

Human Physiology Workshop

3rd December 2022

Hybrid event



Human Physiology Workshop

We are pleased to welcome you to the 7th German Human Physiology Workshop 2022. 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, Claudia Kaiser-Stolz, Alexandra Noll, Friederike Wütscher (German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne)

Katrin Stang, Michaela Girgenrath, Christian Rogon (German Space Agency at DLR, Bonn)

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Jury

Markus Braun	German Aerospace Center (DLR), Space Agency, Research and Exploration/Life Sciences, Bonn, Germany
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Nandu Goswami	Medical University of Graz, Institute of Physiology, Gravitational Physiology and Medicine, Graz, Austria
Bruno Grassi	Department of Medicine, University of Udine, Italy
Markus Gruber	University of Constance, Sensorimotor Performance Lab, Germany
Peter Hodgkinson	King's College London, Centre for Human & Applied Physiological Sciences, UK
Ylva Hellsten	University of Copenhagen, Department of Nutrition Exercise and Sports, Denmark
Jens Jordan	German Aerospace Center (DLR), Institute of Aerospace Medicine, Cologne, Germany
Tobias Kammerer	University Hospital Cologne, Germany
Justin Lawley	University of Innsbruck, Department of Sport Medicine, Austria
Grégoire Millet	Faculty of Biology and Medicine, University of Lausanne, Switzerland
Anja Niehoff	German Sport University, Institute of Biomechanics and Orthopaedics, Cologne, Germany
Jörn Rittweger	German Aerospace Center (DLR), Institute of Aerospace Medicine, Space Physiology, Cologne, Germany
Michele Salanova	Charité Medical University Center for Space Medicine and Extreme Environments, Institute of Physiology, Berlin, Germany
Stefan Sammito	Oberfeldarzt, Zentrum für Luft- und Raumfahrtmedizin der Luftwaffe, Cologne, Germany
Tobias Weber	ESA, Cologne, Germany
Rob Wüst	Vrije Universiteit Amsterdam, Faculty of Behavioural and Movement Sciences, the Netherlands

Program

Saturday, December 3, 2022

9:00 **Welcome** (Markus Braun/Jörn Rittweger, *German Aerospace Center (DLR)*)

Session 1:

Chairs: **Anja Niehoff** (*German Sport University Cologne*), **Markus Braun** (*German Aerospace Center (DLR)*)

09:15 **Renana Bruckstein** (*Charité University Berlin, Germany*)
COOLFLY – cooling as a countermeasure for cardiovascular instability

09:30 **Amir Fathi** (*King's College London, United Kingdom*)
Successful ageing: A longitudinal study on the cardiorespiratory function of master cyclists

09:45 **Thalia Zamora-Gómez** (*Vrije Universiteit Amsterdam, the Netherlands*)
Hindlimb suspension induces skeletal muscle-specific atrophy in mice

10:00 **Anna Hawliczek** (*Medical University of Graz, Austria*)
Investigating altered hemodynamic responses to stand test following sinusoidal vibration - varying frequency and bed tilt compilation

10:15 **Jule Heieis** (*German Aerospace Center (DLR), Germany*)
Curvature of gastrocnemius muscle fascicles as a function of MuscleTendonComplex length and contraction level in humans

10:30–10:45 Break

Session 2:

Chairs: **Rob Wüst** (*Vrije Universiteit Amsterdam*), **Edwin Mulder** (*German Aerospace Center (DLR)*)

10:45 **Carolin Möller** (*University Hospital Bonn, Germany*)
Metabolomics of young and old muscle: Impacts of aging and exercise on muscle metabolism

11:00 **Moritz Eggelbusch** (*Vrije Universiteit Amsterdam, the Netherlands*)
Estimating vastus lateralis muscle volume from a single anatomical cross-section

11:15 **Isabella Wiedmann** (*German Sport University, Cologne, Germany*)
The ESA Parastronaut feasibility project – Investigating need and contents of physical performance tests for parastronaut candidates

11:30 **Elie-Tino Godonou** (*Friedrich-Alexander-University Erlangen-Nuremberg & University Hospital Erlangen, Germany*)
Bone microarchitecture and volumetric bone mineral density after three campaigns of 21-days of 6° head-down-tilt bed rest (HDT-BR)

11:45 **Basak Su Tashan** (*Charité University Berlin, Germany*)
Spatial updating deficits in response to altered gravity conditions

12:00–12:45 Break

Session 3:

Chairs: **Peter zu Eulenburg** (*LMU Munich*), **Stefan Sammito** (*Zentrum für Luft- und Raumfahrtmedizin der Luftwaffe*)

- 12:45 **Anne Hermann** (*Institute for Theoretical Physics, Heinrich Heine-University, Düsseldorf, Germany*)
An in-silico study of fascicle curvature during isometric muscle contractions
- 13:00 **Leonard Brauns mann** (*German Sport University Cologne, Germany*)
Brain hemodynamics during cognitive processing is altered with 60-days of bedrest
- 13:15 **Jochen Zange** (*German Aerospace Center (DLR), Germany*)
The effects of elite sports at high age on volume and fat fraction of hip and leg muscles
- 13:30 **Cyril Tordeur** (*Université Libre de Bruxelles, Belgium*)
Effects of acute normobaric hypoxic exposure and capnia control on myocardial inotropism assessed by kinocardiography
- 13:45 **Maren Dreiner** (*German Sport University, Cologne, Germany*)
Five days of immobilization leads to changes in cartilage biomarker concentrations – first data from the Vivaldi study

14:00–14:45 Break

Session 4:

Chairs: **Jens Jordan** (*German Aerospace Center (DLR)*), **Justin Lawley** (*University of Innsbruck*)

- 14:45 **Ann Charlotte Ewald** (*German Aerospace Center (DLR), Germany*)
Resting energy expenditure in bed rest: Accuracy of predictive equations
- 15:00 **Fabian Möller** (*German Sport University, Cologne, Germany*)
The role of inter-subject variability for the effects of countermeasure exercise training on cardiorespiratory fitness in analog and spaceflight studies
- 15:15 **Lisanne van Osch** (*Vrije Universiteit Amsterdam, the Netherlands*)
Cellular cardiac alterations in size and mitochondrial density after hindlimb suspension in mice
- 15:30 **Vishwajeet Shankhwar** (*Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates*)
Gravitational load modulation bodygear for enhancing muscle activity: A novel countermeasure for maintenance of astronaut health
- 15:45 Invited Talk: Dr. Thomas Berger** (*Team Leader of the Biophysics-Group, Radiation Biology, DLR Institute of Aerospace Medicine*):
The journey of Helga and Zohar – to the Moon and (hopefully) back home – during the NASA Artemis 1 mission

17:00 Awards

17:15 Adjourn

Human Physiology Workshop

Abstracts

1

COOLFLY – cooling as a countermeasure for cardiovascular instability

Renana Bruckstein², Tomas L. Bothe^{1,2}, Michael Nordine^{2,3}, Magdalena Genov², Oliver Opatz²

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Introduction: Ensuring cardiovascular stability is critical for ensured success of human spaceflight. Astronauts and military pilots are exposed to highly dynamic acceleration profiles which leads to peripheral blood pooling, resulting in cardiovascular instability. Peripheral blood pooling provokes sudden decreases in blood pressure, which can lead to sudden loss of consciousness.

The scientific aim of this study was to utilize peripheral cooling to minimise peripheral blood pooling during parabolic flight. We hypothesized that the peripheral cooling would minimise peripheral blood pooling during hyper-gravity. The results of this study will aid in the development of a new countermeasure systems for human spaceflight.

Methods: 6 healthy subjects (3 Men/3 Women) participated in the 39th DLR parabolic flight campaign. During the first 15 parabolas, 1 subject was outfitted with a peripheral skin cooling system on their legs, thighs and waist in an upright standing position. Another subject wore the system for the next 15 parabolas. Each subject served as their own control in a cross-over design. To research the impact of cooling on peripheral venous pooling and the cardiovascular system, continuous non-invasive blood pressure, pulse wave velocity and peripheral perfusion (laser doppler and near infrared spectroscopy) were utilized. Near infrared spectroscopy and Laser Doppler were attached to the legs, while Pulse Wave analysis and pulse transit time were recorded from the left arm. In addition, the hydration status of the subjects was determined using bioelectrical impedance analysis before and after the flight to estimate intra- and extracellular water.

Results: We were able to clearly show beneficial effects of peripheral cooling on blood pressure and overall perfusion stability under hyper-gravity. Cardiovascular reactions were most pronounced in the transition phases into hyper-gravity (pull-up before and after the micro-gravity), with relevant reductions of blood pressure, peripheral, microvascular perfusion, and a prolongation of both the peripheral and central pulse wave velocity. The used system for peripheral cooling was able to stabilize these effects, both by reducing the amplitude of change between micro/normo- and hyper-gravity as by raising the baseline perfusion and importantly blood pressure levels.

Conclusions: This study showed the feasibility of this countermeasure system for use in parabolic flight and that it can be beneficial for astronauts, military pilots, and patients after a longer period of bedrest. Since cooling is nondependent on pharmacological interventions it carries a lower risk concerning side effects or allergic reactions. Two more campaigns of parabolic flights are planned for additional 12 subjects to increase the significance of the results.

Successful Ageing: A Longitudinal Study on The Cardiorespiratory Function of Master Cyclists

Amir Fathi¹, Eloise Milbourn¹, Thomas Francis¹, Esme Newton¹, Niharika Duggal², Janet Lord², Norman Lazarus¹, Ross D. Pollock¹, Stephen D. R. Harridge¹

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² University of Birmingham, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research & Institute of Inflammation and Ageing Research, United Kingdom

Introduction: Space flight (extended stay in microgravity (μG)) is considered an analogue model of [accelerated] ageing secondary to disuse and unloading. The impact of μG on some physiological systems, however, can be mitigated through exercise-based countermeasures. On earth, humans lead increasingly sedentary lifestyles, thus confounding the ageing process. To study the inherent ageing process, a group of master cyclists were tested 9 years earlier¹. This longitudinal study compares changes in cardiorespiratory function of master cyclists who still meet the original health criteria to those who have developed disease over time.

Methods: After nine years of active ageing, we re-tested 41 master cyclists ($\sigma^2=35$, $\phi^2=6$) comprised of: i) those that meet the original health criteria² (MC; $n=21$), and ii) those who no longer meet this criteria (DNMC; $n=20$). Body composition was measured using dual-energy X-ray absorptiometry (DEXA). Resting blood pressure and heart rate (RHR) were measured after 10 minutes of supine rest using a finapres. Respiratory function (Forced Vital Capacity, FVC, Forced Expiratory volume, FEV1) was assessed following the American Thoracic Society guidelines. An incremental exercise test was performed on a cycle ergometer to determine maximal aerobic power ($\text{VO}_{2\text{max}}$), maximum heart rate (MHR), ventilatory threshold (VT), VE/VCO_2 (volume of ventilation (L) per litre of CO_2 exhaled), and O_2 Pulse (volume of O_2 taken up per heartbeat (mls beat^{-1})). Subsequent tests determined O_2 kinetics (TAU). Statistical analysis: two-way ANOVA with post-hoc comparisons (t-test) performed using Bonferroni correction; results are mean(\pm SD).

Results: Self-reported cycling volume declined by 37% and 25% in MC and DNMC, respectively ($p<0.001$). There was no change in total body mass, however, fat mass (FM) increased by 20% in MC and 29% in DNMC ($p<0.001$). Fat free mass (FFM) declined by 9% and 6% in MC and DNMC, respectively ($p<0.001$). Systolic and diastolic blood pressures were unchanged. FVC declined by 29% in both groups ($p<0.001$), whilst FEV1 declined by 20% in MC and 22% in DNMC ($p<0.001$). $\text{VO}_{2\text{max}}$ declined by 16% in MC (44.8 ± 5.6 vs. 37.3 ± 5.3 $\text{ml kg}^{-1} \text{min}^{-1}$; $p<0.001$), and 23% in DNMC (44.3 ± 6.5 vs. 34.3 ± 6.8 $\text{ml kg}^{-1} \text{min}^{-1}$; $p<0.001$). MHR declined by 8 bpm in both groups ($p=0.005$), and VT declined by 7% in MC and 17% in DNMC ($p<0.001$). Surprisingly, only RHR (MC; 55 ± 8 vs. 57 ± 8 bpm ns.; DNMC; 55 ± 7 vs. 60 ± 10 bpm $p<0.001$; $F(1,39)=4.34$, $p=0.04$) and O_2 kinetics (MC 27.3 ± 6.3 vs. 26.5 ± 4.8 ns.; DNMC 22.2 ± 4.0 vs. 30.4 ± 13.1 $p<0.001$; $F(1,37)=9.05$, $p=0.005$) exhibited both a significant change with age and a significant interaction with health status. Despite onset of disease (not associated with inactivity) in DNMC cyclists, they could not be differentiated from their MC counterparts on any other indices of cardiorespiratory function.

Conclusions: These results suggest that master cyclists, who no longer meet the original health criteria, show similar changes in most cardiorespiratory indices over nine years as those who have remained healthy. The exceptions being RHR and TAU. This may relate to treatment modalities and/or to the physiological effects of any co-morbidities.

References:

1. Pollock et al. (2015), Journal of Physiology; 2. Greig et al. (1994), Age & Ageing

Hindlimb suspension induces skeletal muscle-specific atrophy in mice

Thalia Zamora-Gómez¹, Moritz Eggelbusch^{1,2,3}, Lianne van Osch¹, Onno van Driel¹, Wendy Noort¹, Rob C.I. Wüst¹

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Introduction: Forced physical inactivity, such as during microgravity in space, reduces muscle mass and ultimately mitochondrial metabolism. Inactivity is known to differentially affect muscle atrophy, capillarisation, and mitochondrial density (Hendrickse et al., 2022 JCSM in press). However, the muscle-specific effects on these alterations are unknown. Here, we induced physical inactivity by hindlimb suspension in mice to study the time course of changes in muscle fiber size, capillarisation, mitochondrial density and respiration. In this preliminary abstract, muscle weight after short and long periods of inactivity, and pilot work on mitochondrial density will be presented.

Methods: Eighteen male mice (age: 8-10 weeks) were used to induce hindlimb suspension. Eight mice served as controls (CON) and were sacrificed without being subject to inactivity. Ten mice were sacrificed after a short period of inactivity consisting of 3 days (ShortT). Eight mice underwent inactivity for a longer period ranging between 7 and 10 days (LongT). Upon sacrifice, the left and right soleus, gastrocnemius medialis, gastrocnemius lateralis, tibialis anterior, extensor digitorum longus and plantaris were dissected, weighted, and quickly frozen in liquid nitrogen. The right SOL muscle of 4 mice was stained for succinate dehydrogenase activity, as a proxy for mitochondrial density. Images were analysed by ImageJ. Statistical analyses were performed with a oneway ANOVA and Tukey Post Hoc test for multiple comparison between and within groups.

Results: Apart from the tibialis anterior, extensor digitorum longus, all other muscles showed a significant reduction in muscle weight after inactivity (p ranging from <0.001 to 0.027; Figure 1). Mitochondrial density was only performed in the soleus muscles of 2 control and 2 LongT animals. SDH activity was not different between groups: 0.323 ± 0.03 in control vs. 0.350 ± 0.05 after long-term inactivity (Figure 2). Data analysis is ongoing, and a more updated version will be presented at the Human Physiology Workshop

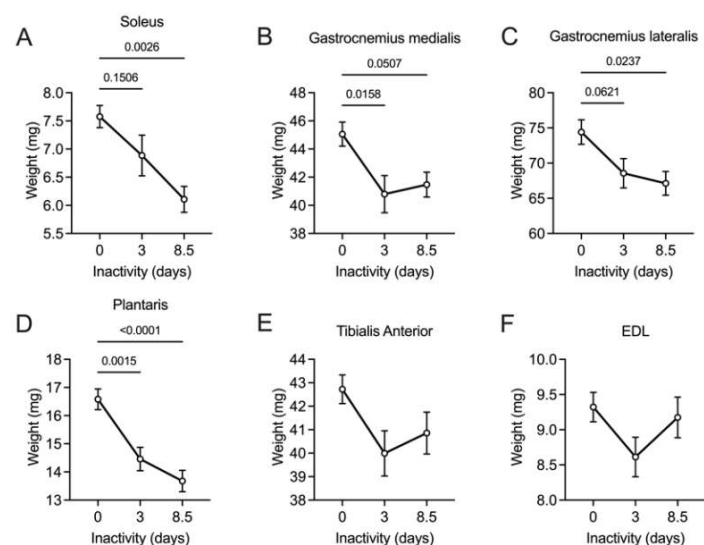


Figure 1. Muscle weights for each muscle with inactivity.

Conclusions: Not all skeletal muscles are affected to the same degree by the physical activity in mice. Since this study is still ongoing, conclusions cannot be drawn yet.

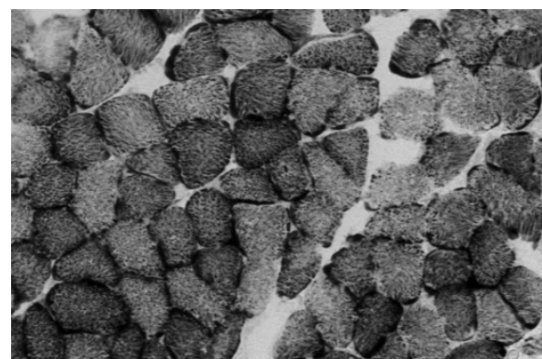


Figure 2. Mitochondrial density analysed through SDH activity in the soleus muscles.

Investigating altered hemodynamic responses to stand test following sinusoidal vibration - varying frequency and bed tilt compilation

Anna Hawliczek¹, Bianca Steuber¹, Karin Schmid-Zalaudek¹, Andreas Roessler¹, Nandu Goswami^{1,2}

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Introduction: Orthostatic intolerance, being the failure to maintain adequate blood flow to the brain, continues to be one of the major issues faced by astronauts upon return to Earth's gravity; however, it also impacts the lives of many older members of the population. The conditions' roots are multifactorial, and the consequences may be serious if the person affected loses consciousness and injures themselves or others. A proposed countermeasure – sinusoidal vibration – has been proven to be a safe solution with proven improvements in areas of muscular and skeletal atrophy, as well as insulin sensitivity. Intriguingly, given how unrelated they seem to be on the surface, both groups – the astronauts, and the elderly - would greatly benefit from addressing these vital concerns. Whole body vibration (WBV) appears to be an attainable mean of training due to low rates of exertion and the passive form of exercise, as improving fitness and cardiovascular fitness by means of standard training is not always an option.

Methods: This research built up on a pilot study carried out at the Medical University of Graz on a group of young and healthy individuals, both male and female; that was a vital element of the study, as females are disproportionately affected by orthostatic intolerance. Four protocols combining two different bed inclines and vibration frequencies were tested: 1. 13 Hz and 15°, 2. 25 Hz and 15°, 3. 13 Hz and 30°, 4. 25 Hz and 30°. The higher bed tilt meant that individual's legs were loaded with a higher percent of their body weight. Two stand tests were performed by the participants – one before and one immediately after the tested vibration protocol – and their physiological responses were monitored throughout. The measurement began with a baseline of 15 minutes (just resting on the bed), followed by the first stand test of 5 minutes. After returning to the bed and recovering for 3 minutes, the vibration platform at the foot of the bed was turned on for 15 minutes. Immediately after the end of the assigned protocol, the participant performed the second stand test. After completing another 5 minutes of unassisted standing, the participant recovered for 10 minutes on the bed.

Results: After analyzing all available data, significant differences were seen for both heart rate and blood pressure responses. When 10-second corresponding epochs were compared (10 pairs), the data showed that the 25 Hz, 30° HUT intervention produced altered responses for pre- and post-vibration values for both systolic and diastolic blood pressure, and the 13 Hz, 30° HUT intervention resulted in lowered heart rate response throughout the vibration phase, as well as increased systolic blood pressure during the vibration and after the stand test. Both vibration protocols were performed on a bed tilted up by 30°, which roughly corresponds to 25% of the participant's body load.

Conclusions: There were changes to the participants' physiological responses to orthostasis following a vibration protocol and a head-up tilt of 30°. It shows that the future optimal vibration protocols might be ruled by the individual's own body-weight loading, and not just the frequency of the vibration. Adjusting the procedure might be crucial for quickly providing the much-needed cardiovascular stimulus for groups at risks of orthostatic intolerance.

Curvature of Gastrocnemius Muscle Fascicles as a Function of MuscleTendonComplex Length and Contraction Level in Humans

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Introduction: In muscle architecture research it is well established to analyze fascicle's length and pennation angle. The most common method is, to expect fascicles and aponeuroses to be straight. However, several studies reported curved fascicles. It has been shown that muscle fascicle curvature increases with increasing contraction level and decreasing muscle-tendon-complex (MTC) length. The analyses in these studies were done with limited examination windows concerning contraction state, MTC length and/or intramuscular position of ultrasound imaging, which makes it difficult to draw conclusions about the cause of fascicle bending. With this study we aimed to investigate the phenomenon of fascicle arching in gastrocnemius muscles in more detail, in order to develop hypotheses about its fundamental mechanism. Additionally, we ventured to study fascicle curvature in relation to potential age and sex related effects.

Methods: Six participants between 20 and 30 years and six between 60 and 70 years of age were tested in five different positions (90°/105°, 90°/90°, 135°/90°, 170°/90° and 170°/75°; *knee / ankle angle). They performed isometric contractions at four different contraction levels (5%, 25%, 50% and 75% of maximum voluntary contraction (MVC)) in each position. Panoramic ultrasound images of both gastrocnemius muscles were collected at rest and during constant contractions. Aponeuroses and up to nine fascicles were segmented in all ultrasound images and the parameters fascicle curvature, deep pennation angle, fascicle length, fascicle strain and intramuscular position have been extracted. Mean curvature of all segmented fascicles within the medial gastrocnemius has been analysed by four different random slope and intercept models with contraction level and MTC strain, mean pennation angle, mean fascicle length or mean fascicle strain as independent variables (models 1a, 1b, 1c and 1d, respectively). The model with best fit (lowest Akaike Information Criterion) was expanded for intermuscular (model 2; + muscle type as fixed two-level factor) and for intramuscular (model 3; + intramuscular position as fixed effect) analysis. Models 1, 2 and 3 have been repeated twice, ones for analysis of sex specific and ones for age specific effects, each by adding the term as fixed two-level factor and interaction term with the independent variable.

Results: Mean fascicle curvature of the medial gastrocnemius increased with contraction (+5m⁻¹ from 0 % to 100 % MVC; p=0.006). MTC length had no significant impact. Mean pennation angle (+0.22m⁻¹ per degree; p<0.001), mean fascicle length (-1.19m⁻¹ per cm; p=0.034) and mean fascicle strain (-0.007m⁻¹ per percent; p=0.004) were correlated with mean fascicle curvature. Best fit and variance explanation of the data was achieved with pennation angle as independent variable. Fascicle curvature as well as the impact of pennation angle on fascicle curvature differed between medial and lateral gastrocnemius (-3.04m⁻¹, p<0.001 and +0.09 m⁻¹, p=0.023). Curvature also varied within the muscle (-3.64m⁻¹ from muscle centre to muscle-tendon-junctions; p<0.001) with additional sex-specific differences in the influence of the intramuscular position on fascicle curvature (-2.20m⁻¹ in males vs - 4.96m⁻¹ in females from muscle centre to muscle-tendon-junctions; p<0.001).

Conclusions: In contrast to previous studies fascicle curvature correlates with contraction level but not with MTC strain. Curvature can be best explained by the deep pennation angle. Similar correlations with pennation angle can also be seen in studies investigating intramuscular fluid pressure. In future studies it would be of great interest to study correlations between intramuscular fluid pressure and fascicle curvature to investigate a potential causal relationship. Additionally, future studies on fascicle curvature should also investigate muscle tendon's mechanics, because of the known influence of sex hormones on tendon stiffness. In general, analyses of muscle architecture should not treat fascicles as straight lines and fascicle curvature should also be included into analysis as architectural parameter.

Metabolomics of young and old muscle: Impacts of aging and exercise on muscle metabolism

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Introduction: Skeletal muscle (SKM) is the largest organ in the human body and plays important roles in exercise and energy homeostasis. SKM is able to secrete factors (myokines) which act in an endo-, para- or autocrine way to influence whole-body metabolism. Aging is associated with loss of muscle mass (sarcopenia) as well as declining muscle quality including reduced oxidative capacity and energy expenditure (Sayer et al., 2008). Diet and exercise are known measures which can lead to healthy aging and decelerated loss of skeletal muscle. In our study we analyzed the secretome of human *soleus* muscle biopsies from young and old participants who were either unactive or active in sports. Also we investigated the effects cAMP-stimulation of these samples.

Methods: We performed untargeted metabolomics to screen for secreted factors of *soleus* muscle biopsies from young (20-35 years) and aged (60-75 years) male participants who were either sportively active or unactive. Tissue samples were stimulated with or without forskolin (30 μM; 30 min.) in order to induce intracellular cAMP signaling, which has been shown to be a positive regulator of muscle metabolism and function (Berdeaux & Stewart, 2012). Subsequently, metabolomics analysis of the secreted factors in the muscle supernatant was performed with UPLC-HRMS (Orbitrap Exploris 120). To cover a broad range of analytes, reversed phase and hydrophilic interaction chromatography were used analyzing a mass range of 40-3000 m/z.

Results: Overall, we could detect 394 different metabolites. The three most abundant metabolite classes were Lipids, Amino Acid metabolism and Nucleotides. Principal component analysis for the two age groups showed an overlap under basal conditions but significant differences after cAMP stimulation. 25 metabolites were significantly upregulated, whereas 11 metabolites were significantly downregulated in all groups. Especially in the young active subgroup forskolin led to significant changes in 12 metabolite profiles.

Conclusions: Under basal conditions young and old; active and unactive muscle show a similar secretome. Regarding the different subgroups, especially, the stimulation of *soleus* biopsies with forskolin from young active participants seems to lead to a distinct secretome, indicating the highest impact of cAMP stimulation in the young active sub-population. In a next step, we will link the metabolomics profiles to expression profiles from RNA Seq data.

Estimating vastus lateralis muscle volume from a single anatomical cross-section

Moritz Eggelbusch^{1,2,3}, Guido Weide¹, Laura Secondulfo⁴, Melissa T. Hooijmans⁵, Richard T Jaspers¹, Gustav J. Strijkers⁴, Peter J.M. Weijs^{2,3}, Rob C.I. Wüst¹

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Introduction: The non-invasive assessment of muscle morphology is valuable in fundamental and clinical research into muscle function. We developed a 3D ultrasound (3D US) technique to reconstruct muscle volume and architecture (Weide et al., 2017 J Vis Exp.129:55943). However, application in large clinical cohort settings is limited due to the time-consuming nature of these measurements. Here, we present an estimation of vastus lateralis muscle volume from a single anatomical cross-sectional area (ACSA) at 50% of muscle length. This would allow muscle volume assessments to be used in largescale clinical studies.

Methods: Twenty-one healthy volunteers (age range: 22-64) with a heterogenous muscle volume participated in this study. Muscle volume of the vastus lateralis (VL) was assessed by 3D US. Intervals of 10% along the muscle length was plotted against relative ACSA, and fourth-order polynomial was made for each individual (Figure 1A). Interpolation of this curve yielded a constant value muscle shape factor to estimate VL volume from muscle length and a single ACSA at 50% of muscle length (ACSA_{50%}, which was in 20/21 subjects the maximum area). A regression model was used to compare equationderived muscle volumes with 3D US values.

Results: Adding the 10% ACSA intervals resulted in a total VL muscle volume of 533 ± 152 (mean \pm stdev) cm³. Estimating VL volume in a heterogenous subset of n=21, using a fourth-order polynomial function (Figure 1A) provided the following equation: $VL_{\text{volume}} = 0.5350(\pm 0.0667) \times \text{Length}_{\text{muscle}} \times \text{ACSA}_{50\%}$. Estimation of VL volume based on this derived equation yielded a mean value of 525 ± 162 cm³, an absolute difference of 37 ± 27 cm³ vs. 3D US assessed volume ($7.3 \pm 5.2\%$ relative difference). According regression to the model, derived our equation explained 91.9% of variance in 3D US scans (Figure 1B). The individual muscle shape factor tended to correlate moderately to muscle volume ($R^2=0.168$, $p=0.07$).

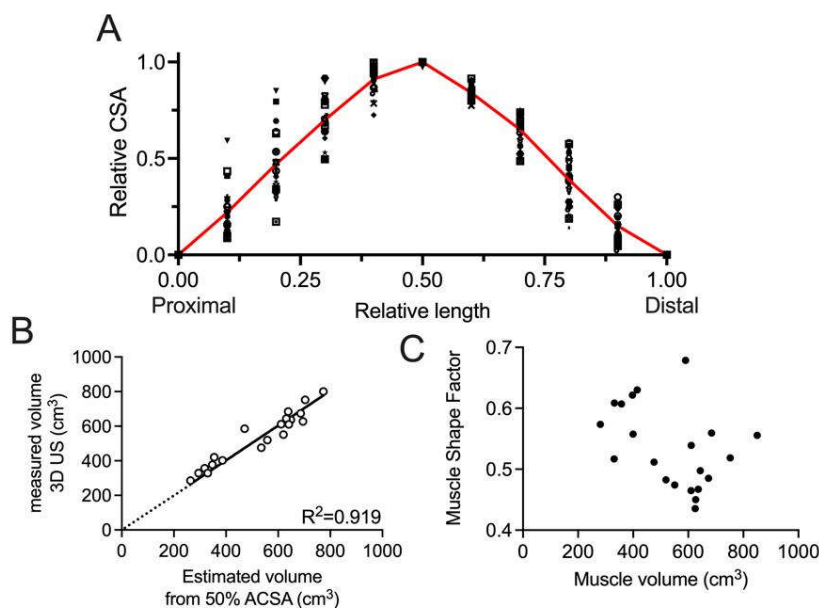


Figure 1. **A:** Fourth-order polynomial function. **B:** estimated muscle volume vs. measured volumes. **C:** muscle volume vs. muscle shape factor.

Conclusions: These results suggest that multiplying muscle length and ACSA_{50%} by a muscle shape factor of 0.535 is a good way to estimate vastus lateralis muscle length. Validation of this method in an independent sample population is in progress.

The ESA Parastronaut feasibility project – Investigating need and contents of physical performance tests for parastronaut candidates

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Introduction: The European Space Agency (ESA) is currently conducting the first astronaut selection in which physically impaired astronaut candidates could become part of the European astronaut corps as part of ESA's "Parastronaut Feasibility Project". Nominal and off-nominal ISS crew activities comprise of a variety of physically demanding tasks in an extreme environment. In previous ESA astronaut selections, astronaut candidates did not have to perform dedicated physical performance tests. Previously, astronaut candidates were only medically tested to assess their health status and to assure their flight readiness. In preparation for, and during the Parastronaut Feasibility Project, the question arose if for parastronaut candidates' additional physical performance tests (PPTs) would be needed to assure their flight readiness, safety, and mission success, and if so which areas of physical performance should be tested to mimic nominal- and off-nominal crew activities during an ISS mission. Moreover, the present study sought to assess if additional PPTs are compatible with the ethical principles of equal opportunities.

Methods: Using a classic digitally conducted Delphi survey with a political slant, a sixty-one-member international panel of experts with expertise in space research, operational human space flight, space medicine, medical ethics and para-sports was interviewed in three rounds. The analysis was carried out by frequency analysis according to quantitative aspects and according to the principles of qualitative content analysis using valence analysis. The results were categorised and included in the results according to their weighting.

Results: A two-third majority of the expert panel was in favour of implementing additional PPTs and recommended to implement additional PPTs for both non-physically impaired and physically impaired astronaut candidates to assure their flight readiness, safety and mission success. Implementing additional PPTs for physically impaired astronaut candidates only was rejected with 89.4% of the votes. According to the expert panel, the skills that should be examined as a priority were first and foremost space mission-specific motor skills of the upper extremities, such as motor learning ability, coordination, dexterity, as well as strength and endurance, also with a focus on the upper extremities.

Conclusions: The main finding of the present study was that additional PPTs were deemed useful to be implemented for both physically impaired and physically unimpaired astronaut candidates to assure their mission readiness. The expert panel suggested that the main aim of these additional PPTs should be to assess physical performance capabilities of mostly the upper extremities. As a preliminary conclusion, mainly due to the wide range of environmental influences and the various forms of protective clothing to which an astronaut is exposed, and to implement the main findings of this study, an adaptation of the "Trondheim Test for Experienced Firefighters" (von Heimburg & Ingulf Medbø, 2013), as well as parts of, and additions to the standardised Astronaut Fitness Test (AFA) (Petersen, Thieschäfer, Ploutz-Snyder, Damann, & Mester, 2015), in combination with assessments from the shallow water training of Eurofighter pilots of the German Air Force is proposed as a starting point to develop a meaningful and valid PPT for future astronaut candidates. Further assessments of nominal and off-nominal crew activities as well as additional validation studies are needed to generate a fair, valid and meaningful PPT for future inclusive astronaut selection campaigns.

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Bone microarchitecture and volumetric bone mineral density after three campaigns of 21-days of 6° Head-Down-Tilt Bed Rest (HDT-BR)

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Introduction: Mechanical stress and unloading are regulators of bone metabolism, structure and density [1–3]. The presented research aimed to study the effects of three campaigns of 21-days of HDT-BR within one year, on bone mineral density (BMD) and micro-architecture at the distal tibia and radius by three-dimensional high-resolution peripheral quantitative computed tomography (HR-pQCT).

Methods: The European Space Agency (ESA)-funded “Medium duration nutrition and vibration exercise” (MNX) study was conducted in a cross-over design with three campaigns (C1 – 3), including 7 days baseline data collection (BDC) days, 21 days of HDT-BR, and 7 days of recovery (R+). A 4-month wash out period separated study campaigns. Twelve healthy male subjects (age 34.2 ± 8.3 years; body mass index 22.4 ± 1.7 kg/m²) participated in the study. The bone evaluation was performed by HR-pQCT (XtremeCT, 82µm, Scanco Medical, Brüttisellen, Switzerland) of the distal radius and tibia (right and left) on BDC-1, R+14 and R+28. After quality control of the images (motion grade ≤ 3) the following parameters were considered: total BMD (Tt.BMD, mg HA/cm³), trabecular BMD (Tb.BMD, mg HA/cm³); cortical BMD (Ct.BMD, mg HA/cm³), cortical thickness (Ct.Th, mm), trabecular separation (Tb.Sp, mm), trabecular thickness (Tb.Th, mm), cross-sectional cortical area (Ct.Ar, mm²), cross-sectional trabecular area (Tb.Ar, mm²). Linear Mixed-Effects Models were used to detect significant changes in bone morphology, including time points and site as predictors, and considering data from BDC of C1 (C1-BDC) and R+28 of C3 (C3-R+28) as time points.

Results: At baseline of C1, the following values (for the distal radius, and tibia, respectively) were measured (mean±standard deviation): Tt.BMD (344 ± 53.8 mg HA/cm³, 340 ± 43.7 mg HA/cm³); Tb.BMD (196 ± 38.4 mg HA/cm³, 214 ± 26.9 mg HA/cm³); Ct.BMD (855 ± 39.0 mg HA/cm³, 868 ± 37.6 mg HA/cm³); Ct.Th (0.9 ± 0.2 mm, 1.4 ± 0.2 mm); Tb.Sp (0.4 ± 0.03 mm, 0.4 ± 0.05 mm); Tb.Th (0.1 ± 0.02 mm, 0.1 ± 0.01 mm); Ct.Ar (73.3 ± 11.8 mm², 154 ± 18.0 mm²); Tb.Ar (280 ± 65.3 mm², 665 ± 122 mm²). At the distal radius (right and left), changes between C1-BDC and C3-R+28 were observed for the following parameters: Tb.Ar (+0.8%; $p < 0.001$), Ct.BMD (+1.6%; $p < 0.001$), Ct.Ar (-1.5%; $p = 0.001$), Ct.Th (-1.6%; $p = 0.005$). Only the change (C1-BDC vs. C3-R+28) in Tb.Sp ($p = 0.031$) was different between hands (radius right and left). At the distal tibia (right and left) changes between C1-BDC and C3-R+28 were observed for: Tb.Ar (+0.6%; $p < 0.001$), Ct.BMD (1.1%; $p < 0.001$), Ct.Ar (-2%; $p = 0.006$) and Ct.Th (-2.9%; $p = 0.001$). The change in Tb.Th ($p = 0.043$) and Tb.Sp ($p = 0.027$) was different between legs (tibia right and left). No significant changes in other bone morphology parameters were noted.

Conclusions: Prolonged bed rest immobilization with three campaigns of 21 days HDT-BR within one-year leads to changes of bone microarchitecture and density, especially in the cortical compartment (Ct. BMD, Ct.Th. Ct.Ar, Tb.Ar).

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Spatial Updating Deficits in Response to Altered Gravity Conditions

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Introduction: Spatial updating, a fundamental skill to continuously update one's body position with respect to its surroundings, is expected to be critically important for operational performance of complex tasks during space missions, such as docking, rendezvous maneuvers, landing, extravehicular activities, and the exploration of new terrains. Previous findings demonstrated deficits in spatial updating performance during micro-gravity when the task was performed in a seated position (Stahn et al. 2020). In preparation for the CIPHER project Spatial Cognition on ISS, we examined the acute effects of alternating gravity levels on spatial updating performance during parabolic flight. The overarching aim of the study was to identify the effects of different gravity levels on spatial updating in a secured free-floating state position. We hypothesized that both, micro- and hypergravity would impair spatial updating performance by altering a fundamental reference frame.

Methods: The experiment was performed as part of the 78th ESA parabolic flight campaign organized by Novespace in Mérignac in March 2022. A total of N=11 healthy subjects (6 female, 5 male), aged 27 to 61 years, volunteered to participate in the experiment. Up to 4 subjects were tested per flight and participants completed a spatial updating performance paradigm during micro- (0 g), normo- (1 g), and hypergravity (1.8 g). As part of this paradigm, participants were asked to encode the positions of two objects followed by either a stationary delay phase or a virtual forward transition. After this delay phase, the participants had to point toward the location of the object. The task consisted of three test conditions (static, short updating, and long updating trials) (see Stahn et al. 2020 for more details). The task was presented using a head-mounted display (Fatshark HD02) while participants were laying in a supine position and fixed with a harness, enabling them to free float 50 cm during the microgravity phase. Each participant performed a total of 90 trials (30 trials per g-level) during 15 parabolas.

Results: During the parabolic flights, all eleven participants were compliant with the task, and a total of 975 responses ($\bar{x} = 88,6$, $R = [86;90]$) were recorded. Preliminary results show that 7 out of 11 participants performed worse during 0 g compared to 1 g for long updating trials although not reaching statistical significance. In the next step, the pre-flight task performance of all participants was considered with respect to in-flight task performance leading to the exclusion of two participants. We reran the analysis and observed a significant decrease in task performance on a group level ($p=0.035$).

Conclusions: This study partly confirms previous findings that report deficits in spatial updating during altered gravity levels (Stahn et al. 2020). Due to the nature of parabolic flights, we have a small sample size, which might have severely influenced our results. Thus, we suspect that the overall poor performances of the two participants masked the g-Level-dependent performance of the other. An additional parabolic flight campaign is planned for November 2022 and will be integrated into further analysis. Following that, we can either show that spatial updating is prone to higher errors in microgravity or suspect that the free-floating position of the subjects reduce the effects of gravitational alterations on spatial updating performance. We speculate that the observed deficits in spatial updating can be attributed to altered vestibular stimulation and distortion of the reference frame associated with Earth's gravity. The significance of these findings relates to the importance of monitoring spatial updating during spaceflights because it is a central component of situational awareness, particularly when vision is poor or unreliable and objects go out of sight, for example during extravehicular activities in space or the exploration of unfamiliar environments.

An in-silico study of fascicle curvature during isometric muscle contractions

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Introduction: Since recent observations showed that fascicles not only shorten during muscle contraction, but sometimes also bend/curve, we developed a theoretical model to reveal the main physical mechanisms of this still unexplored effect. Since the physiological conditions leading to this curvature and also the importance for the inner work of the muscle still need to be researched experimentally, we are trying to test and establish an analogous mechanical muscle model. Mechanical muscle models can be dated back to the pioneering works of Hill [1] or later Zajac [2], which in principle usually treat the entire muscle as viscoelastic elements with active force input. Following the observations of Meyer et al. [3] who used viscoelastic elements to model individual sarcomeres, it was shown that the important value of soft passive tissue provides lateral connectivity of active elements, which are not negligible. But none of these preceding models show fascicle curvature.

Methods: We present a two-dimensional mesh model of muscle fibres that represents fascicles as a sequence of active viscoelastic elements interconnected and nested within passive viscoelastic elements that represent connective tissue and tendon attachments. Based on the observations of the models before, we also include the passive connective tissues and the important connections of the extracellular matrix within our model. The model allows to control the role of partial activation of the muscle tissue and of stiffness changes in the extracellular tissue where the muscle fibres are located. We examine the effects of different model parameters, especially the stiffness coefficient between fascicles and tendon junctions, and tested the hypothesis that in some human subjects, physiological conditions lead to partial muscle activation, which in turn increases the tendency for fascicle curvature.

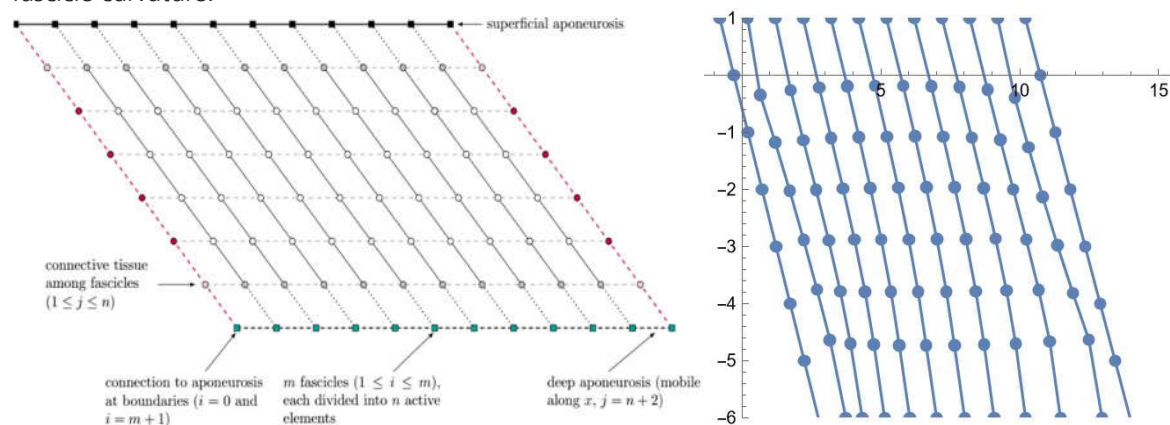


Figure 1. **A. (left)** Schematic of the model of the gastrocnemius muscle, pennated at $\alpha \approx 37^\circ$ of size $(m+2)(n+3) = 12 \times 8$. The fascicles are displayed by solid dark grey lines, while the interconnective tissue among the fascicles is given by dashed lighter grey lines. The fixed aponeurosis is represented by black square nodes; the mobile aponeurosis, which slides along x-direction during muscle contraction, by green squares. The nodes between the aponeurosis and the muscle are grey circles, and those in the connective tissue of the extracellular matrix at the boundary are represented by red circles. The inner $m \times n$ nodes linked by active mechanical components are white circles. **B. (right)** Final state of the simulation with visible fascicle curvature.

Results/Conclusions: The model produces fascicle curvatures and in addition shows that fascicle curvature is dependent on the stiffness coefficient at the tendon junctions, which can be attributed to possible physiological age effects. An example solution in response to force activation shows that there is a non-zero two-dimensional geometric interaction between active contraction and the passive viscoelasticity of the extracellular matrix and tendon junctions.

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Brain hemodynamics during cognitive processing is altered with 60-days of bedrest

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Introduction: Microgravity induces fluid shifts and cardio-vascular deconditioning which may contribute to impaired brain oxygenation and function (De la Torre, 2014), threatening the success of human spaceflight. Long-term hemodynamic changes during real or simulated weightlessness (6° head down bedrest; HDBR) are barely investigated, and the effect of daily artificial gravity (AG) exposure as a countermeasure has not been examined, yet. Based on neurophysiological adaptations to physical exercise, a decrease in neuronal activity (decreased hemodynamic response) during executive cognitive processing in prefrontal areas is suggested to reflect more efficient information processing (VoelkerRehage, Godde & Staudinger, 2011). Hence, we hypothesize that 60 days of HDBR lead to impairments of information processing accompanied by increased neuronal activity (higher oxygenated (O2Hb) and lower deoxygenated hemoglobin (HHb) concentrations) as a compensation mechanism.

Methods: The Artificial Gravity Bed Rest study of the European Space Agency (AGBRESA) was conducted at the :envi hab facility of the German Aerospace Center (DLR) in Cologne, Germany in 2019. 24 participants (aged 33.3 ± 8.9 years, 8 females) spent 60 consecutive days in strict HDBR and were randomly assigned to three experimental groups: intermittent artificial centrifugation (iAG), continuous artificial centrifugation (cAG), and no centrifugation (Ctrl). An ambulatory control group (ambCtrl, $n = 9$, aged 32.5 ± 10.0 years, 3 females) completed the same test battery and schedule at the German Sport University Cologne without HDBR or AG exposure. Hemodynamic changes within the prefrontal cortex were measured during cognitive tasks (auditory and visual attention and working memory tasks) using functional near-infrared spectroscopy (fNIRS; Oxymon, Artinis, Netherlands) at baseline data collection (BDC-4), during head-down tilt bedrest (HDT3, HDT50), and during the recovery phase (R+2, R+13). ANOVA for repeated measures with the factors time, group and their interaction were calculated for O2Hb and HHb, as well as reaction time (RT) and accuracy (ACC) of the cognitive tests. Bonferroni test was used for post-hoc pairwise comparisons. To investigate the acute effect of AG, two HDT sessions took place prior to and directly after centrifugation, respectively.

Results: RT was faster for auditory than visual tasks ($p < .001$), but no difference between modalities in progression over time was observed. A main effect of time ($p = .002$) indicates improvements of RT, which were significant for R+13 compared to BDC-4 ($p = .014$) and HDT3 ($p = .010$) for ambCtrl only. ACC did not change over time, between groups or conditions (all $p > .05$). O2Hb revealed a time effect ($p < .001$) with increased concentrations during bedrest and decreases during recovery for all bedrest groups, which was significant for HDT50 compared to BDC-4, R+2, and R+13 (all $p < .05$). No difference was observed for HHb. No acute effect of AG (pre vs. post AG) was detected within the bedrest groups for O2Hb ($p = .35$), HHb ($p = .14$), RT ($p = .39$), and ACC ($p = .26$). There was no difference between AG protocols (iAG vs. cAG) for O2Hb ($p = .18$), HHb ($p = .15$), RT ($p = .94$), and ACC ($p = .10$).

Conclusions: Performance in attentional cognitive tasks was slightly affected during 60 days of HDBR. These cognitive deteriorations during bedrest may be reflected by increased O2Hb concentration (higher neuronal/oxygen demand) during cognitive task performance. The AG protocols did not seem to sufficiently counteract hemodynamic changes related to HDBR on both physiological and behavioral levels, but did not lead to any deterioration either.

The Effects of Elite Sports at High Age on Volume and Fat Fraction of Hip and Leg Muscles

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Introduction: Intramuscular fat (IMF) of skeletal muscle increases with age, which is associated with a decrease in muscle mass¹. Changes in IMF with age are associated with impaired glucose tolerance and chronic inflammation². We tested the hypothesis that the training of high aged elite athletes participating in sprint and jump competitions not only conserves muscle volume but also reduces the age-related increase in IMF in comparison with age matched controls. We further hypothesized a greater effect of sports on IMF in old age compared with younger age.

Methods: In this study 43 male subjects participated belonging to 4 groups: 10 young athletes (YA, 24±2 years), 12 young controls (YC, 29±5 years), 10 old athletes (OA, 65±4 years), and 11 old controls (OC; 66±5 years). All 4 groups were not different in body height and weight. The athletes competed in sprint or jumping disciplines during national and international events. The controls performed sports only on recreational levels. Continuous axial images from the right leg and the right side of the hips were recorded at 3 Tesla on a Siemens mMR Biograph using a 6-echo DIXON sequence. Segmentation was semi-automatically performed using the advanced muscle segmentation module within the Mimics innovation suite 24 (Materialise, Leuven, Belgium). For each muscle we determined the volume (MV, ml) and the median of the fat fraction (FF; amplitude ratio of 1H signal in CH2 in % of the total 1H signal (CH2 plus H2O)). We analyzed the tibialis anterior muscle and the following muscle groups with their components: the gluteus muscles, the quadriceps muscle, the hamstrings and the triceps surae muscle. For both variables (FF, MV) we used linear mixed effect models to test the effects of age, sport and age*sport. Post-tests of age in each sport group and sport in each age group, respectively, were further performed with Bonferroni correction. The level of significance was a P<0.050.

Results: In all muscles FF was significantly, almost twice higher in the old groups compared with the young groups. Moreover, in both age groups the athlete had less fat than the controls. But in some muscles, especially in the young subjects the effect of sport on FF did not reach significance. For example, in the entire triceps surae muscle we found following FF values (mean ± SE, %): YA 2.6 ± 0.1, YC 3.5 ± 0.4, OA 5.2 ± 0.6, OC 7.0 ± 0.5. LME: age P<0.001, sport P=0.004; post-tests: age in athletes P<0.001, age in controls P<0.001, sport in young P=0.137, sport in old P=0.008. For all muscle groups the volume of athletes was bigger than the volume of controls. However, age affected muscle volume only in the thigh muscles (hamstrings and quadriceps) with smaller volumes found in older subjects. In the gluteus muscles and in lower leg muscles volume was not significantly affected by age. For the triceps surae muscle we measured following values (mean ± SE, ml): YA 801 ± 44, YC 687 ± 36, OA 775 ± 45, OC 715 ± 36; LME: age P=0.856, sport P=0.032; post-tests: all not significant.

Conclusions: The entire gluteus muscles and the triceps surae muscle of elite athletes of high age performing sprint or jumping disciplines almost keep their volume in comparison with young athletes matched for height and weight. The OA only lose little volume in thigh muscles. In all muscles, FF generally increased with age. However, the old athletes could keep FF significantly lower than old controls. FF of old athletes was always on the levels of young controls or only little above. However, it remained an open question, whether the conservation of musculature in terms of volume and FF in OA was only an adaptive consequence of a life-long intensive training, or whether the rare cases of high age elite athletes also profit from genetically determined slower aging processes being a prerequisite of the intensive training in high age.

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Effects of Acute Normobaric Hypoxic Exposure and Capnia Control on Myocardial Inotropism Assessed by Kinocardiography

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Introduction: The increase in cardiac output resulting from acute hypoxic exposure is mainly explained by the increase in heart rate (HR), the stroke volume (SV) remaining unaffected. Kinocardiography (KCG) is a technique based on the combination of ballistocardiography (BCG), reflecting hemodynamic activity, and seismocardiography (SCG), influenced by mechanical cardiac activity at each cardiac cycle. The aim of this randomized complete counterbalanced crossover study is to investigate, via KCG, the cardiac hemodynamic and cardiac mechanical effects of exposure to acute normobaric hypoxia.

Methods: Five exposure conditions were used: three levels (21%, 16%, 13%) of fraction of inspired oxygen (F_{iO_2}) with (isocapnia) and without control of capnia (poikilocapnia) in normobaric hypoxia. Recordings were made on 16 healthy volunteers (26 ± 2 yo), 60 degrees seated, during the first 5 minutes of exposure with a face mask. The volunteers were asked to stay still and not talk while watching the same documentary. Sufficient washout time was allowed between each condition.

Results: A negative correlation between pulse oxygen saturation (SPO2) and the cardiac rotational kinetic energy produced during systole (iK_{QT}^{SCG}) was found ($p = 0.002$). This correlation has a conditional R^2 of 0.95. Under hypoxic exposure with a F_{iO_2} of 13%, switching from poikilocapnia to isocapnia induces an increase in the hemodynamic linear kinetic energy produced during the pre-systolic period (iK_{QT}^{BCG} ; $p = 0.002$) and during systole (iK_{QT}^{BCG} ; $p = 0.003$). In accordance with previous work, SV, measured by finger continuous non-invasive blood pressure, did not change between the different conditions and HR increased with decreasing F_{iO_2} ($p < .001$).

Conclusions: In conclusion, the present results reflect an increase in myocardial contractility in acute hypoxic condition. A stable SV needed to achieve an efficient oxygen transport in hypoxia, requires, depending on the hypoxic stress level, an increase in myocardial contractility and ventricular ejection energy. Also, KCG is a suitable technique to study these adaptations.

Five days of immobilization leads to changes in cartilage biomarker concentrations – first data from the Vivaldi study

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Introduction: Unloading and immobilization induced by dry immersion (DI) reproduces adequately most of the physiological effects of short-term space flight (Tomilovskaya et al., 2019). Articular cartilage is affected by mechanical unloading (Vanwanseele et al., 2002) and the analysis of specific articular cartilage biomarker concentrations was shown to reflect dynamic and quantitative changes in joint remodeling and disease progression at an early stage (Lotz et al., 2013). Previous studies, which examined such biomarkers in response to -6° head-down-tilt bed rest (Liphardt et al., 2017) and microgravity (Niehoff et al., 2016) demonstrated that catabolic processes in articular cartilage were initiated. However, the effects of dry immersion and particularly the impact of unloading models on females regarding changes in cartilage metabolism have not been investigated yet. Therefore, the aim of the study was to analyze the effect of five days immobilization induced by dry immersion on serum concentrations of the articular cartilage biomarkers C2C (type II collagenase cleavage neoepitope), COMP (cartilage oligomeric matrix protein) and TIMP-2 (tissue inhibitor of metalloproteinases 2) in female healthy adults.

Methods: The "Vivaldi" study is part of a research program of the European Space Agency (ESA) and was conducted at the Institut de Médecine et de Physiologie Spatiales (MEDES) in Toulouse (France). Eighteen healthy female volunteers (29±5 years, 165±6 cm, 59±6 kg) completed four days of baseline data collection (BDC), five days of DI and three days of recovery (R). During DI the volunteers were immersed in a water tank for almost 24 hours per day. A special elastic fabric preserves the volunteers from direct contact with water. Due to the surrounding water the volunteers' movements, in particular of the lower extremities, were extremely limited. Fasting, venous blood samples were taken on four days: BDC-1, DI3, R+0 and R+2. The volunteers were immobilized during the measurement time points of DI3 and R+0. Serum concentrations of C2C, COMP and TIMP-2 (sC2C, sCOMP and sTIMP-2) were analyzed with commercially available ELISAs. One-way repeated measures ANOVA, following by LSD post-hoc test, were carried out to detect significant changes in the biomarker concentrations over time.

Results: The sC2C concentration increased significantly from before (BDC-1) to day three of DI (DI3) by +10% (p=0.013). Followed by a further significant increase of +7% from DI3 to R+0 (p=0.02), resulting in an overall increase of +17% compared to baseline (R+0 vs. BDC-1, p=0.002). sC2C levels were still significantly higher during recovery than baseline (R+2 vs. BDC-1, p=0.006). In contrast sCOMP and sTIMP-2 reacted with a significant decrease from BDC-1 to DI3 (p<0.01) and remained reduced until R+0 (R+0 vs. BDC-1, p<0.01). sTIMP-2 showed the highest reduction at DI3 by -7%, while sCOMP indicated the highest decrease at R+0 by -38%. Both COMP and TIMP-2 concentrations increased significantly (p<0.001) after remobilization and reached baseline levels at R+2.

Conclusions: We could show that five days of DI leads to significant changes in the analyzed cartilage biomarkers. These preliminary results suggest that even a short period of unloading and immobilization has significant effects on the articular cartilage metabolism in healthy female adults, which reinforces the need for proper countermeasures on short- and long-term space flights.

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Resting energy expenditure in bed rest: Accuracy of predictive equations

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Introduction: Resting energy expenditure (REE) depicts the amount of energy during stillness of body which is required to sustain all vital functions in individuals at rest. Indirect calorimetry (IC) is considered as gold standard to determine REE by measuring pulmonary gas exchanges. Although various easy applicable predictive equations for estimating REE exist, they do not fully account for different (pathophysiological conditions and individuals. It is still unknown whether certain predictive equations match IC-measured REE in healthy humans confined to bed for a longer time period. To answer this question, we analyzed REE by comparing common predictive equations to IC in healthy individuals exposed to constant bed rest for 60 days.

Methods: REE was measured over 30 minutes under highly controlled conditions via IC (Quark RMR, COSMED Deutschland GmbH, Germany) in 24 healthy individuals (33 ± 9 y, 175 ± 9 cm, 74 ± 10 kg, 8 females, 16 males), participating in the AGBRESA bed rest study. Constant bed rest of each individual lasted for 60 days. Measured REE was compared to commonly used predictive equations determining REE (Harris-Benedict, WHO/FAO/UNU, Müller, Owen, Cunningham, Mifflin) and one created for a sedentary lifestyle (Uchizawa). REE was analyzed before, two times during, and 12 days after bedrest. In addition, fat free mass (FFM) was determined using dual-energy x-ray absorptiometry (DEXA, Prodigy Full Pro device, GE Healthcare GmbH, Solingen, Germany). Body composition measurements were performed twice during baseline data collection, bi-weekly throughout the 60 days of bed rest, and once during the stationary recovery phase correlating REE and FFM changes.

Results: Measured REE was 1643 ± 54 kcal/day at the beginning of the study. REE significantly decreased by 117 ± 25 kcal/d ($p < 0.001$) after 32 days of bed rest. After 55 days of bed rest no further significant decrease could be detected. FFM was reduced by 2.6 ± 0.2 kg during bed rest ($p < 0.001$). 12 days after termination of bed rest REE and FFM reverted back to baseline levels. When comparing predictive equations to IC, nearly all equations overestimated REE in bed rest resulting in a mean accuracy rate of 62%. Only Owen's equation accurately predicted REE for bed rest with a rate of 96%.

Conclusions: REE is reduced during 60 days of constant bed rest in healthy individuals, primarily as a result of decreased FFM. However, Owen's equation seems to be best estimating REE under these conditions. The comparison of measured and predicted REE values during inactivity in our study provides new insights for our society, struggling with a constant decrease in physical activity. Nevertheless, further investigations are necessary determining changes and normalization of REE during and after physical inactivity

The role of inter-subject variability for the effects of countermeasure exercise training on cardiorespiratory fitness in analog and spaceflight studies

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Introduction: Cardiorespiratory fitness (CRF) must be maintained during spaceflight to meet the physical demands of re-entry or future planetary landings in partial gravity (Norsk et al., 2020). Therefore, the efficacy of potential countermeasure exercise training programs is investigated in terrestrial analog studies. With a perspective on future space travel, long-term terrestrial spaceflight simulations are utilized for their operational fidelity and highly controlled environment. However, the lack of microgravity enables CRF improvements, whereas reduced values are still reported after sojourns on the international space station (ISS; Hoffmann et al., 2016). An additional factor requiring consideration is the inter-subject variability of physical fitness between most analog studies' participants and cosmonauts and astronauts. We hypothesize that the magnitude of countermeasure exercise effects on CRF is dependent on premission physical fitness, which might impair the transfer of terrestrial findings to an application in spaceflight.

Methods: CRF was investigated with an exercise test protocol, including moderate-intensity pseudo-random work rate changes to assess heart rate (HR) and oxygen uptake ($\dot{V}O_2$) kinetics and incremental exercise for peak values. The same protocol was implemented in 60 mission days (MD) of head-down tilt bed rest with reactive sledge-jump countermeasure exercise (RSL; N=22; 11 Ctrl; 30±6 years; $\dot{V}O_{2peak}$: 47±9 ml×kg×min⁻¹), 30 MD of simulated spaceflight in isolation with bicycle and strength exercise (HERA; N=16, 40±9 years; $\dot{V}O_{2peak}$: 39±4 ml×kg×min⁻¹), two times 120 MD of simulated spaceflight in isolation with endurance treadmill running and strength exercise training (SIRIUS-19: N=6; 34±6 years; $\dot{V}O_{2peak}$: 41±7 ml×kg×min⁻¹ & SIRIUS-21: N=5; 35±6 years; $\dot{V}O_{2peak}$: 32±2 ml×kg×min⁻¹), and 174±22 MD on the ISS with treadmill and strength training (ISS; N=10; 44±3 years; $\dot{V}O_{2peak}$: 37±5 ml×kg×min⁻¹). The exercise test protocol was performed pre and post (all studies) and at MD 27 (± 4; SIRIUS only), 47 (± 8), 86 (± 4), and 117 (± 1; all SIRIUS & ISS). ANCOVAs with repeated measures on the factor MD were calculated for the variables HR_{peak}, $\dot{V}O_{2peak}$, HR kinetics, and $\dot{V}O_2$ kinetics, including the group variable *study* and pre-mission $\dot{V}O_{2peak}$ as a covariate (CoV).

Results: From pre to post, $\dot{V}O_{2peak}$ showed an MD effect ($P = 0.003$; $\eta_p^2 = 0.193$), a MD*study*CoV interaction ($P < 0.001$; $\eta_p^2 = 0.325$), and a significant between-subject effect for the CoV ($P < 0.001$; $\eta_p^2 = 0.441$). $\dot{V}O_{2peak}$ improved in HERA but was reduced after RSL and ISS. In SIRIUS-19 and SIRIUS-21, improvements in $\dot{V}O_{2peak}$ during the mission were diminished at Post. No effect was found for HR_{peak}. $\dot{V}O_2$ kinetics showed a significant MD*study*CoV interaction ($P = 0.002$; $\eta_p^2 = 0.320$) and effects for CoV ($P = 0.020$; $\eta_p^2 = 0.119$) and CoV*study ($P < 0.001$; $\eta_p^2 = 0.605$) with slowed responses in HERA, RSL, SIRIUS-21, and ISS but accelerations in SIRIUS-19. HR kinetics showed a significant MD*study*CoV interaction ($P = 0.045$; $\eta_p^2 = 0.199$) and a significant between-subject effect for study*CoV ($P < 0.001$; $\eta_p^2 = 0.387$). HR kinetics were accelerated after SIRIUS-19 and SIRIUS-21 but slowed following RSL and ISS.

Conclusions: Countermeasure exercise training effects on CRF differ depending on the study design and the participants' initial level of physical fitness. While the beneficial effects of exercise are mitigated the most by head-down tilt bed rest and microgravity, high outcome variability is also observed in isolation studies. The current findings emphasize the need for individualized exercise prescription and the consideration of individual fitness for participant selection in analog studies. An improved matching might enhance the transfer of findings from analog studies to spaceflight.

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Cellular cardiac alterations in size and mitochondrial density after hindlimb suspension in mice

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Introduction: Humans have evolved to thrive in an environment with gravity. As such, human physiology is altered when exposed to microgravity. One organ that received relatively little attention is the heart. Inactivity has been suggested to lower cardiac volume, but whether this is due to an altered fiber size is unknown. Little information exists about the metabolic changes in cardiomyocytes upon inactivity. Here, we report preliminary data on heart and cardiomyocyte size, and mitochondrial density in mice undergoing hindlimb suspension.

Methods: Inactivity in mice (8-10 weeks old) was induced by hindlimb suspension, either for a short (3 days, N=6), or long term (7-10 days, N=6). Eight mice were sacrificed without inactivity, to serve as controls (CON). After sacrifice, hearts were removed and immediately put into 1mM calcium chloride phosphate buffered saline to pump out remaining blood, and subsequently blotted dry and weighed, and stored in liquid nitrogen. In a subset of two CON and two mice after long-term hindlimb suspension, cross sectional area (CSA) of the cardiomyocytes, and succinate dehydrogenase (SDH) were assessed. Statistical analysis was performed with ANOVA.

Results: Body weight was not different between groups (CON: 22.7±0.8 g, short-term: 21.7±1.3 g and long-term: 22.5±1.4 g). Heart weight was reduced in short- and long-term hindlimb suspension (Figure 1). Cardiomyocyte size was assessed in 2 control and 2 animals after long-term hindlimb suspension. The average cross-sectional area was 443±160 and 308±58 µm² in control and long-term hindlimb suspension respectively. Surprisingly, succinate dehydrogenase activity was higher after long-term hindlimb suspension: 1.22±0.23 vs. 1.55±0.11 x10⁻⁴ A₆₆₀.µm⁻¹.s⁻¹ in control vs. long-term hindlimb suspension respectively.

Conclusions: Heart weight was significantly lower after hindlimb suspension. While experiments are ongoing, the relatively large reduction in cardiomyocyte size suggests that physical inactivity reduces size in mice. What the cause of the higher inactivity-induced mitochondrial density is, is currently unknown. Since data analysis is ongoing, conclusions cannot be drawn yet.

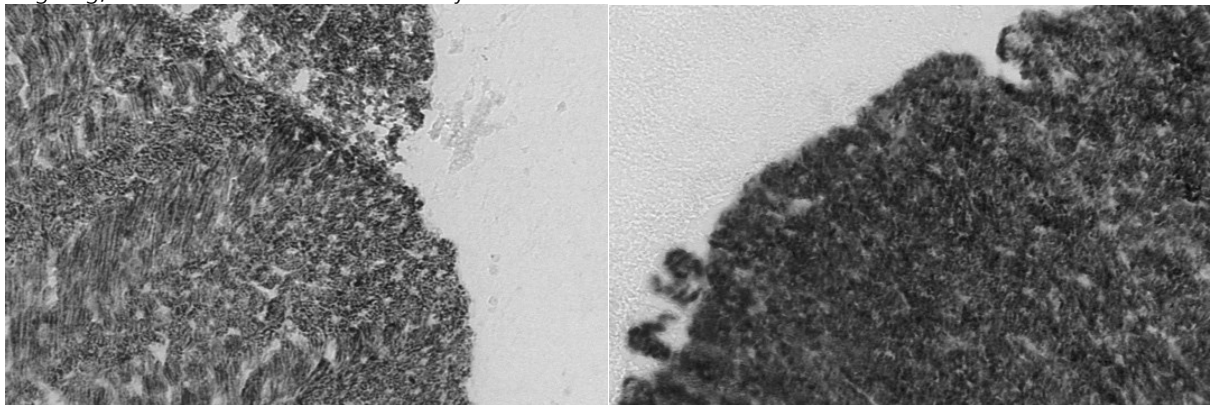
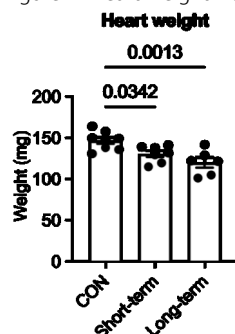


Figure 1: Left: photo of an SDH-stained section of a CON mouse. Right: Photo of an SDH stained section of a mouse after long-term hind limb suspension.

Figure 2: Heart weight was significantly lower after shortterm, and long-term hindlimb suspension in mice compared to control.



Gravitational load modulation bodygear for enhancing muscle activity: A novel countermeasure for maintenance of astronaut health

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Introduction: Muscle atrophy occurs in human muscular system due to inactivity of muscles in microgravity environment. A novel gravitational load modulation bodygear was developed to enhance muscle activity. The bodygear provides axial loading from the shoulders to the feet. In comparison to other countermeasure suits and techniques, the proposed gravitational load modulation bodygear (CGLM bodygear) provides required gravitational loading to the muscles and bones in microgravity. The present study investigated the effects of proposed bodygear, which consists of twenty-four identical stainless-steel springs. The steel springs were used due to their non-magnetic and non-corrosive nature which makes them suitable for long-term use. An adjustable strap was used to adjust the length of the bodygear according to the height of the subject. The force-length relationship of springs was determined according to Hooke's law. Specifically evaluated were the effects of countermeasure gravitational load modulating bodygear on biceps and rectus femoris muscles in volunteers while performing squats.

Methods: Thirty male volunteers between the age of 18-40 years with body mass index (BMI) of 20–30 kg/m² participated. They completed a questionnaire that included specific information on age, physical activity, muscle or bone pain and history of hypertension. The subjects with a history of stroke, head injury, bone injury, neurological and medical disorders were excluded. Effect of bodygear on rectus femoris (RF) and biceps femoris (BF) was evaluated using electromyography (EMG) while performing squat exercises. The Ag/AgCl surface electrodes were positioned on muscles of the right leg according to SENIAM criteria. The study was conducted by performing the squats by subjects with and without bodygear. The unfiltered EMG signals were amplified using a differential amplifier (MP 36 Biopac Systems, USA), sampled at 1,000 Hz, and stored on a personal computer for analysis. All signal processing was performed using MATLAB software. EMG signals were bandpass filtered (zero-lag, fourth-order Butterworth) at 10 Hz to 500 Hz with a notch filter of 60Hz to remove power line interferences.

Results: The time domain results of EMG analysis showed that the bodygear enhanced biceps and rectus femoris muscle activity by 50% and 90%, respectively. The frequency domain results revealed that the rate of fatigue was higher when squats were performed with bodygear as compared to without it.

Conclusions: Bodygear enhances biceps and rectus femoris muscle activity by 50% and 90% respectively and it generates more action potential in lower limbs but without increasing the bodyweight. The muscle activity was increased due to the generation of counterforce against the spring restoration force. Moreover, the results suggest that the bodygear can provide axial loading comparable to the loading observed while standing on Earth (1G). These findings suggest that gravitational load modulation bodygear could be promising exercise countermeasure as well as help in preventing musculoskeletal deconditioning associated with space, aging, and disuse.