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Original Article

Hylonome

Study protocol, implementation, and verification of a short versatile upright exercise regime during 5 days of bed rest

E. Mulder[1], P. Frings-Meuthen[1], M. von der Wiesche[1], G. Clément[2], D. Linnarsson[3], W.H. Paloski[4], F.L. Wuyts[5], J. Zange[1], J. Rittweger[1]

[1]Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany, [2]International Space University, Strasbourg, France, [3]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden, [4]National Aeronautics and Space Administration, Houston, TX, USA, [5]University of Antwerp, Belgium

Abstract

Objectives: This work provides a reference for future papers originating from this study by providing basic results on body mass, urine volume, and hemodynamic changes to 5 days of bed rest (BR) and by describing acute cardio-respiratory/mechanographic responses to a short versatile upright exercise battery. **Methods:** Ten male subjects (mean±SEM age: 29.4±1.5 years; height: 178.8±1.5 cm; body mass: 77.7±1.5 kg) performed, in random order, 5 days of 6° head-down tilt (HDT) BR with no exercise (CON), or BR with daily 25 minutes of quiet upright standing (STA) or upright locomotion replacement training (LRT). **Results:** Plasma volume, exercise capacity and orthostatic tolerance decreased similarly between interventions following 5 days of BR. Upright heart rate during LRT and STA increased throughout BR; from 137±4 bpm to 146±4 bpm for LRT ($P<0.01$); and from 90±3 bpm to 102±6 bpm ($P<0.001$) for STA. **Conclusion:** the overall similarity in the response to BR, and increase in upright heart rate during the LRT sessions suggest early and advancing cardiovascular deconditioning during 5 days of BR bed rest, which was not prevented by the versatile exercise regime.

Keywords: Smith Machine, bed rest, standardization, squat, heel raise

Introduction

Weightlessness causes a loss of mechanical loading of muscles, tendons and bones, and a redistribution of fluids in the body that lead to a gradual reduction of bone, cartilage and muscle mass from the lower parts of the human body, deconditioning of the cardiovascular system, and alterations in metabolism. Kakurin[1] introduced bed rest with head-down tilt (HDT) as a more appropriate model of microgravity than horizontal bed rest. HDT bed rest at an angle of 6° has not only become the standard model for microgravity simulation, but also the model to screen and validate potential countermeasures[2-6].

Physical exercise such as walking and running, can be utilized as effective countermeasures with definite compensatory effects on the cardiovascular and the musculoskeletal systems[7,8]. For bone, the magnitude and frequency of loading are important signals[9,10], whereby 36 cycles of loading per day appear to be just as effective as 360[10]. Static loading is apparently ineffective[11], but fractionation of the training stimulus appears to reinforce the anabolic effect[12]. Given the variety of stimuli that are needed to maintain musculoskeletal and cardiovascular systems, the present study sought to investigate the feasibility and effectiveness of a short-duration exercise battery that aimed at compensating for the lack of habitual locomotion- and gravity-induced stimuli.

The European Space Agency (ESA) started funding bed rest studies in 2001 to complement its program of isolation studies that started in the 1990s. Short-term studies - such as the present study - serve foremost as a first screening of potential promising countermeasures under bed rest conditions. Adaptation and optimization of procedures and protocols are sub-

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Corresponding author: Dr. Edwin Mulder, DLR-Institute of Aerospace Medicine, Department of Space Physiology, Linder Hoehe, 51147 Cologne, Germany E-mail: Edwin.Mulder@dlr.de

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sequently validated using mid-term and long-term studies. If proven to be effective in the present study, the LRT scheme would be implemented in subsequent, longer-duration, ESA bed rest studies, and supplemented by artificial gravity.

The aim of this methodological work is foremost to provide a reference for future publications originating from this broad multisystem study that also examined the responses of the cardiovascular, vestibular, muscular and skeletal systems, by outlining the applied countermeasure and reporting on the acute respiratory and mechanographic responses during upright exercise under strictly standardized study conditions. Basic results on body mass, urine volume and hemodynamic changes to bed rest, as well as changes in plasma volume, exercise capacity and orthostatic tolerance with/without countermeasure are presented as part of the ESA standardized bed rest core data and given for standardized reference purposes. We hypothesized that the versatile upright exercise regime would counter the changes in the abovementioned parameters during 5 days of bed rest, and we based our hypothesis mainly on the work by Vernikos et al.[8]. These authors showed that two and four hours of daily standing or walking partly or completely prevented an otherwise severe impairment of orthostatic tolerance. We reasoned that a more physically demanding combination of gravitational and motor stimuli might have an equal effect even if applied during a shorter time per day.

Methods

Study design

This single-center, crossover study was conducted at the Institute of Aerospace Medicine at the German Aerospace Center (DLR) in Cologne, Germany. A total of three campaigns took place over a six month period. The washout period between the end of campaign 1 and the start of campaign 2 was 50 days; the washout period between the end of campaign 2 and the start of campaign 3 was 94 days. Each campaign consisted of 5 days of baseline data collection (BDC-5 through BDC-1), 5 days of 6° HDT bed rest (HDT1 through HDT5), and 6 days of recovery (R+0 through R+5). All subjects went through three different conditions in a permutated sequence: (a) passive bed rest only (CON); (b) bed rest with 25-min of daily upright quiet standing (STA); and (c) bed rest with 25-min of locomotion replacement training (LRT). At the end of the study, each subject had completed all three interventions.

Each subject was accommodated in a single-person room, equipped with television, telephone, and laptop with internet access. Room temperature within the ward and rooms was kept at 19-22°C. During the bed rest period, the subjects maintained the 6° HDT for 24 h/day. All personal hygiene activities (bowel movement, showering etc.) were performed in the HDT position. Round-the-clock staff monitoring ensured compliance with the protocol throughout the 5-day HDT period. The subjects were not allowed to elevate their heads more than 30° from horizontal. Horizontal displacements were allowed, but static and dynamic muscle contractions were strictly prohibited. During the adaptation and recovery phases, physical activity was restricted to free movement within the ward. The study design was approved by the Ethics Committee of the Northern Rhine Medical Association (Ärztekammer Nordrhein) in Duesseldorf, Germany.

	Age (yrs)	Height (cm)	Mass (kg)
CON	29.7±1.9		77.8±1.5
STA	29.6±1.8	178.8±1.5	78.1±1.5
LRT	29.6±1.8		78.0±1.6

Body fat (%) was determined based on whole body Dual energy X-ray absorptiometry (DEXA).

Table 1. Subject characteristics at baseline.

Subject recruitment

The recruitment process was conducted by the Biomedical Science Support Centre within the Institute for Aerospace Medicine at the German Space Centre (DLR). After the announcement of the study, a telephone screening looked for basic inclusion criteria: male, aged between 20 and 45 years, body mass index between 20-26 kg/m^2, non-smoking, no medication, no competitive athlete, and no history of bone fractures. After passing an information session and a first psychological test, potential volunteers were invited to a medical screening with clearly defined medical exclusion criteria such as: chronic hypertension, diabetes, obesity, arthritis, hyperlipidemia, any hepatic disease, or a disorder of calcium or bone metabolism. Heritable blood clotting disorders (AT III, S-Akt, Lupus-PTT, ferritin, Factor V Leiden, Factor IV, and Factor II) were also screened for and subject exclusion followed if they had positive test results. Volunteers that were medically eligible for the study subsequently underwent psychological screening, involving questionnaires and interviews. The recruitment process was concluded by a dual energy X-ray absorptiometry (DEXA) screening of the bone mineral density of the femur and the lumbar vertebra column and for body composition. Of 213 original volunteers, 55 took part in an information session; 40 were medically screened; 23 participated in the psychological interview; and 16 underwent the screening DEXA scan.

Test subjects

13 volunteers of which 3 were backups passed the screening process successfully and finally 10 were enrolled into the study. One subject discontinued the study on BDC-3 of the first campaign for reasons unrelated to the study. This subject was immediately replaced by a back-up volunteer. The baseline characteristics of the subjects that completed the study are presented in Table 1.

Medical supervision

To ensure safety and well-being of subjects a 24-h medical- and paramedical care was provided. As part of this procedure, blood pressure and heart rate were assessed daily (Intellivue

Exercise type	Repetition rate	No. of repetitions
Bilateral heel raise	1 per 6 s	20
Bilateral squatting (90°):	1 per 6 s	20
Bilateral hopping	3 per s	24 (4 x 6)
		60 s pause (standing)
Unilateral heel raise left	1 per 6 s	12
Unilateral heel raise right	1 per 6 s	12
Bilateral deep squatting (60°):	1 per 6 s	12
Bilateral hopping	3 per s	24 (4 x 6)
		60 s pause (standing)
Unilateral heel raise left	1 per 6 s	12
Unilateral heel raise right	1 per 6 s	12
Bilateral shallow squatting (120°)	1 per 4 s	45
Bilateral cross-hopping	1.3 per s	228
Static squat (90°):	--	1

Table 2. Locomotion replacement training scheme.

MMS X2, Philips, Best, The Netherlands) in the fasting state, immediately following the scheduled wake-up at 6:30 AM (lights off at 10:30 PM). Body mass (BM) was assessed daily following the first urine void of the day (DVM 5703, Sartorius, Goettingen, Germany). All safety parameters (assessed from blood and urine samples, data not shown) were assessed by an independent medical doctor who additionally monitored the subjects' health status during daily ward rounds. The subjects also had to keep a daily log of critical incidents.

Urine collection

Urine was collected daily from 7:00AM (±15 min) to 7:00 AM (±15 min) on the following day. Single voids were stored under darkened and cooled conditions and subsequently pooled to 24-h volumes.

Diet

During the entire study, the subjects received a strictly controlled and individually tailored diet. The individual energy intake (total energy expenditure, TEE) was calculated by multiplying resting metabolic rate, measured by indirect calorimetry (Deltatrac II MBH 200 metabolic monitor, Datex-Ohmeda) by a physical activity level of 1.4 (for light physical activities) during the ambulatory phases (BDC and R) and by 1.1 during HDT, and adding 10% of TEE for energy expenditure associated with thermogenesis from food and beverages. Indirect calorimetry measurements were obtained prior to the start of the first campaign. The assessment of resting metabolic rate was repeated prior to the start of campaign 3 and adjustments in TEE were made, if necessary. 29.7±0.2% of the daily energy intake was consumed as fat, 54.9±2.2% as carbohydrates and protein was taken in the amount of 1.21±0.01 g/kgBM/d. The daily diet was also constant for calcium (1085±62 mg), potassium (3.9±0.3 g), sodium (2.3±0.1 mmol/kgBM) and water (50 mL/kgBM) intake. Additional fluid and energy intake was administered in the form of water and diluted apple juice following physically demanding experiments to compensate for sweat and energy loss. Intake of caffeine, as well as alcohol consumption, was not allowed. The ward is built underground and artificial light illuminated the bed rest facility to maintain a stable day-night-cycle. In order to account for the lack of sunlight exposure, the subjects were supplemented with 1000 IU/d of vitamin D. Vitamins and elements were controlled and standardized as well and achieved as a minimum the recommended Dietary Reference Intakes (http://ods.od.nih.gov). All meals for this study were prepared in a metabolic kitchen where all foods were weighed to ± 0.1 g using laboratory scales. The nutrient content of each prepared meal was calculated, using the PRODI 5.2 software (Kluthe Prodi 5.6 ® expert).

Exercise intervention

Locomotion replacement training (LRT). The subjects were familiarized with the equipment and the proper execution of the exercises during the BDC phase. During HDT bed rest, the subjects were transported in the HDT position from the ward to the exercise physiology laboratory where the daily sessions were carried out in the afternoon. Following 5 minutes of rest in HDT, subjects first sat upright for 2 minutes on the gurney, then stood up and executed the LRT program in the upright position (Table 2).

All sessions were supervised by an exercise physiologist and a medical doctor. A Smith Machine with fixed rails (PTS-1000 Dual Action Smith ™ Cage, Hoist Fitness Systems, San Diego, USA) was used to guide the heel raise and squat exercises (Figure 1). The Smith Machine was implemented not only in order to standardize the movement pattern across subjects, but foremost used to serve as a surrogate sledge system for future short-radius centrifuge applications. The exercises in the present study were therefore performed with a straightened spine, and against body mass only (plus an additional mass of ~15 kg of the barbell), as the current ESA centrifuges do not allow any extra weight application during centrifugation.

Figure 1. An overview of the upright exercises performed during locomotion training replacement (LRT). From left to right: squat and static squat (90°); shallow squat (120°); deep squat (60°); bilateral heel raise; unilateral heel raise (right leg); hopping exercise; and cross-hopping exercise. The squat and heel raise exercises were performed with aid of the Smith Machine. The hopping and cross-hopping exercises were performed outside the Smith machine.

The upright exercises were performed as follows:
- Squats and heel raises against body mass
- The heel raises with straight knees without ankle dorsiflexion.
- The shallow squats continuously for 3 minutes.
- The hopping and the cross-hopping exercises without Smith Machine. The bilateral hops on the ball of the foot (i.e. the heels not touching the ground) at ~3 repetitions per second (metronome: 200 bpm) and fragmented by 15-s rest insertions between each set of 6 hops. Cross-hopping continuously for 3 minutes against ~1.3 repetitions per second (metronome: 152 bpm).

A metronome was used to direct the subjects during all exercises. Except for the duration of the static squat, which increased from 45 s at HDT1 to 70 s at HDT5 for motivational purposes, none of the exercises were progressed during the study. Including the transition between positions and scheduled rest pauses, the LRT sessions were completed in 24-25 min.

In order to evaluate the acute responses to the exercise battery, heart rate was measured during each LRT session by means of a heart rate monitor (FT1, Polar Electro, Kempele, Finland). Oxygen consumption during the LRT session was measured on HDT1, HDT3 and HDT5, using anOxycon Mobile system (Viasys Healthcare GmbH, Hoechberg, Germany) for campaign 1 (4 subjects), and the Cortex Metalyzer system (Cortex Biophysik GMBH, Leipzig, Germany) during campaigns 2 and 3 (6 subjects). For logistical reasons, ground reaction forces were only recorded during the hopping and the cross-hopping exercises on a ground reaction force platform (Leonardo GRFP Mechanography, Novotec medical, Pforzheim, Germany) that was positioned outside the Smith Machine. Pilot testing showed that blood pressure could not be recorded validly during the execution of the exercises. Subjects were asked to rate the perceived exertion of the LRT program using the 15-step (6-20) Borg scale.

Upon signs of orthostatic intolerance (e.g. cold sweat, dizziness, tunnel vision, nausea, slowed responsiveness), or at subject request, the subject were positioned in the HDT position on the gurney. After heart rate and oscillometric blood pressure (Datex-Ohmeda S/5 FM; Datex-Ohmeda Inc., Madison, USA) were checked by the MD the LRT session continued. Pilot testing with a separate set of volunteers before the study showed that the average heart rate response during the initial HDT training session was considerably higher. As the first session was scheduled after only 5-6 hours of HDT, this seemingly elevated heart rate hinted towards a rapid decrease in orthostatic tolerance with bed rest. Considering that we did not have the ambulatory training response before the start of HDT, we implemented an extra training session at R+3 for comparison. All parameters mentioned above, except for oxygen consumption, were obtained during this session, which was otherwise identical to the session performed on HDT5.

Active and passive control conditions

Active control condition: standing (STA). The standing condition was implemented as an 'active' control to elucidate whether the effects of LRT were related to the exercise *per se*, or related to the fact that the exercises were performed in the upright posture. The standing condition was scheduled in the afternoon in the subjects' room. Before the start of each session, the subject was instrumented with the Task Force ® Monitor 3041 (CNSystems Medizintechnik AG, Graz, Austria) to continuously monitor and record heart rate and systolic and diastolic blood pressure. Heart rate was continuously recorded by 3-lead ECG. Each session started with 5 minutes of supine rest in HDT, followed by 2 minutes of upright sitting on the bedside and 25 minutes of quiet standing directly next to the bed. Any type of physical activity other than quietly standing was prohibited. Each session was terminated following 5 minutes of supine rest in HDT. In case of signs of orthostatic intolerance, the same principles as for LRT applied.

Passive control condition (CON). Subjects in the CON group remained in HDT 24h/day for 5 days, where never in the upright posture during this time, and refrained from any type of physical exercise. Resting systolic and diastolic blood

Intervention	Lean mass (kg)	Fat mass (kg)	Body fat (%)
CON	63.9±1.2	14.9±1.1	18.8±1.2
STA	63.6±0.8	14.9±1.1	18.8±1.0
LRT	64.5±1.4	14.6±1.1	18.4±1.3

Table 3. Body composition based on dual-energy X-ray absorptiometry (DEXA).

pressure, as well as heart rate (via blood pressure) were measured in the afternoon in triplicate with 5-min intervals (Intellivue MMS X2, Philips Healthcare, Best, The Netherlands). Mean values were subsequently constructed for further analyses. Subjects remained in their rooms during the assessments and no other experiments were conducted immediately prior, during, or following the measurements.

Intravascular volume

Intravascular volume was determined before bed rest and on the final day of bed rest using the optimized Carbon Monoxide Rebreathing Technique (CORT)[13,14]. In brief, after an initial resting period in the supine position for 20 min during BDC and 6° HDT during HDT5, a baseline 3-ml EDTA blood sample was obtained from an antecubital vein (S-Monovette®, Sarstaedt AG & Co., Nümbrecht, Germany) that was immediately placed on ice for subsequent analyzes. The subjects were then connected to a Krogh-spirometer (Student Spirometer, ZAK, Germany) and started the rebreathing procedure: after a complete exhalation, subjects completely inhaled a ~3 l bag containing pure oxygen. A bolus of ~70 ml CO was simultaneously applied (1.0 ml kg body mass for trained males, 0.8 ml kg body mass for untrained males). After an initial 10 s breath-hold, subjects remained breathing through the mouthpiece until two minutes of CO-rebreathing were completed. Five minutes after the termination of the rebreathing, a second 3-ml EDTA blood sample was obtained. Blood gas and blood count analyses were then immediately performed via routine clinical work using the ABL 520, Radiometer, Denmark, and the ABX Pentra 60 hematology analyzer (Horiba ABX SAS, Montpellier Cedex, France).

Maximal oxygen uptake

Upright testing was performed during BDC and at R+2 using a Lode Excalibur electrically-braked bicycle ergometer. Subjects were instrumented to continuously monitor 12-lead ECG (Padsy, Medset Medizintechnik, Germany) and beat-to-beat blood pressure in the finger (portapres, TNO, Amsterdam, The Netherlands). Oscillometric blood pressure was periodically manually obtained from the brachial artery. Breath-by-breath systemic oxygen uptake (VO2) was obtained using the Innocor system (Innovision, Odense, Denmark). After an initial 5 min of seated rest, subjects were instructed to start (and maintain) a pedaling cadence of 75 rpm against an individual load that initially corresponded to 25% of VO2max (as determined during the initial screening process). After 5 min, the load was sequentially increased to target 50%, and 5 min thereafter to target 75% VO2max. After 15 minutes of cycling the load was increased every minute by 20 Watt until exhaustion. VO2max and maximum heart rate were determined as the mean vale over 30s immediately preceding test termination.

Orthostatic tolerance time

Subjects were instrumented to continuously monitor ECG (Datex Ohmeda, GEHealthcare, Helsinki, Finland) and beat-to-beat blood pressure in the finger (Finometer, TNO, Biomedical Instrumentation, Amsterdam, The Netherlands). Blood pressure in the brachial artery was recorded each 3rd minute throughout the test (Datex Ohmeda, GE Healthcare, Helsinki, Finland). After an initial 20 min of supine rest, the subjects were tilted to 80° head-up tilt (HUT) using an automatic tilt table. The subjects remained in this position for 30 min, or until symptoms of orthostatic hypotension (systolic blood pressure below 70 mmHg, drop in heart rate 20bpm) and/or presyncope (lightheadedness, dizziness, or nausea) occurred. If none of the termination criteria were reached, the 30 min in 80° HUT were followed by the application of lower body negative pressure (LBNP) at -10 mmHg for 3 min with additional increments of -10 mmHg in 3 min stages until termination criteria were reached. Subjects were returned to the supine posture upon termination of the test protocol. Subjects were discouraged from movement, muscle contractions, talking (except to report symptoms), and were encouraged to breathe normally. Orthostatic tolerance time was defined as the time in the 80° upright position, with or without the application of LBNP.

Data processing

Analyses of the LRT sessions. To represent the heart rate for each LRT session, a mean value was constructed based on the heart rate during the squatting, the deep squatting and the shallow squatting exercise. Mean oxygen consumption was calculated for the shallow-squatting exercise, for the cross-hopping exercise and for the session as a whole. For each set during the hopping exercise (2 x 4 sets in total), the mechanographic data were analyzed for peak acceleration and for jump height, using the software provided by the manufacturer (Leonardo GRFP v4.2, Novotec, Pforzheim, Germany). The peak accelerations were normalized for body mass and subsequently expressed in multiples of g. Mean session values for peak acceleration and for jump height were calculated based on the mean value of the 8 sets that were performed during each LRT session. The mean ground reaction peak acceleration during the 3-min cross-hopping exercise was also assessed.

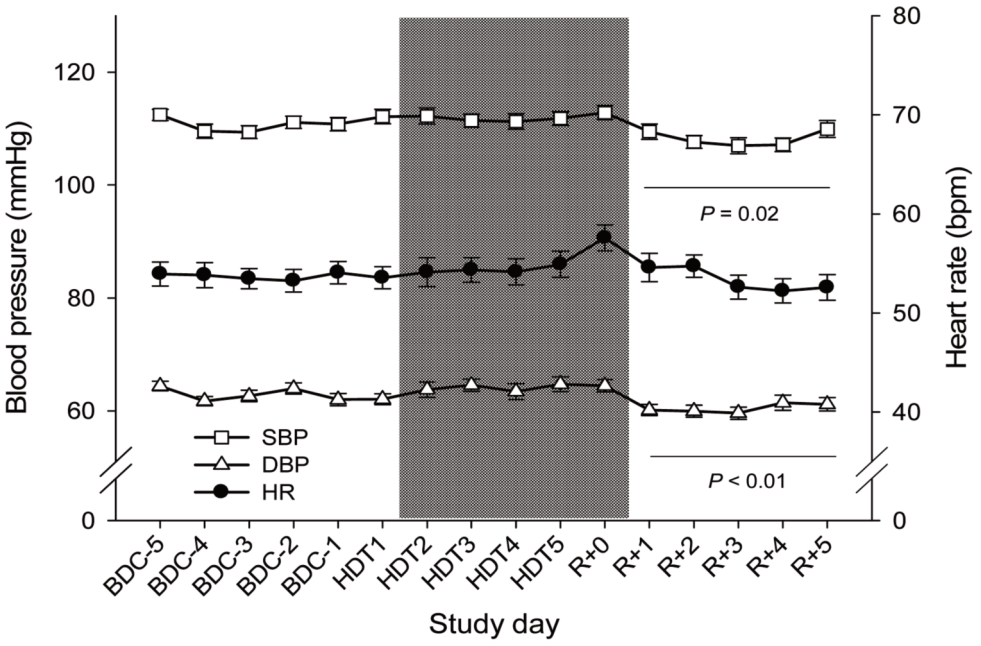

Figure 2. Time course of morning systolic arterial blood pressure (SBP), diastolic arterial blood pressure (DBP) and heart rate (HR) during the study. As no differences between groups were observed, the data are lumped and displayed as means ± standard error of the mean across interventions. The shaded area indicates the HDT bed rest phase. The p-values indicate the difference with BDC.

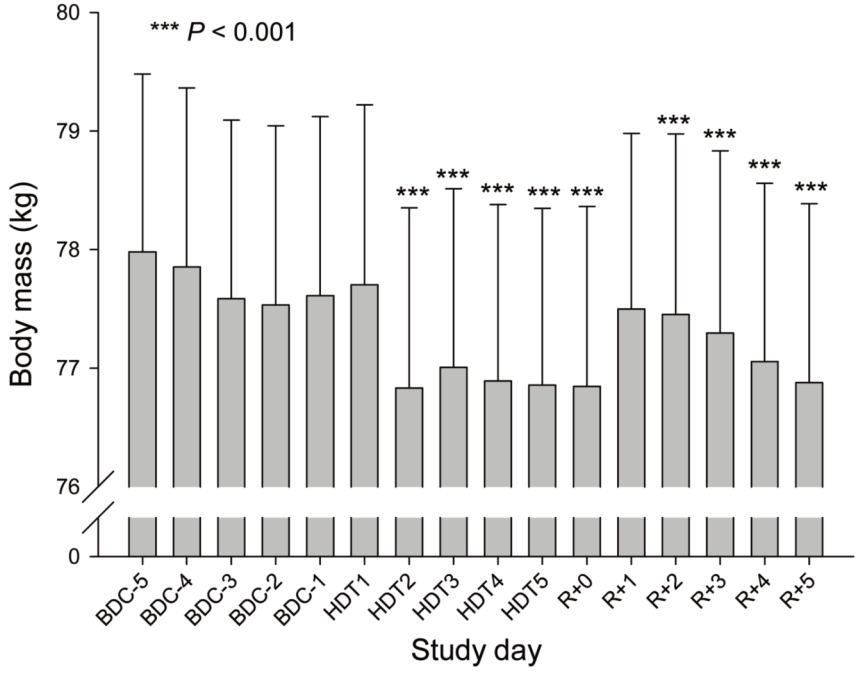

Figure 3. Time course of body mass during the study. As no differences between groups were observed, the data are lumped and displayed as means ± standard error of the mean across interventions. Compared to BDC, mean body mass was lower for all study days (P<0.001) except for HDT1. The shaded area indicates the HDT bed rest phase.

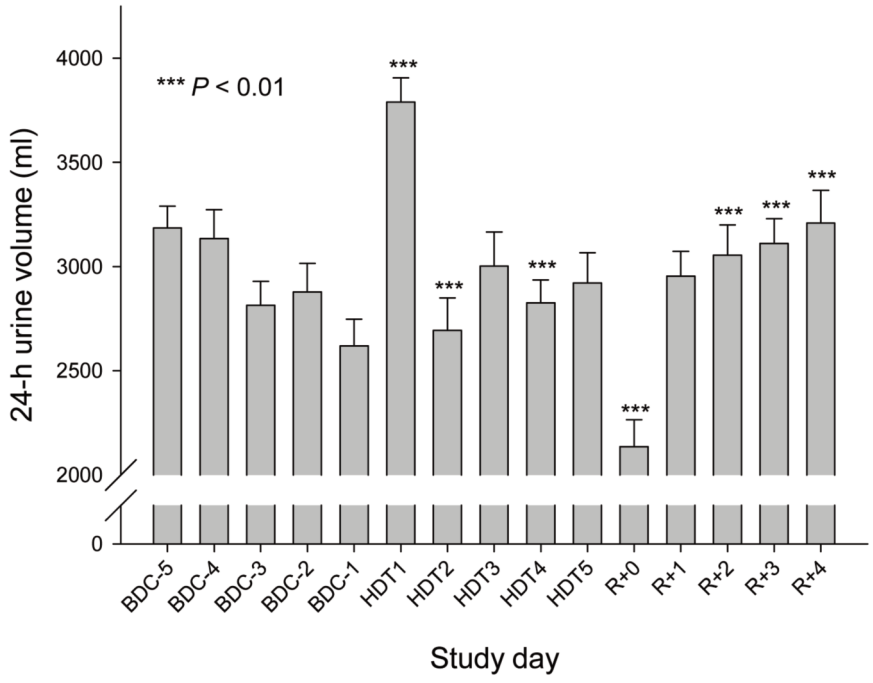

Figure 4. Time course of 24-h urine volume. As no differences between groups were observed, the data are lumped and displayed as means ± standard error of the mean across interventions. *** different (P<0.001) compared to BDC. The shaded area indicates the HDT bed rest phase.

Analyses of the STA sessions. The analysis of the STA sessions was performed by the software provided with the Task Force ® Monitor 3041 (CNSystems Medizintechnik AG, Graz, Austria). Mean heart rate, systolic and diastolic blood pressure were separately analyzed for the HDT, seated and standing phases.

Statistical analysis

Linear mixed effect (LME) models with time and conditions (CON, STA, LRT) as fixed effects and subject ID as random effect were constructed in order to assess condition effects. Variances were allowed to differ between participants and group, and LME models were optimized according to the Akaike information criterion (see p. 353 and p. 652 in [15]). Data were box-cox transformed where indicated by non-linear quantile-quantile plots or in case of heteroscedacity. Initially, all HDT days, as well as the recovery (R) days, were tested against the lumped BDC days. Models were then further simplified in a step-wise manner when no significant intervention effect was found. Firstly, data from all HDT days were lumped together, and so were data from all recovery days to yield the HDT phase and the R phase. When there was still no significant effect, then STA and LRT conditions were pooled to yield CON vs. treatment (TREAT) comparisons. The last step of model simplification was the deletion of intervention effects, so that pure phase effects were analyzed as the simplest model. Statistical analyses were carried out in the "R" statistical environment (version 2.9.2, www.r-project.org). Data are given as means and standard errors (SE) if not indicated otherwise. The level for statistical significance was set to α=0.05.

Results

Bed rest core data

Body composition

Across campaigns, baseline fat mass, lean body mass and percent body fat did not significantly differ between interventions (Table 3; P>0.33).

Morning heart rate and arterial blood pressure

Figure 2 shows the mean values for early morning systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) during the study. No significant differences were observed between interventions for any of the variables and no significant effects of HDT were found during the HDT period. Compared to BDC, however, both SBP and DBP decreased during the recovery phase by 1.2±0.6 mmHg (P=0.02) and 2.0±0.5 mmHg (P<0.001), respectively.

Body mass

Figure 3 shows the time course of body mass during the study. No significant differences between interventions were observed (P>0.10). Compared to BDC, mean body mass (BM) was lower on all study days (P<0.001), except for HDT1 (P=0.85). Reductions in weight ranged between 0.87±0.06 kg on R+0 and 0.22±0.06 kg on R+1.

24-h urine volume

Figure 4 shows the time course of the mean 24-h urine volumes throughout the study. No significant differences between inter-

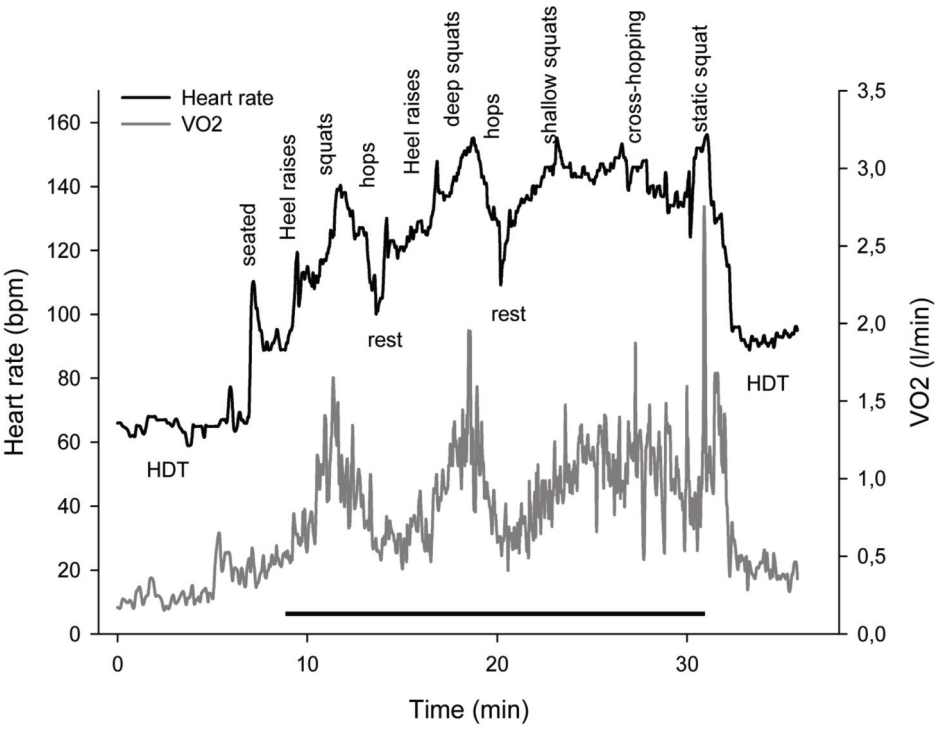

Figure 5. Representative example of the heart rate response (black) and oxygen consumption (VO2, dark grey) during a typical locomotion replacement training (LRT) session for one subject. Depicted are the various exercises, the interposed 1-min rest periods, as well as the start and end conditions in 6° head down tilt (HDT). The black bar represents the ~24 min training period in the upright posture.

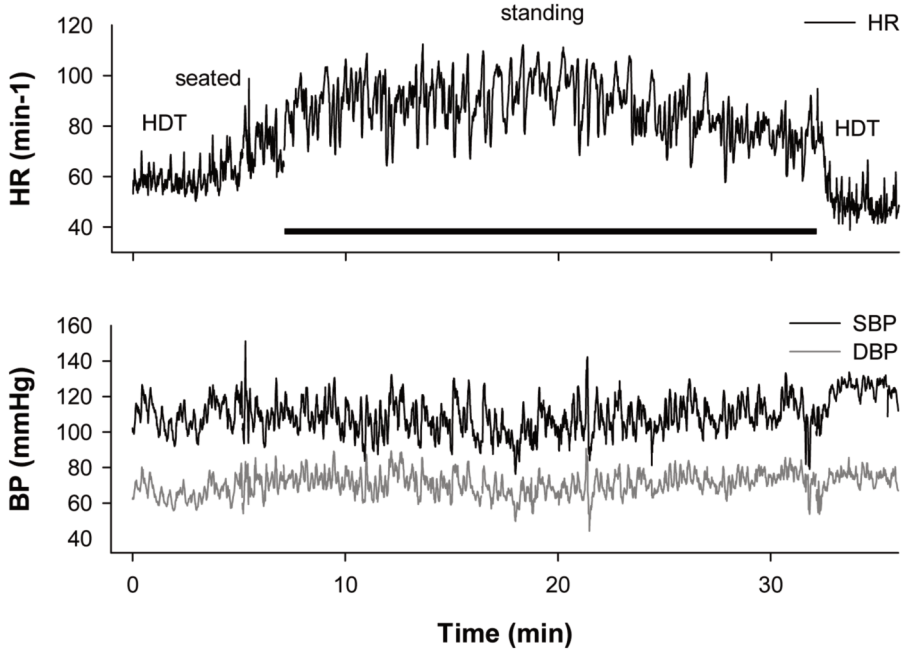

Figure 6. Representative example of the heart rate response (top) and systolic (SBP) and diastolic (DBP) during a typical standing (STA) session. Depicted are the start and end conditions in 6° head down tilt (HDT), as well as the 2-min seated position and the 25-min standing. The black bar represents the 25-min standing period in the upright posture.

	HDT1	HDT2	HDT3	HDT4	HDT5	R+3
Mean LRT heart rate (min-1)	137±4	145±4***	141±4	147±4***	146±4***	124±4***
Oxygen consumption						
- Mean (l/min)	1.06±0.03	--	1.05±0.02	--	1.01±0.02	--
- Shallow squats (l/min)	1.36±0.06	--	1.29±0.04	--	1.26±0.05	--
- Cross-hopping (l/min)	1.64±0.11	--	1.51±0.05	--	1.44±0.04	--
Ground reaction forces						
- Peak acceleration 4 x 6 hops (g)	4.49±0.12	4.40±0.13	4.32±0.07	4.33±0.09	4.18±0.07	4.22±0.07
- Hopping height (cm)	7.51±0.47	7.19±0.37	7.31±0.28	7.52±0.31	7.81±0.38	7.77±0.35
- Mean peak acceleration cross-hopping (g)	1.92±0.10	1.98±0.08	1.78±0.13	1.73±0.14	1.70±0.12*	1.70±0.10*

*Significantly different from HDT1: * P<0.05, ***P<0.001.*

Table 4. Overview of the cardiovascular and mechanographic parameters obtained during the LRT intervention.

ventions were observed. Compared to BDC, 24-h urine volume was elevated at HDT1 (838±123 ml, $P<0.001$), R+2 (151±60 ml; $P=0.012$), R+3 (203±60 ml; $P<0.001$), and R+4 (323±60 ml; $P<0.001$). Compared to BDC, 24-h urine volume was reduced at HDT2 (-237±60 ml; $P<0.001$), HDT4 (-163±60 ml; $P=0.007$), and R+0 (-792±60; $P<0.001$).

Intravascular volume

Total plasma volume decreased significantly from 3828±146 ml at BDC to 3238±79 ml at HDT5 ($P<0.001$), without differences between interventions ($P=0.428$). Likewise, total blood volume decreased significantly ($P<0.001$) from 6427±189 ml at BDC to 5660±126 ml at HDT5, without differences between interventions ($P=0.456$).

Maximal oxygen uptake

Maximal oxygen uptake (VO2max) decreased significantly ($P<0.05$) from BDC (47.06±2.35 ml/kg/min) to R+0 (45.37±1.85 ml/kg/min), without differences between interventions ($P=0.624$).

Orthostatic tolerance

Orthostatic tolerance time (OTT) decreased significantly with bed rest ($P=0.001$); from 33.0±3.1 min during BDC to 19.2±4.1 min at R+0, without differences between interventions ($P=0.128$). The mean OTT for the CON subjects reduced from 35.0±3.1 to 17.2±4.3 min; the mean OTT for the STA subjects reduced from 31.2±3.9 to 19.5±4.6 min, and the mean OTT for the LRT subjects reduced from 33.0±2.8 to 20.9±4.1 min.

Monitoring during exercise and control conditions

Locomotion replacement training (LRT)

Figure 5 shows the heart rate response and oxygen consumption during a single LRT session at HDT1 in one typical subject.

An overview of the cardiovascular and mechanographic parameters collected during the LRT sessions is presented in Table 4. With respect to HDT1, HR was elevated at HDT2, HDT4 and HDT5 (all $P<0.001$) by 7.7±1.94, 9.2±1.90, and 8.5±1.94 min-1, respectively. Mean HR at R+3 was 13.4±1.94 min-1 lower compared to HDT1 ($P<0.001$). Across sessions, VO2 was higher (by 205.7±28.4 ml; $P<0.001$) during cross-hopping than during shallow squatting. Mean session VO2 was 97.9±40.5 ml/min less on HDT5 than on HDT1 ($P=0.035$), but no such difference was found between HDT1 and HDT3 ($P>0.2$). Mean peak acceleration and jump height during the hopping exercise remained unaltered throughout the study (both $P>0.3$). Compared to HDT1, mean peak acceleration during the cross-hopping exercise was 0.22±0.10 g less at HDT5 ($P=0.032$) and -0.22±0.20 g less at R+3 ($P=0.036$).

Borg scale. Subjects rated the intensity of the LRT training higher at HDT4 (13.9±1.9, $P=0.036$) than at HDT1 (12.7±0.9). The rating at HDT5 (13.7±0.4) tended ($P=0.071$) to be higher than the rating at HDT1. The ratings at HDT2 and HDT3 were 13.4±0.5 and 13.4±0.6.

LRT adherence. One subject showed repeated signs of orthostatic intolerance on HDT2 and HDT3 that required a temporary interruption of the LRT session following the shallow squat exercise. At HDT4 and HDT5, the shallow squat exercise was omitted for this subject. For another subject, the LRT was interrupted at HDT4, also following the shallow squat.

Standing condition (STA)

Figure 6 shows a representative example of the heart rate and blood pressure responses during a single LRT session at HDT1.

An overview of the cardiovascular parameters assessed during the STA sessions is presented in Table 5. The data are presented for the different body positions for each session. Across body position, Compared to HDT1, HR increased by 2.48±1.10 min-1 on HDT2 ($P=0.022$), by 3.6±1.39 min-1 on HDT4 ($P=0.010$), and by 7.5±1.5 min-1 on HDT5 ($P<0.001$). Across body positions, no effects of bed rest were observed for both systolic and diastolic blood pressure.

STA adherence. One session was temporarily interrupted, and one session was prematurely ceased at HDT5 due to signs of orthostatic intolerance. A third subject did not complete the

	HDT1	HDT2	HDT3	HDT4	HDT5	R+3
Heart rate (min-1)						
- Supine (head-down)	63.5±2.8	64.1±2.5	62.9±2.5	63.3±2.7	67.6±3.5	66.4±3.3
- Seated	75.5±2.6	78.8±3.0	77.4±2.7	81.9±3.5	82.9±3.9	74.8±3.7
- Standings (mean of 24 min)	89.7±3.2	93.7±3.9	89.9±4.3	95.9±4.4	102.4±6.2	85.9±6.2
Systolic blood pressure (mmHg)						
- Supine (head-down)	117.7±1.8	118.7±3.3	119.7±2.7	119.0±2.2	117.0±2.6	113.1±3.7
- Seated	126.8±5.0	128.2±3.5	131.7±4.7	124.1±4.9	127.8±4.5	123.2±2.7
- Standing (mean of 24 min)	122.4±3.1	119.1±2.9	123.4±3.5	124.0±2.8	117.3±2.7	122.7±2.4
Diastolic blood pressure (mmHg)						
- Supine (head-down)	73.0±2.3	74.3±2.4	76.2±1.8	74.2±2.3	72.2±1.8	67.7±2.7
- Seated	87.1±4.3	89.1±3.3	94.6±4.0	87.7±4.1	87.8±4.1	81.4±1.8
- Standing (mean of 24 min)	83.3±3.3	80.5±2.9	85.5±2.8	82.5±2.9	79.0±2.4	82.2±1.9

Table 5. Overview of the cardiovascular parameters obtained during the STA intervention.

	HDT1	HDT2	HDT3	HDT4	HDT5	R+3
Heart rate (bpm)	57.0±4.3	58.7±2.9	57.3±3.8	63.6±4.9**	60.0±4.4	--
Systolic blood pressure (mmHg)	118.3±2.6	114.8±1.9	115.4±2.7	116.8±2.0	121.8±3.2	--
Diastolic blood pressure (mmHg)	64.3±2.2	63.1±2.6	63.0±3.2	63.7±3.8	68.4±3.4	--
*Significantly different from HDT1: **P<0.01.*						

Table 6. Overview of the cardiovascular parameters during the control condition.

full STA session on HDT5 due to illness unrelated to the test or the bed rest.

Control condition

An overview of the afternoon HR and BP values for CON are provided in Table 6. HR on HDT4 significantly increased by 6.5±1.9 min-1 (P=0.0087) relative to HDT1. Both SBP and DBP tended to increase at HDT5 (P=0.071 and P=0.063, respectively) relative to HDT1.

Discussion

This methodological work foremost aims to provide a reference for future papers originating from this study by a) providing basic results on body mass, hemodynamic changes, and changes in exercise capacity and orthostatic tolerance following five days of bed rest with and without the application of a brief versatile upright exercise countermeasure using the European Space Agency Bedrest Core Data (BDC) and by b) describing the acute cardio-respiratory and mechanographic responses to the exercise battery during 5 days of bed rest.

Locomotion replacement training was tested in a 5-day bed rest study against bed rest only and bed rest with 25 min of upright quiet standing. The results indicated that the time course of morning heart rate, arterial blood pressure, 24-h urine volumes and body mass, as well as the changes in blood and plasma volume, maximal exercise capacity (VO2max) and orthostatic tolerance after 5 days of bed rest were comparable across the three conditions.

During the LRT sessions, the mechanographic and respiratory responses remained virtually unchanged during the bed rest period, suggesting maintenance of functional capacity. However, in light of the observations that the training regime was not beneficial to maintain plasma volume, maximal oxygen uptake and orthostatic tolerance, it is possible that the mechanographic and respiratory responses would have deteriorated following a longer duration bed rest study. Compared to early recovery values at R+3, heart rate during LRT was already significantly elevated during the first day of bed rest (i.e. following 5-6 h or bed rest) and increased further throughout the remainder of the bed rest period. A similar pattern of heart rate increase was observed in the volunteers during quiet standing. In light of the comparable decreases in blood and plasma volume, exercise capacity (VO2max) and orthostatic tolerance across conditions, these acute results indicate that subjects became acutely and less tolerant to the upright body posture during bed rest where both LRT and STA were used.

Bed rest core data

The experimental bed rest model is widely employed to mimic the physical inactivity and the fluid redistribution ob-

served during exposure to real microgravity[3,5,6]. One prominent observation of the present study was the overall similarity in group responses in the so-called ESA bed rest core data; the daily measurements of resting morning body mass, heart rate, blood pressure and 24-h urine volume, as well as the changes in blood and plasma volume, exercise capacity and orthostatic tolerance. In previous studies, changes in resting heart rate and blood pressure as a consequence of bed rest were not always coherent. Convertino et al.[16] found an increase in resting heart rate after 10 days of bed rest in the upright, but not in the supine position. These results concur with the findings of Melada et al.[17], but contrast with the increase in heart rate in 12 subjects following 4 days of bed rest[18]. In the present study, the afternoon resting heart rate was significantly elevated towards the end of the bed rest period at HDT4 and HDT5 (CON and STA), respectively. Longer duration HDT up to 90 days shows a more coherent picture and report a progressive increase in resting heart rate[19,20]. Resting blood pressure did not change throughout the study. These findings concur with previous reports[16,17,19-21], which collectively suggest that blood pressure does not change significantly with bed rest. The abrupt changes in 24-h urine volume and body mass at the start and end of HDT, are presumably related to the fluid shift from the legs to the thoraco-cephalic region in both intravascular and extravascular volumes[22]. The resulting central hypervolemia affects hormonal regulation of fluid excretion and stimulates central cardiac volume receptors, resulting in an increased diuresis[23] and a progressive loss of plasma volume[24,25]. The similarity between groups in changes in the bed rest core data parameters indicates that the LRT intervention and the STA condition applied for 25 min daily did not have any measurable effect on the mechanisms described above.

Monitoring during exercise and control conditions

Upright heart rate increased progressively during LRT and STA throughout the bed rest period, which should be interpreted as a sign of cardiovascular deconditioning[6,26]. The increased heart rate on HDT1 (as compared to R+3) concurs with previous reports that indicate that orthostatic tolerance deteriorates rapidly after the onset of bed rest[27,28]. The necessity to interrupt or prematurely cease the STA intervention in three subjects and the LRT intervention in two subjects due to presyncopal signs also supports this notion. Previously applied daily interventions such as active standing, passive centrifugation, or repeated exposure to lower body negative pressure have been shown to have protective effects against cardiovascular adaptation and orthostatic intolerance[8,22,29-31]. Previously, Vernikos et al.[8] have shown that 4hr of upright walking/standing had a positive effect on orthostatic tolerance, but also that reduced orthostatic exposure had less of an effect. In the present study, the much shorter standing condition was implemented as an active control condition to disentangle the effect of upright body posture and exercise during bed rest. The findings that pre to post bed rest orthostatic tolerance time decreased similarly across conditions implies that the exercise regime was likely too short and also too moderately intense to cause any observable effects under 1g conditions.

Nonetheless, important lessons are still leaned; particularly the occurrence of presyncopal episodes during LRT has implications for designing artificial gravity centrifugation profiles - for which the presently applied exercise battery was designed. Due to the increased gravito-inertial acceleration, blood surely pools more in the lower limbs during hyper gravity centrifugation than under the 1g conditions tested in the present study. Passiveness, and/or exercises that hinder an effective muscle pump during centrifugation, such as quietly standing, and apparently also continuous shallow squatting exercises without rest intervals, would make deconditioned bedridden subjects even more intolerant to gravitational stress than observed in the present study, and hence should be avoided. In contrast, exercises that alternate between activation and relaxation of the targeted muscles and hence have an effective usage of the muscle pump to support venous return, are thought to improve tolerance to centrifugation[32,33]. With respect to the present study, the cross-hopping exercise, which required greater mean oxygen consumption than the shallow squat exercise, was indeed performed with a lower heart rate. Recent evidence suggests that exercise protocols that incorporate large bending of the knees such as squatting can be safely completed without significant after-effects in mediolateral knee position or motion sickness[32,34-36]. The exercises in the present study were executed at a low repetition rate (6 s for one repetition during heel raise and squat, 4 s for one repetition for the shallow squats). As the Coriolis force is proportional to the angular velocity of the rotating environment, the mass of the object, and the linear velocity of the moving object, we expect no deleterious effects for the knee joint during the squatting exercise as performed in the present study. The cross-hopping exercises during the LRT sessions were performed with a repetition rate comparable to those obtained during e.g. cycle ergometry[32] and exercising with a stair stepper[35]. However, unlike such exercises, the knees remain straightened during hopping. We anticipate that the continuous activation of the knee and hip musculature during hopping will stiffen the leg and reject the perturbing Coriolis forces. However, further detailed studies are needed to determine whether hopping exercises can be performed without having deleterious effects on joint integrity on short-radius centrifuges.

In conclusion, daily 25-min bouts of upright locomotion replacement training had no measurable effect on changes in morning heart rate, arterial blood pressure, 24-h urine volume, and body mass throughout 5 days of bed rest. In addition, decrements in blood and plasma volume, exercise capacity and orthostatic tolerance after 5 days of bed rest were also comparable across conditions. Subjects that were daily exposed to upright orthostatic stress showed a rapid and progressive increase in heart rate, and in some occasions episodes of orthostatic intolerance were revealed, suggesting that the prevention of cardiovascular deconditioning to bed rest requires more intense workloads, for longer time periods, and/or a greater number of exposures than those applied in the present screening study. Though it is expected that the training stimulus would

increase with supplementary artificial gravity exposure, an adjustment of the present exercise regime is warranted before commencing its application in a longer-term bed rest study.

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