the so-called master athletes. Hence, these athletes can actively counteract age-related decreases in FFM given a sufficient energy supply. Measuring REE is essential for estimating total energy expenditure, but requires great effort. Therefore, various equations are used to predict REE. However, it is unclear, if these equations, developed for the general population, are also valid for highly active, older master athletes. Therefore, we tested the validity of four common equations as well as one developed for male athletes for predicting REE on older master athletes and also, which factors influence mostly REE in this specific group.

Methods: Within the Master-Athletics-Field-Study (MAFS), Malaga, Spain 2018, we measured REE in 113 test subjects (age: 56±12 years; height: 170±7 cm; weight: 68±10 kg) by indirect calorimetry. The most commonly used equations to predict REE (Harris & Benedict (HB), WHO/FAO, Müller (MÜL), Cunnigham (CUN) and De Lorenzo (LOR)) were tested for their accuracy in a group of older competitive athletes using a Bland-Altman analysis. The influence of age, height, sex, weight, FFM/weight, training hours, phase angle and type of exercise activity on REE of these athletes was determined by a multiple regression model.

Results: All calculated REEs, predicted from equations for the general population, differed significantly from the measured ones (HB, $p<0.001$; WHO, $p=0.001$; MÜL, $p=0.004$; CUN, $p=0.003$). Calculated REE, predicted by De Lorenzo's' equations, showed no significant difference from measured REE ($p=0,750$). The results showed that REE of master athletes is most accurately predicted by using the De Lorenzo equation for athletes. Regarding the other equations, REE of master athletes is most accurately predicted by the Cunningham formula (CUN) whereas all other commonly used formulas underestimated REE of these athletes. "Weight" ($p<0.001$) and "FFM/weight" ($p=0.032$) contributed mainly for predicting REE of both male and female master athletes.

Conclusions: We concluded that due to the higher FFM of these athletes vs. sedentary peers of same age and sex, REE in master athletes remains at rather higher levels independent of age. Our study provides a first estimate of energy requirements for master's athletes in order to cover adequately athletes' energy and nutrient requirements to maintain their health status and physical performance.

17

Evaluation of ultrasound data from the MARES Sinusoidal Perturbation Protocol for the analysis of vibration-induced changes in fascicle length and pennation angle as a function of vibration frequency and muscular preload

Nikolas Thier[1], *Uwe Mittag*[2], *Jule Heieis*[2,4], *Wolfram Sies*[2], *Kirsten Albracht*[5,6], *Benjamin Stäudle*[5], *Kirsten Peters*[2,7], *Jörn Rittweger*[2,3]

[1] Ostbayerische Technische Hochschule, Department of Biomedical Engineering, Regensburg, Germany
[2] German Aerospace Center (DLR), Institute of Aerospace Medicine, Köln, Germany
[3] Department of Pediatrics and Adolescent Medicine, University of Cologne, Cologne, Germany
[4] Fachhochschule Aachen, University of applied sciences, Aachen, Germany
[5] Faculty of Medical Engineering and Technomathematics, University of Applied Sciences Aachen, Aachen, Germany
[6] Institute of Movement and Neurosciences, German Sport University Cologne, Cologne, Germany
[7] Radboud University Medical Center, Nijmegen, Netherlands

Introduction: Vibration exposure, mostly applied as whole-body vibration, has been used more and more frequently in recent years in sports, health, research, preventive medicine and rehabilitation. It has been proven to be a useful training method and examination method in a variety of contexts. In the body, periodic stretching and shortening cycles of the muscle-tendon complex occur during vibration. This is accompanied by periodic changes in fascicle length (FL) and pennation angle (PEN). However, little is known about the extent to which stretching and shortening depends on muscular preload and frequency. Due to visco-elastic properties of the muscle, both factors should influence the stress-strain behavior in a speed-dependent manner. Therefore, the main aim of this study was to get an insight into vibration induced changes in FL and PEN at different frequencies and levels of preload. In addition, the influence of long-term bed rest on FL and PEN excursion is evaluated as well as the changes in static muscle architecture at different levels of contraction.

Methods: 23 subjects performed the sinusoidal perturbation protocol before and after 60 days of bed rest (artificial gravity bed rest study AGBRESA). Subjects were grouped into three countermeasure groups: iAG group (6 x 5 min centrifuge drive per day), cAG group (1 x 30 min centrifuge drive per day) and control group. Ultrasound videos of gastrocnemius medialis were recorded during vibration exposure at 10 different frequencies (4 -16 Hz) and 4 levels of contraction (isometric plantar flexion at 0, 25, 50 and 75% MVC). Muscle architecture and excursion of FL and PEN during vibration exposure was evaluated using a semi-automated tracking software (UltraTrack) and custom-made R-scripts.
The total excursion values were determined by means of perievent histograms and referred as relative values to the initial FL or PEN value. Linear mixed effects models with all possible interaction terms were constructed and stepwise simplified. Significant effects observed in the ANOVA were followed up by a-priori defined treatment contrasts with 4 Hz, 0% MVC, control group and baseline data as references level of contrasts.

Results: Excursion for reference level of contrasts was 1.94 (0.82) % for FL and 1.30 (0.99) % for PEN. Excursion of FL and PEN was statistically significant smaller when muscle was contracted (FL: $P < 0.001$ for 25 and 50% MVC, $P < 0.01$ for 75% MVC; PEN: $P < 0.001$ for 25% MVC and $P < 0.01$ for 50% MVC). In the range from 7 to 10 Hz there was a significant increase in excursion for pre- tensioned muscle, especially for FL. After bed rest, excursion was significantly greater at 9 and 10 Hz vibration ($P < 0.05$) for FL and at 8 Hz, 10 Hz ($P < 0.01$) and 9 Hz ($P < 0.05$) for PEN, respectively. Considering static muscle architecture, mean initial FL was 42.1 (5.6) mm, mean initial PEN was 24.3 (2.6) ° and mean initial muscle thickness (MT) was 17.2 (2.3) mm. There was a statistically significant decrease in FL ($P < 0.001$ for all contraction levels) and an increase in PEN ($P < 0.05$ for 25 % MVC, $P < 0.001$ for 50 and 75% MVC) by increasing contraction level. Minor changes occurred in MT. Compared to baseline data, overall values increased for FL (+1.09 ± 0.56 mm, $P < 0.001$) and decreased for PEN (-2.88 ± 0.7°, $P < 0.001$) and MT (-1.03 ± 0.22 mm, $P < 0.001$) after bed rest at all levels of contraction.

Conclusions: Fascicle excursion decreases as the muscle contracts. However, no further correlation between the level of contraction and the decrease in excursion could be found. The main finding of this study was a significant resonance effect at 7 – 10 Hz vibration in contracted muscle. Resonance was even more evident after bed rest. There was no alteration of resonance frequencies across the levels of contraction. One explanation for this resonance effect would be an equivalent increase in damping properties and stiffness in the muscle as muscle activity elevates. It is furthermore conceivable that the increase in excursion at vibration frequencies in the range from 7 – 10 Hz is a consequence of resonance within the natural tremor frequency. It is possible that the induced vibration causes the physiological tremor to resonate. However, the exact cause of this resonance effect requires further investigation.

18

Skeletal muscle ultrastructural changes in myofibrillar morphology and nutrient storage after short- and long-term bed rest

Moritz Eggelbusch[1,2], Tom Kerkhoff[1], Anita Grootemaat[3], Ifigenia Giakoumaki[4], Bergita Ganse[4], Jörn Rittweger[5], Hans Degens[4] and Rob CI Wüst[1]

[1] Laboratory for Myology, Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Amsterdam Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
[2] Department of Nutrition and Dietetics, Amsterdam Movement Sciences, Amsterdam UMC, location VUmc, Amsterdam, The Netherlands and Faculty of Sports and Nutrition, Center of Expertise Urban Vitality, Amsterdam University of Applied Sciences, Amsterdam, The Netherlands
[3] Core Facility Cellular Imaging, Amsterdam UMC, location AMC, Amsterdam, the Netherlands
[4] Department of Life Sciences, Research Centre for Musculoskeletal Science & Sports Medicine, Manchester Metropolitan University, Manchester, UK
[5] Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany

Introduction: Physical inactivity causes skeletal muscle weakness and induces insulin resistance. The reduction in force generating capacity is attributable to a loss of contractile material, but likely also due to qualitative alterations in the remaining tissue. Long-term inactivity blunts skeletal muscle glucose signaling due to a misbalance in nutrient uptake and utilization rates, likely because of accumulation of glycogen and lipid droplets. Here we determined skeletal muscle morphological and metabolic adaptations during short- and long-term bed rest using electron microscopy.

Methods: In this preliminary work, four control participants (age range 29-46 years, 50% women) from the AGBRESA study (Cologne, Germany) were included. Biopsies were taken from the vastus lateralis muscle before, after 6 (short-), and 55 (long-term) days of bed rest. Z- disk width, myofibrillar structure, and the amount of glycogen and intramyocellular lipid deposits were measured in transmission electron microscopy images.

Results: Long-term bedrest significantly reduced Z-disk width by ~19% compared to baseline (from 0.85±0.12 to 0.69±0.06 µm, $p=0.027$). Myofibrillar structure significantly worsened after short-term bed rest, demonstrated by curved, disordered and torn myofibrils. This myofibrillar disarray was further deteriorated after long-term bed rest. Also, cytoplasmic bodies and autophagosomes were observed after long-term bed rest. Glycogen content and lipid droplets significantly increased after short-term and further accumulated after long-term bed rest.

Conclusions: Bed rest not only results in a loss of muscle mass, but also in progressive deterioration of skeletal muscle ultrastructure and nutrient overload. The myofibrillar atrophy and disarray likely contribute to a lower force generating capacity after bed rest, while the nutrient overload may contribute to impaired cellular glucose metabolism and mitochondrial dysfunction.

19

Cervical intervertebral disc expansion, vertebral compliance modulation and neck pain is induced by 4h of Hyper-Buoyancy Flotation and is only partially reversed by 15 mins re-exposure to gravity

Marcos Lorenzo, D[1], Fernadez Corujo, R[2], Hernandez Bernal, P[2], Gil Martinez, A[2,3,4], Green DA[5,6,7]

[1] PhD student, School of Medicine of Autonomous University of Madrid, Spain
[2] Departament of Physiotherapy, Centro de Estudios Superiores La Salle, Madrid, Spain
[3] Motion in Brains Research Grup, Instituto de Neurociencias y Ciencias del Movimiento.
[4] Unit of Physiotherapiy. Hospital Universitario La Paz, Institute for Health Research, IdIPAZ
[5] Space Medicine Office, European Astronaut Centre, European Space Agency, Cologne, Germany
[6] King's College London, Centre of Human and Applied Physiological Sciences, UK
[7] KBR, Science, Technology & Engineering Group, Cologne, Germany

Introduction: Exposure to microgravity (with periodic axial loading during exercise) is associated with stature increases of up to 7cm (Brown et al., 2003), moderate-to-severe back/neck pain (in 68%) of crew, and an increased risk of intervertebral disc (IVD) herniation including cervical (Johnston et al., 2010). Whilst relatively rapid changes in (Lumbar) IVDs are noted with orientation, unloading and reloading on Earth (Ledsome et al. 1996), very little is known about the pathophysiology of changes inflight, due to the challenges of assessment and the inadequacy of head down bed rest as a ground- based analogue for spinal responses to spaceflight (Green & Scott, 2019). Hyper-buoyancy floatation (HBF) has recently been shown to induce significant stature elongation lumbar IVD expansion and back pain more analogous to spaceflight (Green et al., 2015), but its effect on the cervical spine and the ability of re-exposure to gravity to ameliorate such effects is unknown.
Thus, the aim of the present study was to determine the effect of 4h HBF and subsequent passive 1g axial loading upon stature, cervical IVD height, cervical vertebral compliance, cervical muscle cross- sectional area and muscle thickness, in addition to neck pain.

Methods: 12 (5 male) healthy volunteers (age 30.2±7.2 yrs, 173.4±8.52 cm, 66.13±11.69 kg) provided informed written consent to participate in this study, which received local ethical approval. Participant stature was assessed pre, following 4 hours lay supine on the HBF, and then after 15 mins of upright sitting (SAT) without back support. IVD height was determined by ultrasound at C6-C7 (lower cervical) and C3-C4 (upper cervical), cervical vertebral compliance at the (C0-C7) spinous processes, *Longus Collis* and *Semispinalis Cervicis* cross-sectional area and thickness in addition to neck pain were assessed. All measures were normally distributed (Shapiro-Wilk test) thus PreHBF, PostHBF and PostSAT were compared with paired t-tests.

Results: 4h HBF induced 1.62±0.23 cm stature increments, that was reduced by 0.37±0.10 cm following SAT. Both lower ($t = -4.23$; $p < 0.01$) and upper ($t = -4.78$; $p < 0.01$) cervical IVD height was significantly increased Post HBF ($t = -4.23$; $p < 0.01$) vs. Pre HBF. Significant IVD height reductions were observed PostSAT vs. PostHBF in both upper (t=-2.7; p<0.05) and lower cervical column (t=-2,7; p <0.01). However, lower cervical IVD height remained significantly ($t = -3.44$; $p < 0.01$) greater than Pre HBF, whereas upper cervical IVD height no longer differed ($t = -1.52$; $p > 0.05$). Cervical vertebral compliance was significantly ($t = 4.8$; $p < 0.001$) reduced Post HBF vs. Pre HBF, whereas Post SAT compliance was significantly ($t = 4.009$; $p < 0.01$) increased vs. Post HBF. However, cervical compliance remained significantly (t=3,11; p<0.05) less than PreHBF. HBF, nor post-HBF SAT had any effect on *Longus Collis* and *Semispinalis Cervicis* muscle thickness or cross-sectional area. Significant (t= -6,3; p<0.001) neck pain was induced by HBF, that was reduced but still ($t = -7.40$; $p < 0.001$) greater than Pre HBF.

Conclusions: 4 hours HBF induced increased stature and cervical column IVDs height, both of which were reduced, but not entirely ameliorated by 15 min upright sitting. Cervical vertebral compliance reduced by HBF – indicative of spinal stiffness - but no changes in *Longus Collis* and *Semispinalis Cervicis* muscle thickness or cross-sectional area were following HBF, or Post SAT. In contrast cervical vertebral compliance was increased suggestive of changes in vertebral column stabilisation. HBF- induced mild neck pain was reduced but not ameliorated by upright sitting suggestive of persistent vulnerability upon landing – although associations with anatomical changes remain to be determined. Thus, longer duration HBF and re-exposure to gravity evaluation is warranted to evaluate potential spaceflight-related cervical spinal column pathophysiological risks.

20

Cardiovascular Sex-Differences in Master Athletes Determined by Echocardiography

Savannah V. Wooten[1], *Jens Tank*[2], *Jörn Rittweger*[2], *Hiro Tanaka*[1], *Fabian Hoffmann*[2,3]

[1] Department of Kinesiology and Health Education, The University of Texas at Austin, Austin, TX USA
[2] German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne, Germany
[3] Department of Cardiology, University Hospital Cologne, Germany

Background: Cardiac function and morphology are known to differ between men and women. There is scarce data which addresses the effect of chronic exercise over a multitude of decades. This may further exaggerate or attenuate sex differences seen with echocardiography in older athletes.

Purpose: To identify cardiac structure, function and left ventricular (LV) systolic global longitudinal strain as well as hemodynamic sex differences among masters athletes.

Methods: This cross-sectional study comprises of 164 masters athletes (M=109, 59.8±12.3 years; F=55, 56.8±12.1 years) who participated at the 23rd World Masters Athletics Championship, Malaga, Spain. All athletes underwent state-of-the-art echocardiography including cardiac function, morphology, strain and hemodynamic assessment. Standard views were recorded. Heart rate and blood pressure was captured using an oscillometric upper arm cuff.

Results: LV mass was higher in male versus female athletes (173.7±44.0 vs. 141.1±35.9 g, $p<0.01$) due to greater end-diastolic intraventricular septal, posterior wall and LV diameter. However, LV mass-index did not differ between groups. End-diastolic LV volume and right ventricular area, both indexed to body-surface-area, were greater in men (52.8±11.0 vs. 46.1±8.5 ml/m², $p<0.01$, 9.5±2.4 vs. 8.1±1.7 cm²/m², $p<0.01$). In contrast, women had higher LV systolic global longitudinal strain (-20.2±2.6 vs. -18.8± 2.6%, $p<0.01$) and LV-outflow tract flow velocity (75.1±11.1 vs. 71.2±11.1 cm/s, $p=0.04$). Systolic and diastolic blood pressure, LV ejection fraction, and stroke volume index were found to be similar in both groups.

Conclusion: While sex differences are still present among masters athletes, lifelong exercise does not seem to exasperate morphological difference to a point of cardiac risk or dysfunction in either sex.

Speakers

Aebi, Mathias: Aeromedical Center (AeMC), Swiss Air Force, Dübendorf, Switzerland

Baldassarre, Giovanni: Department of Medicine, University of Udine, Udine, Italy

De Gioannis, Riccardo: Division of Sleep and Human Factors Research, Institute of Aerospace Medicine German Aerospace Center (DLR), Cologne, Germany

De Martino, Enrico: Aerospace Medicine and Rehabilitation Laboratory, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK

Eggelbusch, Moritz: Laboratory for Myology, Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Amsterdam Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Henkel, Sara: German Aerospace Center (DLR), Cologne, Germany

Hinterwaldner, Luis: German Aerospace Center (DLR), Institute of Aerospace Medicine, Köln, Germany

Huber, Judita: Ludwig-Maximilians-Universität München, Graduate School of Systemic Neurosciences, Germany

Klein, Timo: German Sport University Cologne, Institute of Movement and Neurosciences, Germany

Köhlmoos, Anika: Effect of synbiotic intake on liver lipid metabolism and their potential impact on non-alcoholic fatty liver disease

Lindsay, Kirsty: Aerospace Medicine and Rehabilitation Laboratory, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK

Marcos, David: School of Medicine of Autonomous University of Madrid, Spain

Marume, Kyohei: Department of Sports Science, Division of Physiology, University of Innsbruck, Innsbruck, Austria

Monti, Elena: Biomedical Sciences, University of Padova

Schreiber, Kristina: Department of Anaesthesiology, University Hospital, LMU Munich, Munich, Germany

Schubert, Martin: Charité University Medicine Berlin, Institute of Integrative Neuroanatomy, Neuromuscular Signaling, Berlin, Germany

Thier, Nikolas: Ostbayerische Technische Hochschule, Department of Biomedical Engineering, Regensburg, Germany

Tran, Vienna: Adelaide Medical School, The University of Adelaide, Adelaide, Australia

Wooten, Savannah V.: Department of Kinesiology and Health Education, The University of Texas at Austin, Austin, TX USA

Zuccarelli, Lucrezia: University of Udine, Department of Medicine, Udine, Italy

If you have any questions please contact:

Organizers

Jörn Rittweger, Carina Arndt, Friederike Wütscher (German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne)
Katrin Stang, Michaela Girgenrath, Christian Rogon (German Aerospace Center (DLR), Space Administration, Microgravity Research and Life Sciences, Bonn)

→ humanphysiologie-ws@dlr.de